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REVIEW

Longevity genes across species: conservation versus evolvability

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Abstract

The search for longevity genes has greatly developed in recent years basing on the idea that a consistent part of longevity is determined by genetics. The ultimate goal of this research is to identify possible genetic determinants of human aging and longevity, but studies on humans are limited by a series of critical restrictions. For this reason, most of the studies in this field have been, and still are, performed on animal models, basing on the assumption that fundamental biological mechanisms are highly conserved throughout evolution and that, accordingly, extrapolation from model systems to humans is quite reasonable. Indeed, many comparative data obtained on single genes or gene families fit with this assumption. However, it is also clear that, despite such a basic conservative scenario, major changes also occurred in evolution, particularly regarding biological regulatory processes and integration between and among pathways. This consideration raises the fundamental question of the *transferability* of the results obtained from model systems to humans. In this review, we discuss the differences between animal models and men regarding the genetics of aging and longevity, and the possible reasons that can explain such discrepancies, with a particular emphasis on the phenomena of conservation and evolvability of biological systems. Finally we will suggest a possible strategy to identify putative longevity genes basing on their position inside conserved metabolic structures.

Key words: genetics of longevity; animal models; conservation; evolvability

Introduction

In biological studies, the reductionist approach has been very successful, and the use of simple experimental models allowed the researcher to obtain an exceptional number of results. The most popular example of this approach is represented by the bacteriovorous nematode *Caenorhabditis elegans*, a small worm composed of 959 cells in the adult hermaphrodite form. A defined subset of 131 cells undergoing programmed cell death has allowed to the identification of specific apoptosis-related genes, the so called CED genes (Hengartner and Horvitz, 1994). Cloning of these *C. elegans* genes has revealed that nematodes and mammals share a common pathway for programmed cell death. The same reductionist approach has led to the identification of longevity genes in *C. elegans* since 1988 (Friedman and Johnson, 1988). In 1992, Johnson and Lithgow wrote: "If we are fortunate and aging processes exhibit evolutionary conservation, many exciting possibilities await". At least in some cases, human homologs of invertebrate longevity genes have been found (in particular genes belonging to the IGF-1 signalling pathway, see Barbieri *et al*., 2003). Such similarities are striking and suggest that the insulin/IGF-I regulatory system arose early in evolution and that the fundamental mechanisms that control aging and longevity may be evolutionarily conserved from invertebrates (and even yeast) to mammals, not excluding humans.

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Table 1 Comparison between animals and humans as experimental models for genetic studies on aging and longevity. *shorter life span and genetic inbreeding can be both advantageous and disadvantageous. For example, it is questioned whether the effect of the accumulation of mtDNA mutations on aging can be seen in mice that live only two years at maximum (Santoro *et al.*, 2006). Genetic inbreeding is generally advantageous, but it has hampered to discover the effects of mtDNA haplogroups on longevity, that can only be seen in outbred populations as humans are (De Benedictis *et al.*, 1999).

	Animal models	**Humans**
Advantages	● shorter life span* ● genetically inbred* ● possibility to use very high number of subjects ● controlled environment ● simple organisms (especially if invertebrates) ● subjects can be easily genetically manipulated	● Many genes involved in longevity has been discovered only in human studies
Disadvantages	● shorter life span* ● genetically inbred* ● genetic and epigenetic differences with respect to humans ● different level of complexity with respect to humans	● long life span ● complex organisms ● no possibility of genetic manipulation ● large population-specific genetic differences ● profound influence of the environmental background ● presence of cultural factors

Other than the relative simplicity of the organism to be studied, *C. elegans* has many other evident advantages over different experimental models: for example the short life-span, that allows the execution of experiments in short times (experiments that otherwise will be intolerably longer in animal species with a life span of many years, like primates). Another advantage is the great number of animals that can be studied with a relatively small effort, and most importantly, the possibility to easily manipulate the genome of such animals, in order to obtain data on the role of particular genes in the aging process or in life span control. These and other pros and cons of invertebrates experimental models are listed in Table 1. The main problem with these experimental models is that, for a series of reasons, we were not so fortunate as hoped by Johnson and Lithgow. Indeed, many data obtained on animal models were not confirmed on humans or, on the contrary, some contributions to the genetics of longevity were discovered in humans and not always have been replicated in animals (Franceschi *et al.*, 2007). In this review we will briefly summarise some paradigmatic cases in which results found in animals have not been reproduced in men and *vice versa,* and we will discuss the possible reasons that can explain these differences, with a particular emphasis on two striking features of the conserved biological structures, i.e. conservation and evolvability.

Genetics of aging and longevity 1: the case of p66^{Shc} gene

Possible reason for the discrepancies between data obtained from animal models and humans can be the profound differences in the environment where experimental animals and humans live in. Only recently it has been considered by researchers that the animals used for experiments live all their entire life in safe and pathogen-free environment. They do not have to cope with predators or pathogens, they do not suffer famine, they even do not have to struggle for food, nor to compete for reproduction. This setting assures basic requirement of scientific research, *i.e.* the possibility to *replicate* the results in different laboratories, but of course it is a strongly unnatural situation that may lead to confounding results, especially when studying the genetics of aging and longevity. An example of such situation is given by the discovery that the ablation of p66^{Shc} gene leads to an enhancement of longevity in mice (Migliaccio *et al.*, 1999). A fraction of p66^{Shc} has a mitochondrial localization where it can oxidise cytochrome c and give rise to the production of radical oxygen species (ROS) (Giorgio *et al.*, 2005), and the localization to mitochondria seems to be regulated by PKC-β and Pin-1 (Pinton *et al.*, 2006). Thus, it seems that p66^{Shc} gene impinges upon aging and longevity by regulating the resistance to oxidative stress-mediated apoptosis (Trinei *et al.*, 2002). Then, if p66^{Shc} is a *bona fide*

pro-aging gene, one should expect that long living animals do express this gene at low levels. To date, this hypothesis has not yet been tested. However, we studied $p66^{Shc}$ expression in humans, and, unexpectedly, we found that centenarians have higher levels of $p66^{Shc}$ with respect to young people (Pandolfi *et al.*, 2005). These findings lead to the conclusion that the effect on longevity of $p66^{Shc}$ is either species-specific, or highly dependent on the environmental conditions. Supposing that such effects are not species-specific, it has to be considered that all animal species including humans are evolved and still live in an environment that is profoundly different from that of a laboratory (for example it is very dirty from an immunological point of view), thus it can not be excluded that $p66^{Shc}$ has important effects for the fitness of the organism in the wild that are not necessary in a cage. To say, it is possible that $p66^{Shc-/-}$ animals can have some disadvantages in survival with respect to wild type animals, and that such effects only become evident in the environment where genetic selection occurred. Finally, it must be also considered that the ablation of $p66^{Shc}$ gene can lead to an altered stress response that may be the real responsible for the increased life span observed in $p66^{Shc-/-}$ animals.

Genetics of aging and longevity 2: the case of mitochondrial DNA

The contribution to longevity of the inherited variants of mitochondrial DNA (mtDNA) has been neglected until studies performed on humans addressed the question. In particular, some haplogroups of the mtDNA have been found to be more represented among centenarians and long-living subjects with respect to younger people (Tanaka *et al.*, 1998; De Benedictis *et al*., 1999). This discovery would have never come from inbred animals which share the same mtDNA molecule (mtDNA does not recombine and it is inherited only from the mother). The involvement of mtDNA inherited variants in longevity has not yet been confirmed in animals, but other very elegant experiments in mice have indicated that such variants can modulate functions that are likely important for survival, such as memory and learning (Roubertoux *et al.*, 2003). A possible positive role in longevity for specific somatic mutations of the mtDNA has also been postulated basing on studies on humans (Zhang *et al.*, 2003; Niemi *et al.*, 2005; Rose *et al.*, 2007). The C150T mutation in the D-loop of the mtDNA molecule in particular has been found to be more represented in long-living people with respect to younger counterparts. Such a mutation seems to cause an alternative origin of replication of the mtDNA strands and would be thus advantageous for mtDNA pool maintenance (Zhang *et al.*, 2003). More recently it has been reported that the tendency to accumulate somatic mutations at the mtDNA seems to be genetically controlled, since centenarians and their offspring and nephews share the same levels of mtDNA heteroplasmy at the level of D-loop, which differ from that of unrelated people (Rose *et al.*, 2007). These figures indicate that, despite their usefulness, animal models are unable to identify all the genetic determinants of human longevity, because of intrinsic limitations of the model, or for specific differences between animals and humans. The opposite situation is also possible, that is, results obtained in animal models that can not be reproduced in humans. For example, recent findings indicate that mtDNA point mutations can accumulate in organs and tissues without affecting aging in mice (Vermulst *et al*., 2007), but still the levels of mtDNA mutations found in such a model ($Polg^{mut/+}$ mice) are much higher than that found in aged human colonic crypts (Taylor *et al*., 2003), thus suggesting that the functional impact of mtDNA mutations is likely different in mice and humans (Khrapko and Vijg, 2007).

Genetics of aging and longevity 3: population heterogeneity, heterozigosity and homozygosity

A peculiar contribution to the study of the genetics of aging and longevity has come from observations on the levels of heterozygosity and homozygosity of genetic loci of interest. It is generally accepted that natural selection awards heterozygosity as a way to attain high levels of fitness during the reproductive period. Nevertheless, it was not known whether the levels of heterozygosity were also fitting with longevity. By studying people of different ages including centenarians we observed that, contrary to what it was expected, the level of homozygosity of a locus in chromosome 1p35 was increased in centenarians with respect to young people (Bonafè *et al*., 2001). In a following study, we observed that this locus, rich in Alu sequences, contains a gene called YTHDF2 that shows an increase in homozygosity in centenarians (Cardelli *et al.*, 2006). In this study we genotyped 412 participants of different ages, including 137 centenarians, and we confirmed the increased homozygosity in centenarians at this locus, and observed a concomitantly increased frequency of the most frequent allele and the corresponding homozygous genotype. Remarkably, the same genotype was associated with increased YTHDF2 messenger RNA levels in immortalized lymphocytes. Thus, these data suggest a possible role of this locus in human longevity, and more interesting, they suggest the counterintuitive concept that increased homozygosity can contribute to human longevity. These results has been obtained thanks to the study of a genetically heterogeneous population, as humans are. In animal models, different strains are used, but the animals of each strain are rather homogeneous from a genetic point of view, thus making this discovery almost impossible in such models. When these results will undergo replication in animal models, genetically heterogeneous animals must be used.

The role of the environment 1: the antigenic load

An important environmental factor impinging upon longevity is the antigenic load, *i.e.* the number and intensity of antigenic stimuli to which everybody is exposed during lifetime (De Martinis *et al*., 2005). Besides the life-threatening risks of the exposure to highly pathogenic microorganisms, it is becoming

evident that also the mild chronic exposure to antigenic stimuli (for example to viruses such as Cytomegalovirus, CMV) for a period of time largely unpredicted by evolution profoundly affects the possibility to attain longevity (Pawelec *et al.*, 2006; Vescovini *et al.*, 2007). This life-long exposure to antigenic stimuli is one of the main causes of modifications occurring with age of the immune system, leading to the phenomenon known as immunosenescence and to an increase in inflammatory reactions that favours the onset of many age-associated diseases which do share an inflammatory pathogenic background, such as type II diabetes, cardiovascular diseases, neurodegenerative diseases and many types of cancer. This age-related increase in inflammatory markers has been termed "inflammaging" by our group (Franceschi *et al.*, 2000). The effect of such (acute or chronic) antigenic exposure is forcedly skipped out in most experimental models, where animals live in a pathogen-free environment. In particular, the role of chronic antigenic burden on longevity is extremely difficult to assess in animals living in a clean or even sterile environment. Furthermore, the immune responses are clearly very important in order to attain longevity, and not only the immune system of a mammal is much more complicate than that of an invertebrate, but also the characteristics of immunosenescence, i.e. the age-related modifications of the immune responses, can be strikingly different among species (Pawelec *et al*., 2002). Such a confounding factor can overcome the effect of the gene of interest, thus leading the researcher to the conclusion that such a gene does not play a role in longevity.

The role of the environment 2: the dietary restriction

As mentioned at the beginning of this review, a striking difference between men and animal models regards metabolic regulatory processes. To date, the so called directionality theory distinguishes between species according to the ecological constraints they have to face within their ecological niche (Braeckman *et al.*, 2006). According to this theory, two groups of species are recognized: equilibrium species and opportunistic species. The first group is composed mainly by large mammals, like humans, that spent the most part of their evolutionary history in a stationary growth phase, with a limited but roughly constant amount of resources. The latter group includes many small mammals like rodents, insects and worms like *C. elegans*. These species are subject to fluctuations in size because of irregular availability of resources (periods of abundance and scarcity). It is very likely that species belonging to the two groups, despite the fact that they can share genes or even entire metabolic pathways, may have a different behaviour in terms of survival in face of environmental challenges. For example, the best known treatment that extends life span in animal models (mainly opportunistic species, according to the directionality theory) is dietary restriction (DR), i.e. the reduction in food intake. Studies on *C. elegans* have showed that DR increases life span of such animals (Klass, 1977; Lakowski and Hekimi, 1998; Houthoofd *et al.*, 2002). The same has been demonstrated for other "opportunistic" species that are classical animal experimental models, such as *Drosophila* and mice, but contrasting results have been reported on long living mammals and humans (Kayo *et al*., 2001; Roth *et al*., 2004; Everitt and Le Couteur, 2007). Many data suggest that DR acts as a low intensity stressor and increases metabolic stability (Masoro 1998, Butov *et al.*, 2001). Equilibrium species, like humans, already have a strong metabolic stability, and thus DR would have a relatively small effect on such species (Braekman *et al.*, 2006; Demetrius, 2006). From survival studies on overweight and obese people, it is estimated that long-term DR could add 3 to 13 years to human life expectancy (Holloszy and Fontana, 2007), quite far from the dramatic effects observed in worms and rodents, and in any case much lower than the effects of improved life-style conditions.

One of the effects of DR is the reduction of the insulin/IGF-1 signalling pathway (Clancy *et al*., 2002), and, as mentioned, this pathway appears to be conserved throughout evolution from yeast to mammals. Genetic studies on humans have indicated that some polymorphisms of genes involved in insulin/IGF-1 pathway (IGF-1R, PI3KCB) are associated with longevity (Bonafè *et al.*, 2003). These polymorphisms are correlated to low levels of IGF-1, and this finding fits with the data obtained in experimental models, confirming that lower activity of the insulin/IGF-1 pathway is a major determinant of longevity and that genes involved in such a pathway can be considered longevity genes. It is to note however that these and other genes, such as mTOR and $p66^{Shc}$, increase longevity when they are (at least partially) turned off, and their ablation often produces dwarf animals with a series of defects such as obesity and decreased fertility (Longo and Finch, 2003). Moreover, it has been reported that high levels of IGF-1 and low levels of pro-inflammatory cytokines such as IL-6 are beneficial for muscle mass maintenance and are associated with decreased risk of mortality in old people (Barbieri *et al.*, 2003). Thus, there is a clear trade off between early life fitness and longevity, and every species is the result of a million-years evolution that tuned between these two contrasting goals, reaching a peculiar equilibrium for any species. Hence, comparison between species in which this trade-off has led to a different equilibrium is risky. However, a series of possible pharmacological treatments have been envisaged to mimic the pro-longevity effects of IGF-1 deficiency escaping in the same time the detrimental side-effects (obesity, dwarfism, infertility, sarcopenia) of this deficiency (Longo and Finch, 2003).

Conservation and evolvability

Recent conceptualizations stressed the importance of *robustness* as one of the fundamental properties of living organisms, from cellular (Stelling, 2004) to organismal level (Kitano and Oda, 2006; Kitano, 2007), a characteristic acquired during evolution which allow them to be error-tolerant and to easily respond to external perturbations (Jeong *et*

al., 2000). Metabolic stability appears to be genetically determined and respond to the requisites of robustness. On the basis of available data obtained from different organisms, especially yeast and Invertebrates, a *"bow-tie" organizational architecture* is likely to be a common feature of highly organized, robust systems (Csete and Doyle, 2002, 2004), which enables them to accommodate perturbations and fluctuations on many temporal and spatial scales. A bow-tie model is present when many inputs converge on, and are integrated by, few elements, and many different outputs come out as the product of the integration. The elements composing the core ("knot") of the bow-tie in a biological system are "hub proteins" and the genes that encode them are likely to be in many cases robustness genes. Such a structure has also an inner fragility, because few enzymes responsible for robustness can be easily hijacked by pathogenic microorganisms or used to amplify pathological processes (Csete and Doyle, 2002; Kitano and Oda, 2006; Kitano, 2007). Much of the core of a bow-tie is often conserved throughout evolution, but this conservation does not prevent, but rather facilitates the variability of the possible outputs. Thus, a bow-tie structure allows both robustness and evolvability (Gerhart and Kirschner, 1997; Caporale, 2003). Longevity could be intended as a consequence of the robustness of the animal system. Indeed, it is conceivable that the outputs of a bow-tie structure include elements that ultimately control the life-span of the animal. If these elements can vary, as a feature of the bow-tie evolution, this variation may explain, at least in part, why knot genes, yet conserved, not always result to be involved in longevity on evolutionarily distant animals according to classical association or functional studies, thus casting some doubts on their role as real "longevity genes".

Conclusions

Homo sapiens is one of the most long-living species among animals, but his longevity has had a dramatic increase in recent times, at least in western Countries, due to profound modifications in lifestyle. Thus, human longevity has two major components, whose effects are very difficult to distinguish, genetics and culture. This raises an insurmountable obstacle, since no animal species depends on culture as humans do. Thereafter, results obtained on animals studies not only must be always confirmed on humans, but they are also not completely satisfactory for a series of reasons: these studies do not consider neither the genetic and epigenetic differences existing between men and animal species, even those much close to humans, nor the influence of culture on the duration of the life after the age of reproduction, *i.e.* after the period influenced by natural selection. As an example of specific genetic differences between man and animals, it can be mentioned the case of TP53 gene. This gene is crucial for a series of biological processes such as apoptosis, cell senescence, DNA repair, and energy metabolism, and is considered a longevity gene in both animals and humans (Donehower, 2005). In humans TP53 gene harbours a common functional polymorphism at codon 72 which alters the protein' functions (Thomas *et al.*, 1999; Dumont *et al.*, 2003; Bonafè *et al*., 2004). This polymorphism has been and still is under investigation for its possible involvement in longevity (Bonafè *et al*., 2002; Van Heemst *et al*., 2005), and such effects, if any, can be detected only in humans, because this polymorphism does not exist in Chimpanzee, while in mice it seems to have no effects (Phang and Sabapathy, 2007). Moreover, our data indicate that the effects of such a polymorphism become evident as the age of the studied subjects increases (Bonafè *et al*., 2004; Salvioli *et al*., 2005) thus suggesting that the role of such a gene does change with age. This could be a case of antagonistic pleiotropy, that is, a gene that has a positive effect for fitness at young age could turn to be detrimental later on, in a period of time not selected for reproductive fitness, or *vice versa*. The genetics of human longevity has thus been proposed to be described as a post-reproductive one (De Benedictis and Franceschi, 2006). It is at present unknown whether also laboratory animals have a post-reproductive genetics, and which similarities it has with the human one. In conclusion, animal models remain an irreplaceable tool to shed light into the genetics of aging and longevity, but the transferability of the results to humans is always an issue.

As discussed all along this review, longevity genes are classically identified by their association with an extended life-span, but for a series of reasons this strategy is not always successful, and results can not always be reproduced in different experimental models. Here we propose the idea that longevity genes could be identified by an alternative strategy. As mentioned, conservation and evolvability are inherent features of the bow-tie structures, that determine robustness. If robustness is important for longevity, it can be hypothesised that longevity genes should participate with a core position to a bow-tie metabolic structure. We propose that a new way to identify putative genetic determinants of longevity could be the conservation of their products in bow-tie structures all along evolution, rather than for their association with prolonged life-span. In this perspective, studies of comparative biology associated with a systems biology approach could be useful tools to get some insights also into the genetics of human longevity.

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