A study into genes encoding longevity in humans

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CHAPTER 9

Genes Encoding Longevity; from Model Organisms to Man

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Abstract

Ample evidence from model organisms has indicated that subtle variation in genes can dramatically influence lifespan. The key genes and molecular pathways that have been identified so far encode for metabolism, maintenance- and repair mechanisms that minimize age related accumulation of permanent damage. Here, we describe the evolutionary conserved genes that are involved in lifespan regulation in model organisms and have been studied for association with lifespan in humans. The accumulated data reveal that when moving up the evolutionary ladder, together with an increase of genome complexity, the impact of candidate genes on lifespan becomes smaller. The presence of genetic networks makes it more likely to expect impact of variation in several interacting genes to affect lifespan in humans. Extrapolation of findings from experimental models to man is further complicated as phenotypes are critically dependent on the setting in which genes are expressed while laboratory conditions and modern environments are markedly dissimilar. Finally, currently used methodologies may have only little power and validity to reveal genetic variation in the population. In conclusion, although the study of model organisms has revealed potential candidate genetic mechanisms determining ageing and lifespan, to what extent they explain variation in human populations is still uncertain.

Introduction

Over the last century, mean life expectancy in Western societies has increased dramatically (Oeppen and Vaupel, 2002). In Japan for instance, mean life expectancy has increased from fifty to eighty years in no more than six decades. It is unlikely that changes in the population genome over this time-period can explain for the observed increase in lifespan which is more likely to be attributable to the improvement of environmental conditions and medical care. The increase in mean life expectancy of the total population, however, has left the marked inter-individual variance in lifespan unaltered. Socio-economic factors can in part explain this phenomenon, but ample evidence suggests that genetic factors are also at play. Studies of twins and long-lived families have estimated that 20 to 30 per cent of the variation in human lifespan is determined by genetic factors, which impact becomes more important for survival at older ages (Herskind et al., 1996; Hjelmborg J. et al., 2006; Mitchell et al., 2001). Furthermore, siblings of centenarians have a significantly higher chance of becoming a centenarian themselves when compared to other members of their birth cohort (Perls et al., 2002). The survival benefits of family-members of these long-lived subjects are lifelong and persist up to the highest age-categories (Hjelmborg J. et al., 2006; Perls et al., 2002). Offspring of long-lived sibling pairs have a lower mortality risk already at middle age, whereas their spouses, with whom they have shared in part a common environment, do not show this survival benefit (Schoenmaker et al., 2006).

As for lifespan, ageing is under moderate genetic control influencing the rate at which stochastically induced damaged molecules accumulate. Such damage is caused by various endogenous and exogenous biological- and biochemical stresses. As a result, over the life course there is a constant rise in vulnerability of the body, leading to a continuously increasing risk of disease and death. Longevity and the maintenance of health in old age can be ensured via two principally different strategies that minimize the risk of permanent damage to occur i.e. by a decrease of environmental hazards or an increase of the durability of the body. Pathways that influence metabolism, maintenance and repair mechanisms, and prevent the accumulation of permanent damage thus represent key molecular candidates for the preservation of health and longevity.

Experiments in model organisms have demonstrated that a series of induced mutations in various genes that make up an integrated molecular pathway can dramatically increase lifespan. The most prominent example includes the *Caenorhabditis elegans daf-2* and *clk* double mutants that live nearly five times longer than wild-type worms (Lakowski and Hekimi, 1996). Most of the genes of model organisms are evolutionarily conserved and present in humans. Here, we will briefly review the genes and mechanisms that have been shown to regulate lifespan in model organisms, but limiting ourselves to those genes, which have human homologues, and have been studied for association with human health and/or longevity (Table 1). In addition, it is addressed whether we can expect to find single genes or molecular pathways that substantially affect lifespan in humans; whether the information obtained from model organisms can be translated to variation in lifespan in humans; and whether present genetic surveys are able to pick up this genetic variation.

The evidence

Insulin/IGF-1 signaling (IIS)

The first evidence for genetic regulation of lifespan came from studies with *C. elegans*. It was discovered that worms with mutations in the dauer formation (Daf) genes, such as *daf-2* and *age-1*, were able to bypass dauer formation, and become long-lived adults (Larsen, 2001). The molecular characterization of the *daf-2* and *age-1* genes revealed that these show homology to the mammalian genes encoding Insulin Receptor (IR) and Insulin-like Growth Factor 1 Receptor (IGF-1R) (Kimura et al., 1997), which are evolutionary conserved. Next, it was shown that, similar to *C. elegans*, reduced insulin signaling extends lifespan in *Drosophila melanogaster*. The increase in lifespan was observed for flies with mutated insulin-like receptor (InR) or its substrate (chico), and for flies with ablated insulin-producing cells (Giannakou and Partridge, 2007). In the latter case, the adult flies also exhibited increased storage of lipids and carbohydrates, reduced fecundity, and increased stress- and starvation resistance.

In vertebrates, the insulin signaling system is more complex and contains separate receptors for insulin (IR) and IGF-1 (Navarro et al., 1999). Data from mice indicate that both these receptors, IR and IGF-1R, are involved in lifespan regulation. The IGF-1 branch, which acts through growth-hormone-releasing hormone (GHRH), growth hormone (GH) and IGF-1, influences body composition and is involved in the regulation of gonadal function (Bartke, 2005). The long-lived Ames and Snell dwarf mice, which are deficient in growth hormone, thyroid hormoneand prolactin are infertile (Brown-Borg et al., 1996). A similar phenotype is observed for GH receptor knockout mice (Bartke, 2005). Furthermore, mice mutated for the IGF-1 receptor hint at a direct role for reduced IGF-1 signaling in mammalian longevity: Igf1r+/- females, but not males, exhibit a long-lived phenotype (Holzenberger et al., 2003). In contrast, complete disruption of the IR gene leads to insulin resistance, diabetes and shortened lifespan (Okamoto and Accili, 2003). Likewise, tissue specific IR knockout mouse models develop obesity, insulin resistance and impaired glucose regulation, with the exception of the fat-specific IR knockout mice (FIRKO) (Okamoto and Accili, 2003). These mice have reduced fat mass, are protected against age-related obesity, and live longer than their littermates. Taken together, the evidence in mouse models shows that reduced insulin/IGF-1 signaling can extend lifespan also in mammals.

In humans, there is some evidence that long-lived subjects, such as centenarians, have decreased plasma IGF-I levels and preserved insulin action, thus indicating that insulin responsiveness influences human longevity (Paolisso et al., 1997). For instance, a polymorphisms in the IGF-IR locus, that has been associated with lower plasma IGF-1 levels was shown to be enriched among Italian centenarians (Bonafe et al., 2003). This finding was not replicated in a prospective follow-up study of elderly Dutch subjects, but in the same study it was found that a polymorphism in the *GH1* gene, which controls IGF-1 activity, associates with longevity (van Heemst et al., 2005a). In addition, a combined effect of variation at the GH1, IGF-1 and IRS1 loci was observed, demonstrating an additive effect of multiple variants associated with reduced IIS signaling on human longevity.

Table 1. Selected example of genes identified to influence lifespan in model organisms (in alphabetical order)

Symbol	Gene name/description	Function	Organism*	Reference
age-1	phosphatidylinositol kinase	insulin signaling	C. elegans	(Morris et al., 1996)
Cat	catalase	antioxidant activity	D. melanogaster	(Orr and Sohal, 1994)
Chico	insulin receptor substrate	insulin signaling	D. melanogaster	(Clancy et al., 2001)
daf-2	insulin receptor-like gene	insulin signaling	C. elegans	(Kimura et al., 1997)
daf-12	nuclear hormone receptor	regulation of metabolic and developmental pathways	C. elegans	(Larsen et al., 1995)
daf-16	forkhead transcription factor	regulation of metabolic and developmental pathways	C. elegans	(Ogg et al., 1997)
Gh	growth hormone	insulin signaling, tissue proliferation	M. musculus	(Bartke, 2005)
Klotho	beta-glucuronidase	inhibits IIS signaling	M. musculus	(Kuro-o M et al., 1997)
Mei-41	phosphatidylinositol kinase	DNA repair	D. melanogaster	(Symphorien and Woodruff, 2003)
Pcmt	protein carboxyl methyl-transferase	protein repair	D. melanogaster	(Chavous et al., 2001)
p53	tumor protein p53	tumor suppression	M. musculus	(Tyner et al., 2002)
Sir2	NAD(+)-dependent deacety-lase	regulation of metabolism, stress resistance	S. cerevisiae	(Kaeberlein et al., 1999)
Sod1	superoxide dismutase	antioxidant activity	D. melanogaster	(Parkes et al., 1998)
Sod2	superoxide dismutase	antioxidant activity	D. melanogaster	(Sohal et al., 1995)

*Organism, in which the gene was first shown to influence lifespan

Klotho

The *Klotho* gene, which was identified in mouse models (Kuro-o M et al., 1997), encodes a mammalian specific hormone that negatively regulates the activity of IR and IGF-1R through repressing their autophosphorylation (Kurosu et al., 2005). In mice, genetic variation in the *Klotho* gene results in an early onset of various age-related disorders, including ectopic calcification, skin- and muscle atrophy, osteoporosis, atherosclerosis, and pulmonary emphysema (Kuro-o M et al., 1997). On the other hand, over-expression of Klotho in mice leads to inhibition of insulin and IGF1 signaling and increased lifespan (Kurosu et al., 2005). In humans, a haplotype allele called KL-VS, which contains six sequence variants that are in complete linkage disequilibrium, has been associated with *KLOTHO* expression and shown to be underrepresented in elderly individuals (Arking et al., 2002). Additional studies have demonstrated that the survival advantage is only present in heterozygous KL-VS allele carriers, whereas in homozygous allele carriers a disadvantage for HDL-cholesterol levels, systolic blood pressure, stroke, and longevity was observed (Arking et al., 2005).

Forkhead transcription factors

In *C. elegans*, the IIS pathway negatively regulates the activity of DAF-16, which is its main downstream target. The long-lived phenotype of the IIS mutants depends on the presence of an

active DAF-16 protein (Mukhopadhyay and Tissenbaum, 2007). In mammals, the DAF-16 homologues are forkhead transcription factors (FOXOs): FOXO1a, FOXO3a, FOXO4 and FOXO6 (Furuyama et al., 2000). Similar to DAF-16 in *C. elegans*, in mammals the FOXO proteins relay the effects of insulin on lifespan, influence fertility, and play a role in complex diseases such as diabetes (Carter and Brunet, 2007). In humans, only few studies have analyzed the role of FOXO proteins in the development of age-related diseases, fertility and lifespan. In most studies, no associations between genetic variance in the *FOXO1a* and *FOXO3a* genes and lifespan have been detected (Bonafe et al., 2003; Kojima et al., 2004). These findings, contrast with recent studies where genetic variance in the *FOXO1a* gene was linked to increased glucose levels (Karim et al., 2006; Kuningas et al., 2007a) increased risk of diabetes and decreased lifespan (Kuningas et al., 2007a). Support for these findings has come also from the Framingham Heart Study (personal communications). For genetic variance in the *FOXO3a* gene, associations were observed with increased risks of stroke and mortality, but not with fertility (Kuningas et al., 2007a).

DAF-12

In *C. elegans*, DAF-12 is a member of the evolutionarily conserved Nuclear Hormone Receptor (NHR) super-family (Mangelsdorf et al., 1995) and it has been implicated in dauer diapause, developmental timing, metabolism, fertility, and longevity. Current data have positioned DAF-12 downstream of the insulin- and germline signaling, as the long-lived phenotype of germline ablated mutants and of some IIS mutants depends on DAF-12 activity, but the exact position is still unknown (Rottiers and Antebi, 2006). In humans, the NHRs most similar to DAF-12 are the Liver X Receptors (alpha and beta), which have cholesterol breakdown products (oxysterols) as ligands. Upon activation, LXRs regulate various processes that result in cholesterol excretion from the body (Zelcer and Tontonoz, 2006). Recently, a common haplotype of the *LXRA* gene was associated with increased survival, predominantly due to lower mortality from cardiovascular causes and infection (Mooijaart et al., 2007a). A possible mechanism through which LXR could lead to the observed beneficial effects includes involvement of its target gene Apolipoprotein E (APOE). ApoE is an anti-atherosclerotic protein involved in the efflux of cholesterol from macrophages. Genetic variation in *APOE* has consistently been associated with cognitive decline and cardiovascular disease mortality. Moreover, we have recently shown that independent of genetic variation in *APOE*, high plasma apoE levels associate with increased risk of stroke (van Vliet et al., 2007), increased risk of cardiovascular mortality (Mooijaart et al., 2006) and decreased cognitive functioning (Mooijaart et al., 2007b). These data support previous observations that lipoprotein metabolism is critical for exceptional longevity. It has been shown that families of Ashkenazi Jewish centenarians have larger particles of HDL and LDL, which are associated with a decreased incidence of metabolic syndrome, cardiovascular disease and hypertension (Barzilai et al., 2003). Also in the Dutch Caucasian population, offspring of long-lived sibling pairs have larger LDL particles than their age-matched partners, again suggesting that larger LDL particles confer a survival benefit (Heijmans et al., 2006).

Sirtuins

The Sirtuins represent an evolutionarily conserved family of Silent Information Regulator 2

(Sir2) NAD-dependent protein deacetylases that interact with and influence the activity of various transcription factors and co-regulators (Bordone and Guarente, 2005). Increased expression of the *Sir2* gene, either due to an extra copy of the gene or to caloric restriction, prolongs lifespan in various model organisms (Haigis and Guarente, 2006). In mammals, there are seven Sir2 homologues (SIRT1-7), of which SIRT1 is the most closely related to Sir2 (Frye, 2000). In mouse models, SIRT1 and SIRT3 have been studied the most. SIRT1 has been associated with glucose and fat metabolism, stress resistance and cell survival (Haigis and Guarente, 2006), whereas SIRT3 regulates the activity of acetyl-CoA synthetase (AceCS), and thereby the entry of carbons from acetate into central metabolism (Haigis and Guarente, 2006). In humans, polymorphisms within *SIRT1* and *SIRT3* genes have been analyzed for association with age-related diseases and longevity. In case of *SIRT1*, no associations have been found (Flachsbart et al., 2006; Kuningas et al., 2007b), whereasof *SIRT3*, a G477T polymorphism and a variable number of tandem repeats (VNTR) have been associated with increased lifespan (Bellizzi et al., 2005; Rose et al., 2003). These results demonstrate that at least one member of the SIRT family is involved in human lifespan regulation.

Antioxidative enzymes

Antioxidative enzymes, such as catalase and superoxide dismutase (SOD), prevent damage from reactive oxygen species (ROS), but the evidence from model organisms on the beneficial effects of antioxidative enzymes on lifespan has been controversial. Studies with *D. melanogaster* have demonstrated that overexpression of CuZn-SOD (SOD1), Mn-SOD (SOD2), and catalase lead to lifespan extension (Orr and Sohal, 2003; Sohal et al., 1995). Additional experiments, however, showed that this effect depends on genetic background of the used lines (Orr and Sohal, 2003). Likewise, the extended lifespan of *C. elegans* by administration of synthetic SOD/catalase mimetics was shown to depend on laboratory conditions (Keaney and Gems, 2003; Melov et al., 2000).

In mammals, one catalase and three SOD genes have been characterized; SOD1, SOD2 and SOD3, of which Catalase and SOD2 seem to influence lifespan. In mice, disruption of the *SOD2* gene is lethal due to neurodegeneration and to damage to the heart (Li et al., 1995; Melov et al., 1998). In contrast, overexpression of *SOD2* leads to increased lifespan (Hu et al., 2007), as does overexpression of *Catalase* targeted to mitochondria (Schriner et al., 2005). Mice heterozygous for the mitochondrial form of *SOD2* showed high levels of DNA oxidation in multiple organs. In spite of their abnormally oxidized DNA, these animals showed no decline in lifespan and no acceleration in the hallmarks of aging, such as cataracts, immune dysfunction, and protein modifications (Van Remmen et al., 2003). These data suggest that mice can live reasonably long and healthy lives despite unusually high levels of oxidative damage.

The evidence for the role of antioxidative enzymes in the preservation of human health is not well established. It has been shown that RNA interference (RNAi) of SOD1 induces senescence in human fibroblasts (Blander et al., 2003), which suggests that SOD1 may play a role in the regulation of cellular lifespan. However, genetic variants in the *SOD1* gene have never been studied for that relationship. In contrast, genetic variants in the *SOD2* gene have been studied and associated with a number of phenotypes including increased risk for prostate- and breast cancer, immunosenescence profile, and DNA damage (Liu et al., 2004; Taufer et al., 2005), but

not with mortality (De Benedictis et al., 1998; van Heemst et al., 2005a). Likewise, no associations between genetic variants in the *Catalase* gene and mortality have been found (Christiansen et al., 2004).

Macromolecule repair mechanisms

Defects in mechanisms that repair damage to cellular components, such as DNA, proteins and membranes have been shown to reduce lifespan in various model organisms. Even though these mechanisms are evolutionarily conserved (Eisen and Hanawalt, 1999), systematic comparative genomic analyses across species have not been conducted. In addition, within species, there are many studies demonstrating detrimental effects of impaired repair systems on lifespan, but only few demonstrating beneficial effects of increased repair capacity on lifespan. The only evidence for the latter has come from experiments with *D. melanogaster*, where the absence of *mei-41* excision repair reduces lifespan, whereas flies with one- or two extra copies of the gene have significantly increased lifespan (Symphorien and Woodruff, 2003). Likewise, overexpression of protein carboxyl methyltransferase (*PCMT*), which is a protein repair enzyme, has been correlated with enhanced longevity in a temperature-dependent manner (Chavous et al., 2001). Both of these genes, *mei-41* and *Pcmt* have homologues in mammals, which are ataxia telangiectasia and Rad3 related (*ATR*) and *PCMT*, respectively. In mice, the disruption of the *ATR* gene leads to chromosomal fragmentation and early embryonic lethality (Brown and Baltimore, 2000), and in humans to a rare Seckel syndrome (Casper et al., 2004). The *Pcmt1*-null mice, on the other hand, display a fatal seizure disorder and retarded growth (Kim et al., 1999), and die at a mean age of 42 days (Lowenson et al., 2001).

Compared to the other repair mechanisms, DNA repair has been studied the most in relation to ageing and lifespan. The DNA repair deficient mouse models that have been generated, display a common phenotype of segmental premature ageing (progeria), or cancer predisposition, or both, and have a reduced lifespan (Hasty et al., 2003). Similarly, in humans, all mutations identified in DNA repair genes severely compromise health. For instance, mutations in the components of transcription coupled repair (TCR) have been associated with the premature ageing syndromes of Cokayne syndrome (CS) and Trichthiodystrophy (TTD) (Cleaver, 2005; Hoeijmakers, 2001). Likewise, mutations in the RecQ-like DNA helicase genes, *WRN*, *BLM* and *RecQ4* lead to the premature ageing syndromes of Werner-, Blooms-, and Rothmund–Thomson-Syndrome, respectively (Navarro et al., 2006). In contrast to the strong phenotypes associated with mutations in RecQ helicases, common polymorphisms in these genes do not seem to influence the aging-trajectories and survival in the general population (Bohr et al., 2004; Castro et al., 2000; Kuningas et al., 2006). The RecQ helicases are highly conserved throughout evolution, but in higher eukaryotes, the different homologues seem to have distinct functions because failure of one given RecQ gene cannot be complemented by another RecQ gene. These observations underpin the importance of DNA repair in all organisms. The key question that has yet to be answered is whether subtle variants in the DNA repair genes contribute to different lifespans and whether above average repair makes for a lifespan extension.

Cellular responses to damage

In response to unrepaired damage, cells trigger either apoptosis or cell cycle arrest. The most well known protein implicated in the maintenance of genomic stability is p53. Recently, p53 homologues were identified in *C. elegans* and *D. melanogaster*. In contrast to mammalian p53, which elicits apoptosis or cell-cycle arrest (Attardi, 2005), the p53 in *C. elegans* and *D. melanogaster* affects only apoptosis (Brodsky et al., 2004; Derry et al., 2001; Schumacher et al., 2001). Nevertheless, in all of these organisms, reduced p53 activity leads to lifespan extension (Bauer and Helfand, 2006). In mammals, this extension comes at the cost of increased cancer risk (Campisi, 2003). In humans, it has been shown that Pro/Pro carriers of the TP53 codon 72 polymorphism have a significantly lower apoptotic potential than Arg/Arg carriers, both in p53-inducible human cell lines (Dumont et al., 2003; Pim and Banks, 2004; Sullivan et al., 2004) and in normal diploid fibroblasts (Bonafe et al., 2004). Later on it was shown that despite an increased mortality from cancer, carriers of the same polymorphism have a significantly increased survival at old age in line with the experimental models (van Heemst et al., 2005b). Altogether, these observations support the hypothesis that reduced p53-mediated induction of apoptosis can have beneficial effects on lifespan if tumor formation can be avoided. This might hold true also for other genes that mediate cellular responses to damage.

Discussion

Experiments in model organisms have demonstrated that changes in genes can dramatically increase their lifespan. In some cases, mean and maximum lifespan is extended up to fivefold. The equivalent life extending effect in humans would result in an average lifespan of 400 years and maximum lifespan of over 600 years. Many of the pathways regulating lifespan in model organisms are conserved throughout evolution. Why then, have we not yet identified genetic determinants that could increase human lifespan by more than a few years? Are we looking at the right genes? Is it fair to expect such dramatic effects? Do we have the tools to observe genetic determinants of human lifespan?

Increased complexity

In vertebrate organisms novel genes and signaling components have appeared during evolution, contributing to increased complexity of the genomes (Long, 2001). Among several molecular mechanisms, gene duplication plays a major role in genome evolution (Britten, 2006; Long, 2001). Often, a mammalian genome contains several homologues of a single invertebrate gene with similar or distinct functions and expression patterns. This can hinder the assessment of the role of a specific candidate gene, since genetic variance in duplicated genes is likely to have less dramatic effects than in the original single gene in invertebrate organisms (Conant and Wagner, 2004; Gu et al., 2003). Furthermore, since vertebrates have acquired pathways that are more diverse, the homologous genes in phylogenetically distant animals could be involved in different pathways.

The genomes of vertebrate organisms also contain genes, which have no homologues in

invertebrates. The appearance of novel genes, or "add-ons", probably contributed to the arise of elaborate systems that regulate the activity of the conserved or "shared" genes (Levine and Tjian, 2003). The GH control over IGF-1 and the regulation of IR and IGF1-R activity by KLOTHO have arisen relatively recently in vertebrate evolution (Forsyth and Wallis, 2002; Mian, 1998). Genetic variance in these genes has been associated with lower IIS signaling and with increased lifespan, corroborating the findings from invertebrates. However, in these organisms this phenotype was attained by modifying the "shared" genes. This demonstrates that in humans, we should not limit ourselves to studying the evolutionarily conserved genes that have been implicated in lifespan regulation in invertebrates, but also include, and maybe even focus on, "add-ons" that regulate their activity. Taken together, the increased complexity of signaling systems in vertebrates adds robustness to the signaling pathways, which is the ability to maintain its functions despite changes in its components or environment (Lenski et al., 2003; Soyer and Bonhoeffer, 2006). In that respect, lifespan regulation can be regarded as a complex genetic trait, for which it is unlikely that single alterations in the genetic machinery will have dramatic effects. Furthermore, genes that have appeared later in evolution, "add-ons" are interesting candidates to determine longevity in vertebrate organisms.

Environmental influences

Environmental influences have played a major role in shaping and patterning the genomes of all organisms throughout evolutionary history. Changes in environment can lead to different expression of genotypic information, and thereby complicate the comparability of results between model organisms and humans. Most research on model organism has been performed under laboratory conditions, where temperature, presence of pathogens, food availability and population density are tightly controlled. In most cases these conditions poorly mimic the evolutionary niche in which the genes come to expression and it may therefore be questioned how well the results obtained in these condition are applicable to species under "natural" conditions (Clancy et al., 2001; Marden et al., 2003; Walker et al., 2000).

Even for humans, the environment in which the genome effectively evolved has changed. The genes that were originally selected for survival in adverse environments are now expressed under completely new, affluent conditions. For instance, the IIS system was selected and fine-tuned in times when food abundance and famine alternated. The genotypes that increased the efficiency to store energy in times of abundance and use these storages in times of famine had a survival advantage. In modern Western societies, where food is constantly abundant these "thrifty genotypes" lead to increased prevalence of storage diseases, such as obesity and diabetes (Neel, 1962). This reinforces the idea that our population genome has been optimized to increase fitness under adverse conditions whereas this need not to be the case under modern affluent conditions, the outcome of these new interactions are both unknown and unpredictable. Taken together, caution should be taken in extrapolating results on genetic variation obtained in model organisms to the human situation, since the environments in which the genes come to expression are often markedly different. Moreover, research into human ageing should include various environmental conditions that can explain for different phenotypes despite an equal genetic background

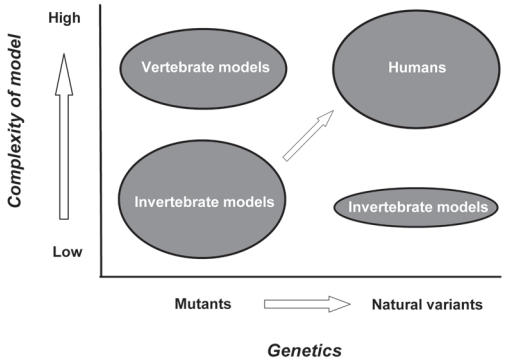

Figure 1. The amount of information obtained from different organisms on genetic mechanisms on ageing and longevity. The amount of available information on the effect of genetic variation on life span in organisms of different complexity is indicated by the size of the four quadrants. As can be seen, in humans most information on the effect of genetic variation on longevity is being obtained by studying naturally occurring variants, while in model organisms, most information on the effect of genetic variation on longevity has been obtained by studying the effect of mutations in invertebrate models, and, to a lesser extent, in vertebrate models. In contrast, only few studies have analyzed the contribution of standing natural genetic variation on lifespan in model organisms. The direct translation of the importance of candidate genes identified in mutant invertebrate models for variation in human life span is complicated by two possible discrepancies. The first includes the influences of mutations and standing (subtle) genetic variations in the candidate loci and the second possible discrepancy includes the differences in genome complexity between the organisms.

Mutants versus natural genetic variants

The majority of candidate genes of longevity have been identified by studies with mutant model organisms, such the worm, fruit fly and mouse models. These approaches are extremely powerful to disentangle biological pathways. However, to date it is largely unknown to what extent these mutations affect fitness in natural environments and whether these candidate loci contain genetic variation, which would contribute to phenotypic variance for lifespan in natural populations (Figure 1). These questions are of importance since not all candidate loci with major effects on longevity in laboratory conditions may exhibit variation in natural populations. Currently, only few studies have tried to disentangle this question. For instance, it has been shown that the long-lived mutant fruit fly methuselah (*mth*) underperforms in most cases under conditions that resemble more a natural situation (Baldal et al., 2006). This illustrates that the mth locus would never have been identified to influence lifespan if natural populations of the fruit fly would have been analyzed. A similar observation is obtained for the *Chico* variant that outlives the wild type under food affluence but becomes short lived when exposed to food restricted conditions (Clancy et al., 2002). In case of humans, most information on the effect of genetic variation on longevity is being obtained by studying naturally occurring variants in candidate genes identified in mutant model organisms. Hence, the discrepancy between the data from

mutant model organism and standing genetic variance in natural populations contributes to the difficulty of translating results from model organisms to humans (Figure 1). Therefore, to facilitate translation of the results from mutant model organisms to humans, the analysis of standing variation in the same loci in natural populations of model organisms should be encouraged.

Methodological considerations

The lack of very strong effects of the evolutionarily conserved genes in humans can have other reasons than those suggested above. Some of these include study design and methodology in human studies. Most of the genetic association studies of longevity have been performed in case-control settings, where genotype- or allele frequencies between elderly and a younger population are compared. The main advantage of case-control study design is that cases are readily obtainable and can be efficiently genotyped and compared with control populations. Cases can be nonagenarians, centenarians or long-lived sibpairs. By collecting long-lived sibpairs instead of long-lived singletons, an enrichment of genetic factors contributing to longevity in this population can be expected, while the likelihood of having reached a long life because of exceptional environmental conditions or chance is lower. The difficulty of case-control studies is in selecting controls. In longevity studies, the ideal control group should be composed of participants from the same birth cohort who were not long-lived, to minimize the effect of environmental differences caused by cohort differences. However, obtaining such a control group is impossible in a retrospective study design. Such case-control studies are only possible if one is studying in long-lasting population surveys. The longest ongoing studies (e.g. Framingham Heart Study) now allow a difference of 30 years between subjects that survived and those that did not. However, in most studies so far, controls have been selected from the general population of younger generations. This, can lead to biases where allele frequency differences between cases and controls can appear as an association, even if they only reflect the results of changes to the source population due to changes in environment, migratory history, gender differences, or other independent processes (Cardon and Palmer, 2003; Manolio et al., 2006). An alternative approach would be prospective cohort studies, which suffer less from population stratification but are more expensive and time-consuming (Manolio et al., 2006).

Another consideration for the methodology includes the selection and analysis of genetic variants in the candidate genes. Commonly, a selected number of polymorphisms from the coding region of candidate genes are analyzed, leaving aside genetic variants in regulatory regions. In addition, besides analyzing the individual polymorphisms only few studies have undertaken haplotype analyses. Given the amount of information that has recently become available through the International HapMap project, polymorphisms that tag common haplotypes can easily be identified. The analysis of haplotypes can be more powerful since this analysis captures the joint effect of all unknown gene variants that are in linkage disequilibrium with the markers forming the haplotype (Johnson et al., 2001).

Finally, similar to other association studies, also the results from longevity association studies have been rarely replicated. Explanations for lack of reproducibility include poor study design, small sample size, incorrect assumptions about the underlying genetic architecture, and over interpretation of the data. In addition, for a number of associations (mainly negative) no replication has been undertaken, leaving open the reproducibility. Replication of even negative

results is necessary, since the lack of associations in the first study could likewise have been due to poor study design, population stratification or other reasons. Therefore, before discarding a candidate gene from the list of possible candidates, replication in different cohorts with more thorough genetic analysis is necessary.

Future directions

Pathway analyses and epigenetic variation

Given the current feasibility of high throughput genotyping and increasing knowledge on cellular mechanisms, pathway analyses instead of analyzing individual loci separately could be performed. The appropriate tools are likely to be available soon, since the analysis of complex traits, which are under the influence of multiple and possibly interacting genes, has become a subject of new statistical methodological research (Kristensen et al., 2006). However, besides genetic variation, other mechanisms influence the expression of genomic information. For instance, epigenetic modifications, which are differences in gene expression that cannot be accounted for by changes in the primary DNA sequence, have a significant impact on gene function, and may explain how iso-genetic organisms are phenotypically very distinct. Likewise, a different level of transcriptional and post-transcriptional control through RNA interference, or other mechanisms, can account for phenotypic differences. These and other modifications contribute to the differences in lifespan between and within species, and this type of information from model organisms and humans is essential for the extrapolation of results.

Genes and pathways for future

A number of interesting candidate genes, with or without homologues in model organisms remain to be investigated in humans. The most interesting and so far not very thoroughly analyzed genes in respect to longevity include those involved in fertility. In model organisms, fertility and lifespan are closely linked (Partridge et al., 2005). In *C. elegans*, ablation of germline precursor cells of the gonad abolishes reproduction and extends lifespan (Hsin and Kenyon, 1999), as do mutations that reduce germline proliferation (Arantes-Oliveira et al., 2002). In *D. melanogaster*, a reduction in fecundity extends lifespan in females (Sgro and Partridge, 1999) and long-lived heterozygous chico mutant females exhibit reduced fecundity, with the homozygotes being almost sterile (Clancy et al., 2001). In mice, Ames and Snell dwarfs are long-lived and sterile (Bartke, 2005). The observational studies in historical human populations living under pre-affluent conditions have provided similar evidence. In the English and Finnish aristocracy, women with the longest lifespan had the smallest number of offspring (Korpelainen, 2000; Westendorp and Kirkwood, 1998). Despite this evidence, the genetic determinants for the trade off between fertility and lifespan are unknown.

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