Gene therapy or interventions in ageing

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Abstract

Studies performed on various experimental model systems indicate that genetic interventions can increase longevity, albeit in highly protected laboratory conditions. Generally, such interventions required partial or complete switching off of the gene and inhibiting the activity of its gene products which normally have other well defined roles in metabolic processes. Overexpression of some genes, such as stress response and antioxidant genes, in some model systems also extends their longevity. Such genetic interventions may not be easily applicable to humans without knowing their effects on human growth, development, maturation, reproduction and other characteristics. Studies on the association of genetic polymorphisms with human longevity have identified several gene combinations which increase the chances of survival to ages beyond ninety years. Whether genetic redesigning can be achieved in the wake of numerous and complex epigenetic factors that effectively determine the life course and the lifespan of an individual still appears to be a mission impossible.

Keywords: anti-ageing, gerontogenes, longevity, homeostasis, homeodynamics, molecular damage, stress response

Introduction

The inevitable consequence of ageing is death. Whatever the duration of “old age” before the final demise may be, this period is generally fraught with increasing chances of occurrence and emergence of one or more diseases. Ageing is the underlying cause of almost all major human diseases, such as atherosclerosis, cancer, cardiovascular defects, cataract, diabetes-2, dementia, macular degeneration, neurodegeneration, osteoporosis and sarcopenia.[1,2] Although the optimal treatments for each and every disease, irrespective of age, is a social and moral necessity, preventing the onset of age-related diseases by intervening in the basic process of ageing may be the ideal solution for maintaining and/or improving the quality of human life and its dignity in old age. Since genes are the fundamental units of information for biological processes, a simplistic view is that gene therapy can be the absolute anti-ageing treatment. While some people have suggested that gene therapy and other interventions for ageing will

be soon able to achieve exceptionally long and healthy lifespans, others have judged such pronouncements as the extreme arrogance of anti-ageing medicine![3]

In this article, we review and evaluate the scientific basis for developing potential therapies and modes of intervention in the process of ageing, which may or may not involve tinkering with genes. In order to fully appreciate these arguments, we first provide a brief overview of the biological understanding of ageing, which is considered to be no longer an unresolved problem in biology.[4,5] This is followed by a critical analysis of various genetic and non-genetic anti-ageing interventions being tested using a variety of experimental model systems but having the aim of improving the quality of human life in old age and of extending human health-span.

Recapitulating the biological basis of ageing

Biogerontology, the study of the biological basis of ageing, has so far unveiled mysteries of ageing by describing age-related changes in organisms, organs, tissues, cells and macromolecules. The large body of descriptive data has led two of the pioneers of modern biogerontology, Leonard Hayflick and Robin Holliday, to declare that ageing is no longer an unsolved problem in biology.[4,5] This declaration does not mean that there are no remaining descriptive data on ageing and that every piece of information about ageing in every biological system has been gathered. The bold assertion by Hayflick and Holliday underlines the fact that biological basis of ageing are well understood and a distinctive framework has been established, which will not get altered significantly with additional descriptive data. Based on the large body of descriptive data, certain general principles of ageing and longevity can be clearly formulated, and these can be the basis for translational research and interventions towards achieving a healthy old age (Table 1).

Thus, ageing has many facets and almost all the experimental data suggest that ageing is an emergent, epigenetic and a meta-phenomenon, which is not controlled by a single mechanism. Although individually no tissue, organ or system becomes functionally exhausted even in very old organisms, it is their combined interaction and interdependence that determines the survival of the whole. A combination of genes, milieu and chance determine the course and consequences of ageing and the duration of survival of an individual.[13]

As regards the role of genes in ageing, there are some misunderstandings that need to be clarified. For example, a lack of specific gerontogenes according to the "non-genetic principle" does not imply that no genes have any influence on survival, longevity and ageing. As will be discussed in a later section below, there is ample evidence from studies performed on yeast, fungi, nematodes, insects, rodents and humans that mutations in certain genes can either prolong or shorten the lifespan in various animals, and cause premature ageing syndromes in humans.[14-16] What is most important is to realise that these genes did not specifically evolve to cause age-related accumulation of damage or to kill the organism. Since their involvement in influencing ageing and longevity is only indirect, they have been termed "virtual gerontogenes",[7,9,17] or "longevity genes",[14] There have been suggestions to further categorise ageing- and longevity-associated genes from evolutionary, biological and physiological angles, which can have a bearing on the prospective of interventions in ageing.[12,18]

Genes that do influence ageing and longevity have evolved in accordance with the life history of a species for assuring the essential lifespan (ELS – see Table 1) long enough for the continuation of generations. The period of survival after ELS, and everything that happens during that period, falls into the evolutionary shadow as it does not determine survival of the species as such.[19-21] There is much supporting evidence for the theory that survival and longevity of a species are a function of the ability of its maintenance and repair mechanisms to keep up with damage and wear-and-tear. The complex processes of maintenance and repair involve hundreds of genes whose products and their interactions give rise to a "homeodynamic space", or "buffering capacity", which is the ultimate determinant of an individual's chance and ability to survive and maintain a healthy state.[8,17] Table 2 gives a list of the key molecular pathways and processes operating in cells, which are quintessential components of the homeodynamic machinery. Theoretically, a fully functional and well maintained homeodynamics could make a biological system immortal.[22]

Treatment or prevention of ageing?

Ageing, diseases and eventual death are the consequences of imperfect homeodynamics. Ageing is characterized by a progressive shrinkage of the homeodynamic space, mainly due to stochastic occurrence and specific accumulation of molecular damage.[8,17] A shrinking

homeodynamic space implies a declining buffering capacity and increased vulnerability, impaired functionality and increased chances of one or more diseases. While most anti-ageing approaches are targeted against the treatment of specific age-related diseases, and these are often more effective and immediate, they do not address the process of ageing itself.[5,47] Furthermore, in the case of a disease, for instance cancer, a treatment will ideally eliminate cancerous cells and restore the affected organ or tissue to its original disease-free state. A similar approach to "cure or treat ageing" is paradoxical. How would such a "treatment" of ageing look like and to what original "age-free" stage should one be restored?

Therefore, rational anti-ageing strategies based on scientific evidence aim to slow down the ageing process by preventing and/or delaying physiological decline and regaining lost functional abilities. Such approaches may be either piecemeal or long term. For example, piecemeal interventions include organ replacement, stem cell injections, and external supplementations to regain youthful levels of hormones, enzymes and micronutrients. Although some of these therapies have demonstrated some clinical benefits in alleviating problems associated with severe deficiencies in old age, none of these piecemeal interventions really act on and modulate the ageing process itself.[23,48-52]

Long term or near permanent prevention of ageing will require effective gene-based interventions to increase the homeodynamic space and to decrease the rate of its shrinkage due to accumulation of unrepaired molecular damage. What follows is a critical review of studies identifying and manipulating genes, directly or indirectly, in the context of their role in modulating ageing and longevity.

Genetic modulation of longevity in model systems

One of the earliest experimental studies which demonstrated that an induced mutation in a single gene can increase the lifespan of an organism was the discovery of the so-called *age-1* mutant in the nematode *Caenorhabditis elegans.*[53,54] Since then hundreds of putative gerontogenes or longevity genes have been reported in *C. elegans, Drosophila* and rodents, which when mutated result in the extension of average and maximum lifespan of the organism. The methods used for the identification of such genes include induction of mutations and deletions by irradiation and chemical mutagens, alterations in gene expression by knockout, homologus recombination, or by gene addition, and reduction in gene

expression by RNAi-induced abrogation of translation (for the latest information on such genes, refer to various online databases, for example: http://genomics.senescence.info/genes/longevity.html, http://wormbase.org/db/misc/site_map?format=searches, http://sageke.sciencemag.org/index.dtl

Table 3 gives a list of genes whose manipulation extends the lifespan of animal model systems including nematodes, insects and rodents. Some genes in other systems, specially in the yeast *Saccharomyces cervisiae*, have also been reported, but are not included here.[55,56]

It is important to note that in several of these studies, and extension of lifespan occurred when one or multiple interventions resulted in the reduction or total inhibition of the activity of one or more genes. Some of the main pathways whose "loss of function" is shown to associate with extended period of survival are: (i) energy generation and utilization in mitochondrial respiratory chain; (ii) nutrition and hormonal sensing and signalling including insulin/insulin-like growth factor-1 and its target forkhead transcription factor FOXO, transcriptional silencing by sirtuin-mediated histone deacetylase; and (iii) translational interference through target of rapamycin (TOR).[82-88] Similarly, there are other examples which show that several mutant mice strains with defects in growth hormone (GH) pathways including deficiencies of GH levels and GH receptor have extended lifespans.[89-91] Application of RNAi technology is further identifying genes whose normal levels of activities are lifespan restricting.[92,93]

In contrast to the above, studies have also been performed in which the effects of adding one or multiple copies of various genes, that leads to the increased expression of their gene products, has resulted in the extension of lifespan. Some such transgenic manipulations in model systems include the addition of gene(s) for one of the protein elongation factors,[94] antioxidant genes superoxide dismutase (SOD) and catalase,[66,95-97] sirtuin dSir2,[98] forkhead trascription factor FOXO,[73], heat shock proteins (Hsp)[62,63,99] heat shock factor (HSF),[64,100] protein repair methyltransferase,[68] and klotho, which is an inhibitor of insulin and IGF1 signalling.[101]

Although theses studies have demonstrated longevity-extending effects of various genes in controlled laboratory conditions, there is very little information available on

the basic process of ageing in terms of the rate and extent of occurrence and accumulation of macromolecular damage and its physiological consequences in these animals. There is also almost no information available as to what is the physiological price paid for inactivating such genes whose normal function is a part of the general metabolism and signaling.[102] There is some evidence that laboratory-protected longevity mutants in *C. elegans* have reduced Darwinian fitness when competing with the wild type worms under nutritionally challenging conditions.[103-106] Similarly, klotho-induced insulin resistance and the paradox of the insulin/IGF-1 signalling pathways in longevity extension seriously question the practicality of such gene manipulations in humans[102,106,107]

Another system in which genetic interventions have been tried as potential anti-ageing therapies is the Hayflick system of limited proliferative lifespan of normal diploid differentiated cells in culture.[108] Almost all the genetic interventions by transient or permanent transfection and ectopic expression of various genes on this model system have focused on extending the replicative lifespan of cells by bypassing the cell cycle check-points.[109-111] One of the most widely used genetic interventions in extending indefinitely the replicative lifespan of normal cells has been the ectopic expression of telomerase in a wide variety of cells.[112,113] However, continuous proliferation by such genetically modified cells often leads to their genomic instability, transformation and cancer-forming activity when injected *in vivo*.[114,115] In the case of animals, whereas telomerase negative mice show reduced lifespan and some other abnormalities after six-generations,[116] overexpression of telomerase in the skin increases myc-induced hyperplasia[117] without any extension of lifespan. Thus it appears that genetic interventions to bypass the Hayflick limit of restricted proliferative potential of normal cells (including stem cells) may lead to carcinogenesis.

Identifying human longevity genes

Although several single gene mutations are known which lead to accelerated ageing, early onset of various age-related disorders and significantly reduced lifespan,[15,18] no gene mutations in humans have yet been identified which increase the lifespan. Another approach that has been developed and adopted for identifying virtual gerontogenes or longevity genes in human beings is by studying association of genetic polymorphisms in candidate genes with human longevity.[118] The evidence that genes do influence lifespan in human beings has

mainly come from the studies performed on centenarians and their siblings, twins and long living families.[119-123] The value of the genetic determinant of longevity was calculated from studies on Danish twins, and it was shown that the heritability of longevity in men and women was 0.26 and 0.23, respectively.[124]

Once it is accepted that genes do exist in humans that can explain some variability in lifespan, the next step is to select candidate genes and a suitable approach to study the association of those genes with longevity. One can either select a candidate gene based on the knowledge about its biological function, or perform a genome wide scan looking for the target areas associated with longevity. The candidate gene approach requires understanding of the related trait and *a priori* assumptions about the biological processes pertinent to the gene, and a hypothesis behind selection of the gene. This is followed by the study of variations in single nucleotide polymorphisms (SNPs) in the candidate gene in order to see if the gene variant co-segregates with the phenotype of long life in a family-based linkage analysis; or if the frequency of a gene variant is more common in a group of old cases as compared to young controls, in a population-based analysis. If the gene variant is shown to be associated with longevity then the next step is to demonstrate how the gene variant alters its function, and how the altered function manifests itself into exceptional longevity.

On the other hand, the genome-wide scan approach does not make any *a priori* assumptions about the pertinent biological processes. In this approach, whole genome scan can be performed to map genes that are linked to longevity using both linkage and association analyses. Then the genes within that target area are studied to localize specific candidate genes. High-throughput genomic technologies have made it possible to perform such studies. For example, a genome-wide scan has linked marker D4S1546 on chromosome 4 with human longevity.[125] However, most of the data available currently on the genetics of human longevity come from the candidate gene approach measuring the frequencies of different alleles, genotypes and haplotypes between old cases and young controls.[118,126,127]

The success of identifying longevity genes depends on the choice of the candidate gene, availability of appropriate samples (study design), and the choice of methodologies with high statistical power. Although polymorphisms in more than 80 genes have been reported which are associated with human longevity in various populations internationally, no reproducible association, except for the APOE gene involved in the

cholesterol metabolism, has been found across the globe.[14,121,128] The lack of reproducibility in results is due to non-compliance to the above three criteria. The choice of candidate genes for human longevity studies has been inspired by the homologous genes in lower organisms where single mutations have been found to modulate lifespan (see Table 3). But the enhancement of lifespan in model organisms via single gene mutation cannot necessarily be translated into humans whose lifespan is much longer and who have a much more complex pathophysiology of ageing.

Much of the available data on the genetics of human longevity come from study designs where gene variation frequencies are compared between long-lived individuals (LLI) and young individuals. This case-control approach has drawbacks with respect to sampling bias. While comparing the two groups for differences in gene frequencies care should be taken that the two groups are similar in all the other factors, such as environment, gender and ethnicity, and should only differ with respect to the phenotype for which they are being studied, which in this case is ability to live long. Hence the real controls for such studies would be those individuals who were born in the same year and were brought up in the same environment as the old individuals but who died before the arbitrary age decided as the longevity cut point. Furthermore, the choice of methodology is crucial for studying the genetics of human longevity. Because longevity is a polygenic trait, instead of comparing the frequencies of alleles and genotypes of one gene between individuals of two groups, one should study a group of variations together either in one gene or many genes. Recent advances have been made in the development of more sensitive methods and study designs for studying genetics of human longevity.[129]

Some genes whose allelic variations have been shown to associate with long lifespan are components of the immune system and the human leukocyte antigen (HLA). These include the haplotypes within the major histocompatibility complex (MHC) region, inflammation and cytokines.[118,121,130,131] Genetic variation in cholesteryl ester transferase protein (CETP) have been reported for long lived Askenazi jews.[132] Some of the genes originally identified in animal model systems, such as human homologue of *Drosophila* gene Indy,[133] Sir2 homologue *SIRT3*,[134] but not *SIRT1*[135] and a functional variant of klotho[136] have also been associated with human longevity. Recently, network of genes involved in cellular and molecular repair and defense mechanisms, such as DNA repair genes and heat shock

protein (HSP) genes, have been analysed for their association with human longevity. Whereas no association of 7 polymorphisms in 4 DNA repair genes with longevity was observed,[137], association of HSP gene variants with longevity have been reported[138-140]

One of the most common limitations of gene association studies with longevity is the lack of data for the relevance at the level of physiological or cellular function. It is not clear as to what extent variations in the frequency of occurrence of certain alleles in LLI translate into the amount, activity and other parameters of molecular function of their respective gene products. However, such analyses are now beginning to be performed, and one of the first studies to do so reported an association of reduced heat shock response in human mononuclear cells with CC genotype of *HSP70-1* gene.[139,141] This gene was also reported to be negatively associated with survival and longevity in Danish LLI including centenarians.[139,141] Therefore, it is very important that polymorphic variations in genes are also studied for their functional effects at the level of gene products and other biological markers. This is extremely important for developing any potential gene therapeutic interventions in terms of which alleles are most effective and desirable.

Issues concerning gene interventions for extended longevity

As discussed above, longevity is a polygenic trait, and at present there is no information how many genes and their variants determine the potential longevity of a species or that of an individual within a species. One of the reasons for this is that, until now, the vast majority of studies in the field have focused on individual genes/proteins, without adequately addressing the possible role of interactions between them. It has now become increasingly clear that biological systems function as complex networks,[142,143] and the cooperation of gene activities may occur at the protein level via protein-protein interactions (PPIs) and eventually by forming PPI networks as a possibility.[144,145] Network-based systems biology approaches for the analysis of the links between longevity-associated genes and genes involved in age-related diseases are being developed,[144,145] which will be necessary to select potential targets of intervention.

Considering that the molecular cause of ageing is the progressive accumulation of macromolecular damage and increased molecular heterogeneity,[8] there are at least three major targets for anti-ageing genetic interventions: (1) increasing the repair of damaged

macromolecules, for example DNA repair pathways; (2) increasing the removal of damaged macromolecules, for example proteasomal and lysosomal pathways; and (3) decreasing the source of damaging agents, for example reactive oxygen species, other free radicals, and reactive sugar metabolites. Whereas the first two targets basically imply achieving genetic enhancement or genetic improvement, the third target requires resetting the metabolic pathways.

All of the above targets for anti-ageing interventions involve hundreds of genes, gene clusters and gene products, whose expression and action are evolutionarily highly regulated in a cell-type-specific manner.[146,147] Although there are several approaches in development for gene-based enhancement of physical strength, endurance, appearance and memory, there are serious technical limitations and ethical and safety concerns that remain to be resolved.[148] Preventing or treating one or more age-related diseases by gene therapy, including stem cells, are at best the piecemeal treatments which often are temporary or become unsuccessful since these are overshadowed by the systemic ageing of the whole body.[149,150] Even in the case of gene therapy as an approach to correct or replace defective genes "responsible" for diseases, there are many methodological steps yet to be perfected. Gene therapy for ageing requires a significant and "intelligent" redesigning, beginning already at the level of the zygote, for better maintenance and survival of the body without having to trade-off with growth, development and reproduction.[151,152] In order to do that it is also necessary to know what combination of genetic variations and their permutations are optimal for indefinite survival. Chances of such an "intelligently redesigned" and directed evolution to succeed in competition with the Darwinian natural selection from much larger random variations and combinations are practically none.

Epigenetic anti-ageing interventions

The next level of complexity for gene manipulation is the crucial role of epigenetics, which is the sum total of interactions of genes and the milieu in which genes happen to operate.[13] Both the "regulated epigenetics", such as 5-methylation of cytosines, histone code through acetylation and non-coding RNAs,[153-156] and "stochastic epigenetics", such as numerous modifications of DNA and RNA nucleotides and of proteins,[157-162] effectively determine the success and failure of a gene action.

Non-genetic factors comprising regulated and stochastic epigenetics influence the gene expression and gene-product-activity determine the course of an individual's life history and its consequences including the occurrence of diseases and the length of lifespan. These epigenetic factors start acting already from an embryonic state and continue throughout the life. Some of the major such factors comprising epigenetic modulators are pre-natal maternal health, nutritional and hormonal status, and post-natal access to nutrition, and exposure to viruses, bacteria and other germs especially until and around the age of puberty.[86,163-171] All these events practically determine the extent of the homeodynamic space (discussed above),[8,17] which is the ultimate determinant of an individual's capacity to maintain health, avoid diseases, and survive as long as possible.

Therefore, an ideal strategy for the prevention or modulation of ageing for extended health-span incorporates genetic and epigenetic interventions. Whereas clearly defective or inefficient genetic pathways which either lead to the emergence of specific diseases or significantly enhance the chances of a disease surely require successful application of the main stream gene therapy approaches, an improvement of the homeodynamic space for better maintenance and survival will be mostly dependent on epigenetic interventions.

A recent approach in epigenetic ageing intervention and prevention is by targetting the homeodynamic space by repeated challenge through mild stress. It is based on observations that low doses of otherwise harmful conditions can challenge and stimulate homeodynamic adaptive responses that benefit cells and the whole organism. The theory behind it, that low doses of toxic or harmful exposure have a protective effect, is known as hormesis. Although the hormesis concept has been defined in different contexts, such as pharmacology and toxicology,[172,173] hormesis in ageing is characterized by the beneficial effects resulting from cellular responses to mild repeated stress.[174] Exposing cells and organisms to brief periods should therefore slow down ageing since the hormetic response to the stressor not only defends the organism against stress but also removes other accumulated damage in cells and tissues. The paradigm for hormesis is exercise, a stressful and damaging activity due to the production of free radicals, acids, stress hormones and cell and tissue breakage. But as an inducer of repair and maintenance processes, the hormetic effect of this strenuous activity has a wide range of health-promoting effects. Some hormetic conditions which have been reported to delay ageing and to prolong longevity in various experimental

organisms include temperature shock, irradiation, heavy metals, pro-oxidants, acetaldehyde, alcohols, hypergravity, exercise and caloric restriction and various phytochemicals such as resveratrol, curcumin and other so-called hormetins.[175] Although there are several unresolved issues regarding the practicality of applying hormesis as an epigenetic intervention in ageing, there is enough evidence in establishing the proof of the principle.[175]

Conclusions

Gene therapy or interventions in ageing are, both in principle and in qualitative terms, different from the more familiar approaches of gene therapy against one or more diseases. Ageing and longevity are polygenic traits for which neither the number of genes involved, their variants, and the extent of interactions are known, nor is the complexity of the epigenetic factors that influence these traits are elucidated at present. Ageing at the molecular level is a progressive increase in molecular heterogeneity leading to interrupted, incomplete and illegitimate macromolecular interacting PPI networks,[8,17] whose exact nature and implications are yet to be understood.

Yet, studies performed on various experimental model systems do indicate that ageing and longevity are amenable to modulation, albeit in highly protected laboratory conditions. Even in those cases, the elements of epigenetic and chance events often determine the quality and duration of lifespan.[176] Furthermore, majority of the putative longevity genes identified so far in model systems require to be switched off or their activities to be highly reduced in order to extend longevity. However, all these genes are part and parcel of normal metabolic processes evolved for normal growth, development and survival. Similarly, other genes whose enhanced expression by genetic interventions leads to extended longevity are also poorly understood for the evolutionary trade-off and for the metabolic price to be paid for tinkering with them. While genetic interventions for slowing down ageing and extending health-span and longevity may be technically a relatively less problematic issue, epigenetic modulators of ageing and longevity bring forth a level of complexity yet to be comprehended.

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Table 1 General principles of ageing and longevity derived from modern biogerontological research

- *Life history principle:* Ageing is an emergent phenomenon seen primarily in protected environments which allow survival beyond the natural lifespan of a species, termed 'essential lifespan' (ELS).[1,6,7]
- *Differential principle:* The progression and rate of ageing is different in different species, in organisms within a species, in organs and tissues within an organism, in cell types within a tissue, in sub-cellular compartments within a cell type, and in macromolecules within a cell.[1,6,7]
- *Mechanistic principle*: Ageing is characterized by a progressive accumulation of molecular damage in nucleic acids, proteins and lipids. The inefficiency and failure of maintenance, repair and turnover pathways is the main cause of age-related accumulation of damage.[4,8]
- *Non-genetic principle:* There is no fixed and rigid genetic programme which determines the exact duration of survival of an organism, and there are no gerontogenes whose sole function is to cause ageing and to determine precisely the lifespan of an organism.[9-12]

Table 2. Main molecular pathways of maintenance and repair which comprise the homeodynamic space

Biological pathway	*Selected references*
1. Antioxidative and enzymic defences against reactive oxygen species.	[23-27]
2. Stress response.	[28-32]
3. Protein repair and chaperoning.	[33-36]
4. Removal and turnover of defective proteins and other cellular components.	[37-40]
5. Nucleic acid repair.	[41-46]

Table 3. A list of putative gerontogenes whose manipulation extends lifespan in animal model systems

Organism	*Gene name (symbol)*	*Normal function*	*Genetic intervention*	*Effect on lifespan*	**Reference**
Caenorhabditis elegans	AGEing alteration (age-1)	Phosphoinositide-3-kinase; a central component of insulin like signaling pathway; lying downstream of daf-2 and upstream of daf-16.	Mutation	Loss of function; a recessive allele of age-1 increases lifespan up to 100%; mutants have a 40-65% increase in mean lifespan and a 65-110% increase in maximum lifespan.	[53,54]
	Abnormal DAuer Formation DAF-2 (daf-2)	Insulin-like/IGF-1 tyrosine kinase receptor; regulator of dauer formation.	Mutation	Loss of function; increase in lifespan by more than 100%; lifespan extension requires the activity of daf-16	[57]
	Abnormal DAuer Formation DAF-16 (daf-16)	Forkhead transcription factor; acts in insulin mediated pathway to affect dauer formation.	Mutation	Suppresses life-extension caused by mutations in daf-2.	[58]
			Overexpression	Modestly increases lifespan (~20%).	
	TachyKinin Receptor family (tkr-1 or old-1)	Tyrosine kinase receptor.	Overexpression	Increased resistance to environmental stress and increased lifespan by 65%.	[59]
	EATing: abnormal pharyngeal pumping (eat-2)	Nicotinic acetylcholine receptor subunit.	Mutation	Loss of function; extends lifespan up to 20-30%.	[60]
	Coenzyme Q7 homolog (clk-1)	Required for ubiquinone biosynthesis.	Mutation	Loss of function; increases adult lifespan by 30%; Clk-1 and daf-2 mutants have five-fold increased lifespan as compared to the wild type.	[61]

	hsp70F	Stress protein	Overexpression	Extends lifespan by 45%.	[62,63].
	Heat shock transcription factor 1 (hsf-1)	Transcriptional factor regulating heat shock response.	Overexpression	Extends lifespan by 22%.	[64].
Drosophila	Insulin receptor substrate-1 (Chico)	Insulin receptor substrate that functions in an insulin/insulin-like growth factor (IGF) signaling pathway.	Mutation	Loss of function; extends fruit-fly median lifespan by up to 48% in homozygotes and 36% in heterozygotes.	[65]
	Superoxide dismutase (SOD1)	Encodes the oxygen radical metabolizing enzyme CuZn superoxide dismutase (SOD1).	Overexpression	Overexpression of a single gene, in a single cell type, the motorneuron, extends lifespan by up to 40%.	[66]
	Superoxide dismutase 2 (SOD2)	SOD2 encodes a mitochondrial manganese superoxide dismutase.	Overexpression	Overexpression in motor neurons extends lifespan by 30%	[67]
	Protein-L-isoaspartate (D-aspartate) O-methyltransferase (pcmt)	Protein carboxyl methyltransferase important for repair of abnormal protein aspartyl residues.	Overexpression	Extends lifespan by 35%.	[68]
	Methuselah (mth)	Member of the seven-transmembrane domain (7-TM) protein superfamily; homology to several guanosine triphosphate-binding 7-TM protein-coupled receptors.	Mutation	35% increase in average lifespan and enhanced resistance to various forms of stressors.	[69]
	I'm not dead yet protein (Indy)	Sodium-dependant citrate transporter; homologous to mammalian sodium dicarboxylate	Mutation	Five independent P-element insertional mutations in a single gene resulted in a near doubling of the average adult lifespan.	[70]

		co-transporter.			
	Insulin-like 1 receptor (InR)	Insulin/IGF receptor; regulates cell growth and proliferation through the dP13K/dAkt pathway.	Mutation	Loss of function; yields dwarf females with up to 85% extension of adult longevity, and dwarf males with reduced late age-specific mortality.	[71]
	Heat shock protein 70 (hsp70)	Heat shock response chaperone.	Overexpression	Increases lifespan by 20-30%.	[72]
	Forkhead box, sub-group O (dFOXO)	Transcriptional regulator.	Overexpression	Increases lifespan and decreases fecundity.	[73]
Rodents	Prophet of Pit1 (Prop1)	Transcription factor involved in hormonal regulation and development.	Knockout	Loss of function; yields dwarves who live approximately 1 year longer than controls. Homozygous Prop1 df/df (Ames dwarf) male and female mice have 49% and 68% increases in lifespan, respectively.	[74]
	POU domain, class 1, transcription factor 1 (Pit1)	Pituitary specific transcription factor. Pit1 is required for normal development of the anterior pituitary.	Knockout	Loss of function; homozygous Pit1 dw/dw (Snell dwarf) mice show a 42% increase in mean lifespan.	[75]
	Growth hormone releasing hormone receptor (Ghrhr)	Growth hormone releasing hormone receptor.	Knockout	$Ghrhr^{lit/lit}$ mice have a 20% increased lifespan.	[75]
	SHC (Src homology 2 domain containing) transforming protein 1 ($Shc1/p66^{shc}$)	Regulation of intra-cellular redox levels, signal transduction, and apoptosis.	Knockout	30% increased lifespan.	[76]
	Klotho (Kl)	Calcium metabolism; involved in the suppression of several aging phenotypes.	Mutation	Reduced lifespan that resembles premature ageing in humans.	[77,78]

	Transformation related protein 53 (Trp53)	Tumor suppressor/DNA-binding transcription factor important for apoptosis.	Mutation	Partial deletion of gene shows signs of premature aging and reduces lifespan.	[79]
	Growth hormone receptor (Ghr)	Growth hormone receptor/growth hormone binding protein.	Knockout	Loss of function; mice homozygous for disruption of Ghr have a lifespan that is 40-50% longer than wild type.	[80]
	Insulin-like growth factor 1 receptor (Igf1r)	Insulin-like growth factor; homologous of tyrosine kinase receptors InR and DAF-2.	Knockout	$Igf1r^{(+/-)}$, heterozygous knockout mice live on average 26% longer than their wild-type	[81]