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Chapter 2

Genetic Factors Associated with Longevity in Humans

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Abstract

Introduction: Life expectancy and the rate of survival into old age have risen dramatically throughout the past century. The positive ageing outcomes may be due to a variety of factors including healthy lifestyle behaviors, but it is clear that longevity has a genetic basis, with heritability estimate of 20–35%. In this contest, it was emerged that human longevity seems strongly influenced by gender defined as the combination between biological sexual characteristics and factors related to behavior, social role, lifestyle and life experiences. Body—research methods: Successful ageing seems to be related to gene involved in different pathways of regulation, such as immune-inflammatory responses and oxidative stress. The aims of the present review are to discuss recent findings and highlight the genetic basis of longevity. For these reasons we are aimed to describe the most important underpinning which is the gender differences in longevity between males and females. Conclusion—key results: It appears clear that longevity may represent a complex polygenic trait that is influenced by the interaction of multiple genetic variants, as was demonstrated by several genetic studies conducted in the last years. Furthermore, epigenetic and environmental factors actin on the longevity phenotype.

Keywords: longevity, genetic basis, healthy lifestyle, inflammation, oxidative stress, epigenetics

1. Introduction

Human life expectancy has increased over the last two centuries worldwide [data shown by the World Health Organization, 2012], and this is mainly due to improvement of health care, lifestyle and nutrition. Considering the continuous increase of lifespan and the consequent growth of the elderly population, research on ageing has been continuously increasing in the last decades. The age threshold used to define "longevity" varies by study but is typically ≥85 years old and "exceptional longevity" may be considered at age ≥ 95 years old [1].

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Many epidemiological data conducted in different populations indicate the presence of a strong familial component of longevity. These studies demonstrate that parents, siblings and offspring of long-lived subjects have significant survival advantage. In particular, it seems that these subjects had higher probability to become long-lived persons and to have a lower risk to undergo the most important age-related diseases, such as cardiovascular diseases (CVD), diabetes and cancer. For these reasons, most of the human ageing studies concentrate around long-lived families, including highly and middle-aged members.

The presence of strong familial component of longevity led to hypothesize the presence of a genetic basis, most likely expected to be polygenic, and was demonstrated a heritability estimates of 20–35% [2]. In fact, human lifespan is a complex trait which is assumed to be determined by many genes with small individual effects [3].

The gerontogenes, genes controlling ageing and longevity, are highly interconnected and related to stress response [4]. Prolonged or severe stress exposure exhausts the defence mechanism accelerating the process of ageing by accumulation of mistakes and physiological abnormalities. Nevertheless, on the other hand, it is known that moderate stress could have beneficial effects stimulating innate defence resources of the body thereby by expression of gene responsible for stress resistance. This stimulation increases the body ability to cope with higher levels of stress and slows down ageing in the so-called lifespan hormesis effect [5].

Genomic studies into ageing thus far focus on the determinants of human lifespan variation by using age at death, prospective survival, disease-free survival or exceptional longevity as outcome. From a genomic perspective, individuals from long-lived families are assumed to be characterized by a decreased prevalence of disease-promoting variants and an increased prevalence of variants conferring maintenance of health and protection from disease, when compared to population controls [6]. However, in 2010 a study conducted by Beekman et al. [7] tested whether a set of alleles increase the risk of coronary artery disease, cancer and type 2 diabetes for compatibility with human longevity, but they found that longevity is not compromised by the cumulative effect of this set of risk alleles for common disease.

Studies in the field of genetics aim to decipher the impact of variation in the DNA structure that either can be inherited and are therefore found in the germ line DNA or that arises during an individual's lifespan. Core end points in the field of genetics are (1) single nucleotide polymorphisms (SNPs), which are single base variations in the DNA structure which are found in 1% of the population or more and (2) copy number variations, which are segments of DNA that vary in copy number between genomes of different individuals ranging from one kilobase to several megabases in size.

Genome-wide association study (GWAS) of human longevity has accumulated lots of data. In order to provide insight into the process of ageing, the applications of integrative genomic may to evaluate heritability of transcripts and identify sequence polymorphism can be useful. Very few copy number variations (CNVs) have been found to be linked with successful ageing and remain the area of active investigation in immune-related genes involved in ageing. A comprehensive consciousness of the interactions between genetic factors involved in the regulation of immune system will help know their roles in longevity and age-related diseases. They also will provide guidance for personalized efforts to intervene in the ageing process.

During ageing, vital bodily function, such as regeneration and reproduction slowly declines and the loss of essential body function leads to age-related pathologies, which ultimately cause death [8]. A large part of ageing phenotype is explained by an imbalance between inflammatory and anti-inflammatory response, which result in "inflammaging", a low-grade chronic pro-inflammatory status of ageing. Successful ageing seems to be related to pointing out that polymorphisms for the immune system genes, which are involved in the regulation of immune-inflammatory responses, may play a key role in the genetics of ageing. Another important pathway implicated in longevity concerns the oxidative stress. The balance between pro-oxidants and enzymatic antioxidant systems may be of particular importance in the elderly. In this scenario, nutritional deficiencies and sedentary lifestyle concur with a depletion of dietary antioxidants and increased susceptibility to oxidative stress.

Women live longer than men, and this difference in life expectancy is a worldwide phenomenon indicating that human longevity seems strongly influenced by gender defined as the combination between biological sexual characteristics and factors related to behavior, social role, lifestyle and life experiences [9].

The aims of the present review are to discuss recent findings, highlight the genetic basis of longevity and describe the most important underpinning which is the gender differences in longevity between males and females.

2. Insulin-like signaling pathway

The most studied pathway that regulates the ageing process is the insulin-like pathways. Briefly, upon insulin-like growth factor-1 (IGF-1) binding to IGF-1 receptor (IGF-1R), the intracellular phosphoinositol-3-kinase (PI3K) is activated, leading to formation of the downstream intermediate phosphoinositide-3,4,5-trisphosphate. The latter binds to 3-phosphoinositide-dependent kinase 1 (PDK-1), which, in turn, phosphorylates and activates the kinases Akt/PKB and *s*erum- and *g*lucocorticoid-inducible *k*inase (*SGK-1*) that control regular growth processes in the cell. Together, the stress resistance factors, among which forkhead box gene, group O (FOXO) transcriptional factor, are activated [10]. This signaling activity is reduced in long-lived subjects of different species, such as nematode, mice and humans.

Gene encoding for protein involved in this pathway contains mutation correlated to longevity. In particular, mutation in IGF-1R gene in humans was associated to longer survival compared to usual [11]. Furthermore, in animal models, mutation in genes encoding for substrates of insulin receptors 1 and 2 results in the extended lifespan [12], and mutations in genes encoding kinases PI3K, AKT/PKB and PDK are associated with a prolonged life [13].

Insulin-like signaling inhibits the mechanisms of stress response regulated by FOXO transcription factor [14]. In mammals, there are four FOXOs (FOXO1, FOXO3, FOXO4, FOXO6) that regulate different genes in different cell types [15]: FOXO3 may undergo a greater decline with ageing. The mechanism by which longevity-associated alleles of FOXO3 reduce age-related mortality is currently of great clinical interest. FOXO3 has been associated with longevity in multiple candidate gene association studies in diverse groups including German, Italian

and Chinese centenarians [16–18]. The precise mechanism by which FOXO3A influences longevity may be due to its effects on oxidative stress, insulin sensitivity and cell cycle progression [2]. A recent GWAS meta-analysis observed only a modest association of FOXO3 with survival to ≥90 years of age [6]. Further, in a genome-wide linkage analysis among nonagenarians, linkage to FOXO3 and another forkhead box gene, FOXO1, was not detected [19]. The lack of an association observed in these studies may be due to small sample sizes of exceptionally long-lived individuals, as the association of FOXO3 with longevity is stronger in persons aged ≥ 95 years and especially in centenarians [17, 18].

3. Lipid metabolism

Lipid metabolism is downregulated with time, leading to age-dependent diseases, such as metabolic syndrome and atherosclerosis. As previously demonstrated, dyslipidemia is associated with altered activity in a number of genes.

Three major genes of lipid metabolism are involved longevity, the genes encoding for apolipoprotein E (APOE), cholesteryl ester transfer protein (CETP) and peroxisome proliferator-activated receptor (PPAR).

In prior candidate gene association, variants of APOE have been consistently associated with longevity. The APOE gene encoded for the apolipoprotein E, a protein that combines with lipids in the body to form lipoproteins, and are responsible for packaging cholesterol and other fats and carrying them through the bloodstream. Maintaining normal levels of cholesterol is essential for the prevention of disorders that affect the heart and blood vessels (CVD), including heart attack and stroke, and consequently it contributed to good ageing.

The APOE gene has three common polymorphic alleles, leading to six possible genotypes [20], called ε2, ε3 and ε4. The most common allele is ε3, which is found in more than half of the general population. Both ε4 and ε2 alleles have been associated with cardiovascular disease (CVD) risk [21]. The associations with CVD may be related to the involvement of these isoforms in inflammation, elevated lipid levels and oxidative stress [22]. Some studies have observed that APOE2 occurs at a higher frequency in the elderly and centenarians, suggesting an association with longevity [23]; on the contrary, APOE4 may be less common in these groups and associated with early mortality [24]. However, a study conducted in Italian subjects showed that the ε2 allele is associated with an increased likelihood of longevity. Interestingly, all of the Italian centenarians of the study were free of cognitive impairment and major age-related diseases, suggesting an association of ε2 allele with successful ageing [25].

A particular chromosomal region, 19q13.11–q13.32 showed linkage with longevity, as shown in a large genome-wide linkage scan among nonagenarian sibling pairs of the European ancestry [19]; subsequent association analyses using GWAS data found that APOE4 and APOE2 alleles explain linkage at this region. Further studies are needed to elucidate the role of rare APOE variants on longevity and healthy ageing.

Variants in the CETP gene, which is involved in the regulation of high-density lipoprotein levels, were previously suggested as markers of exceptional longevity and healthy ageing in Ashkenazi Jews and Japanese-American men, respectively [26, 27]. However, in a recent case-control study among Han Chinese long-lived individuals, none of the four SNPs in the promoter region of the CETP gene was associated with longevity [28]. Additionally, a meta-analysis of eight studies did not observe an association between CETP polymorphisms and longevity [29].

Peroxisome proliferator-activated receptors (PPARs) are ligand-inducible transcription factors that belong to the nuclear hormone receptor superfamily. PPAR ligands comprise fatty acids and their derivatives. PPARα is activated by fatty acids, eicosanoids, 15-d prostaglandin and oxidized fatty acids. PPARα function antagonizes the metabolic syndrome and ageing in general [30], through regulation of genes promoting lipid oxidation and metabolism of lipoproteins, such as main apolipoprotein of high density, Apo A-1.

4. Inflammation

Inflammation forms the basis of many physiological and pathological processes. Much is known about how inflammation is initiated, develops and resolves over the short term. But less is known about the causes and consequences of chronic inflammation. Chronic inflammation, by contrast, is a prolonged, dysregulated and maladaptive response that involves active inflammation, tissue destruction and attempts at tissue repair. Such persistent inflammation is associated with many chronic human conditions and diseases, including allergy, atherosclerosis, cancer, arthritis and autoimmune diseases [31].

A large part of the ageing phenotype is explained by an imbalance between inflammatory and anti-inflammatory networks, which results in the low-grade chronic pro-inflammatory status of ageing, called "inflammaging" [32], which appears accelerated in many age-associated diseases. The source of the age-associated chronic inflammation was mainly attributed to the progressive activation of immune cells over time and to the acquisition of a specific senescent cell phenotype [33]. Thus, the accumulation of senescent cells in aged subjects could contribute to the perpetuation of "inflammaging", and the systemic chronic inflammatory status could, in turn, contribute to the disease development.

Data on case-control studies suggest that the presence of pro-inflammatory genotype is unfavorable for the achievement of extreme longevity in good health and, in addition, it likely favors the onset of age-related diseases, such as CVD and Alzheimer's disease, major causes of mortality and disability in the elderly. In the contrary, it was shown that centenarians have an increased level of inflammatory mediators in comparison to old subjects, but they also have a high level of anti-inflammatory cytokines together with protective genotypes.

Genes implicated in inflammatory pathways may be associated with longevity, as was demonstrated in a case-control study in which a homozygous genotype of the RAGE gene was more frequently found in male long-lived subjects [34]. A study in German long-lived cases

and younger controls observed that cases were less likely to be deficient in complement C4 long genes, suggesting a potential role of immunity in lifespan [35].

The cellular communication has a fundamental role in regulating the reaction of the immune system to a possible danger [36]. In this scenario, a key role may be played by Toll-like receptor 4 (TLR4) that initiated both innate and clonotypic immunity to Gram-negative bacteria and to other agents. A SNP in TLR4 gene, ASP299GLY, was known to regulate the receptor signaling that the presence of 896G allele seems to be attenuated with a minor risk to develop carotid atherosclerosis and less intima-media thickness in the common carotid artery. In addition, 896G TLR4 allele shows a significantly lower frequency in patients affected by acute myocardial infarction with respect to controls, whereas centenarians show higher frequency [37]. This is in agreement with the hypothesis that genetic basis of inflammation might play an opposite role in CVD and in longevity because people genetically predisposed to a weak inflammatory activity less likely develop CVD and, at the same time, without any serious infectious disease complication, more likely live longer [38].

TLR4 activates the inflammatory cell via the NF-kB pathway by inducing the expression of a variety of cytokines; some of these have been shown to be involved in atherosclerosis and reciprocally in longevity. In a previous study, conducted by Candore et al. [39], it was demonstrated that the 896 G allele carriers produce low levels of the pro-inflammatory cytokines IL-6 and tumor necrosis factor (TNF)-α and a higher level of the anti-inflammatory cytokine IL-10.

Cytokines are the expression of a network involving genes, polymorphisms and environment, and are involved both in inflammation and anti-inflammation. Pro-inflammatory cytokines seem to play a pathogenic role in age-related diseases, and in previous study, it was demonstrated that genetic variations located within their promoter regions may influence the susceptibility to age-related diseases, by increasing gene transcription and therefore cytokine production [40, 41]. Conversely, successful ageing seems to be associated to genetic variations determining increased production of anti-inflammatory cytokines or decreased production of pro-inflammatory cytokines, suggesting a role for the control of the inflammatory state in the attainment of longevity.

IL-1, IL-2, IL-6, IL-12, IL-15, IL-18, IL-22, IL-23, TNF-α and interferon (IFN)-γ were described as pro-inflammatory cytokines, while IL-1Ra, IL-4, IL-10 and TGF-β1 as anti-inflammatory cytokines. High levels of IL-1, together with IL-6, tumor necrosis factor (TNF) and interferon (IFN)-γ, are associated with increased risk of morbidity and mortality in the older subject. Two studies have investigated the role of genetic variability of IL-1 gene cluster and a possible association with longevity [42, 43]. Neither study showed statistically significant differences comparing the allele frequencies, genotype frequencies and haplotype frequencies between long-lived patients and youth nor between males or females.

Also, IL-6 cytokine polymorphisms have been linked to longevity. Several data suggest that IL-6 −174C/G locus variability seems to modulate individual susceptibility to common causes of morbidity and mortality among the oldest subjects (e.g. type 2 diabetes, CVD and dementia) and therefore interferes with an individual's ability to reach the extreme limits of human lifespan [44–47]. A meta-analysis, conducted in Europeans, analyzed that data regarding long-lived subjects and controls from eight case-control studies showed no association

between the IL-6 polymorphism and the probability of achieving a very old age. However, in Italian centenarians the IL-6 −174GG genotype appeared to be negatively associated with longevity and reduced the chance for male GG carriers of achieving centenarian status [48]. Also, a Turkish study, conducted by Kayaaltı et al. [49], found an association between IL-6 −174G/C promoter region polymorphism and longevity.

As regards TNF polymorphisms, it was found that there was no association between distribution of TNF-α −308 genotypes and longevity [43, 50]. However, GA genotype was associated with decreased prevalence of dementia in centenarians, and, in centenarians, the AA genotype was associated to higher mortality risk and higher plasma levels of TNF-α [51].

Studies evaluating IFN-γ polymorphisms in longevity showed no association [52, 53]. A gender study evaluated the distribution of +874T → A IFN-γ polymorphisms in 174 Italian centenarians and showed that +874T allele was found less frequently in centenarian women than in centenarian men or in control women, whereas no significant differences were observed in the distribution of the two alleles between male and female controls. These data seem to strengthen the idea that gender may be a major variable in the biology of the ageing process [54].

According to the best of our knowledge, there is no data in the literature to support significant associations between polymorphisms in the anti-inflammatory cytokines and longevity.

Accumulated data strongly suggest that besides chronic up-regulation of pro-inflammatory genes and cytokines, also cyclooxygenases are induced during the ageing process. In fact, cyclooxygenases (COX-1 and COX-2), key enzymes in the conversion of arachidonic acid to the precursors of bioactive lipid mediators, prostaglandin, thromboxane and prostacyclin, and lipoxygenases (LOX) enzymes that catalyze the stereospecific insertion of molecular oxygen into various positions in arachidonic acid, were intimately involved in inflammation. Previous studies have shown that –765GC and –1708GA SNPs in the promoter region of COX-2 gene and 5-LOX genes, respectively, resulting in a significant lower promoter activity, were found to be associated with reduced risk of severe atherosclerosis [55]. In centenarians the frequencies of these pro-inflammatory alleles were significantly lower, whereas age-related controls were higher [56].

Ageing and longevity are complex traits resulting not only and not exclusively from genetics but rather from the interactions between genetics, environment and chance.

5. Oxidative stress

The role of the oxidative stress response in healthy ageing and longevity is a hot topic in the field of human ageing studies.

The free radical theory of ageing, proposed in 1956, suggests that free radical-induced accumulation of damage to cellular macromolecules is a primary driving force of ageing and a major determinant of lifespan [57]. Under normal conditions ROS (including NADPH oxidases (NOX), mitochondria, xanthine oxidase, monoamine oxidase and nitric oxide synthase)

were maintained at the physiological levels by several endogenous antioxidant systems, such as superoxide dismutatase (SOD), catalase, glutathione peroxidases and glutathione reductase (GR). Other antioxidant systems involving thiol-disulfide oxidoreductase systems include the cytosolic proteins thioredoxin (TRX) and glutaredoxin (GRX). ROS at physiological levels can interact with redox state and play a role in mediating cell signaling, while at pathological levels can result in oxidative damage to cellular components that activate several cell death pathways.

The close interrelationship of redox balance to oxidative stress has in recent years become a more prominent aspect of the free radical theory of ageing that was extended to implicate the mitochondrial production of ROS [58]. In fact, studies in long-lived species showed the presence of reduced oxidative damage [59], reduced mitochondrial free radical production [60], increased antioxidant defences [60] and increased resistance to oxidative stress both in vivo and in vitro [61]. However, a lack of correlation of oxidation with lifespan [62], or even an increase in oxidative damage/stress associated with long lifespan, has also been reported [63].

In addition, in support of the importance of genetic factors in the ageing organism's ability to counteract the negative effects of oxidative stress, genetic modifications of the stress response with age have also been reported. These modifications may minimize health risks, and they may increase the individual's possibilities of achieving a longer life [64].

Several studies suggest that both genetic factors and modifiable lifestyle habits have major impact on the oxidative stress response, but the relative contribution of genes and lifestyle in promoting an efficient stress response in cells is difficult to estimate. Data collected in the literature shows that in experimental organisms and in exceptionally long-lived individuals, among the antioxidant enzymes, a major role in longevity seems to be attributed to genes SOD2 and GPX [65].

Knowing that oxidative stress accelerates telomere loss, the genes encoding the telomere maintenance pathway, mainly telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC), are also important to happen [66, 67]. In addition, in support of gender difference in longevity, some genes involved in the stress response pathway, like heat shock protein A1A (HSPA1A) or paraoxonase 1 (PON1), show sex-specific effects [68].

A study in Chinese centenarians and nonagenarians and younger controls identified significant genotype differences in the GNB3 and eNOS genes, whose variants have been implicated in hypertension and vascular function via nitric oxide (NO) generation, respectively [69]. Another study found that variants of two NO synthase genes, NOS1 and NOS2, decrease the probability of attaining longevity, suggesting that NO production and signaling may be involved in ageing [70].

Mitochondrial genetic variability, both germ line and somatic, influences the stress response and is associated with human ageing/longevity. In both physiological and pathological conditions, a strict coordination between nuclear and mitochondrial genomes is necessary to ensure the biosynthesis and functional activity of mitochondria [71]. In normal conditions, signals from the nucleus to mitochondrion are essential for maintaining an adequate mitochondrial structure and function. The mitochondrial replication and transcription were modulated by

several nuclear-encoded transcription factors and coactivators, such as transcription factors which bind to the promoter regions of mtDNA (transcription factors A (Tfam) and B (mtTFB) that enhancing the rate of transcription initiation of mtDNA genes and mitochondrial biogenesis) and nuclear respiratory factors NRF-1 and NRF-2 and the peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1) family coactivators (PGC-1α, PGC-1α,and related coactivator PRC) [71–73].

Although many association studies explored the effect of genetic variability at candidate genes belonging to the oxidative stress pathway in relation to age-related clinical conditions [74], as well as with human longevity, few papers investigated their role on the quality of human ageing and in particular on the functional decline characterizing human senescence. Most of the studies investigate the association of genes related to oxidative stress with cognitive ability and cognitive ageing in healthy older people [75–79] and found an involvement of apolipoprotein E (APOE), catechol-O-methyltransferase (COMT), brain-derived neurotrophic factor (BDNF) and dystrobrevin-binding protein 1 (DTNBP1) genes in cognitive ability in older people.

A recent work conducted by Dato et al. [80] investigated the association between 311 SNPs at 38 genes belonging to the oxidative stress pathway with functional status at very older age. They found associations for TXNRD1 variability with activities of daily living and walking speed, NDUFS1 and UCP3 with handgrip strength and walking speed and GCLC and UCP2 with walking speed. They also found that the association between genetic variability in the pro-oxidant-antioxidant pathway and functional status at old age is influenced by sex and in particular in nonagenarian females. From these data it is possible to speculate that pro-oxidant-antioxidant pathway is able to modulate physical and cognitive performance after the ninth decade of life, finally influencing extreme survival.

Thus, the balance between pro-oxidants and enzymatic antioxidant systems may be of particular importance in the elderly, whose nutritional deficiencies and sedentary lifestyle concur with a depletion of dietary antioxidants and increased susceptibility to oxidative stress.

6. Gender and longevity

The impact of gender difference in ageing has been extensively assessed, but the precise mechanisms of interaction between a series of fundamental aspects, such as hormonal, immunological and metabolic pathways as well as genetic background remain largely unknown. The high prevalence of women among centenarians suggests that men and women follow different trajectories to reach extreme longevity. In particular, females benefited from healthier lifestyles and favorable environmental conditions in the past century [81]. Moreover, the differences in longevity between genders are related to free radical production because mitochondrial oxidative stress is higher in males than females. Estrogens in women confer better protection against ageing, through an up-regulated expression of antioxidant longevity-related genes [82]. In support of this, there are also some available epidemiologies of age-related diseases confirming substantially different between genders and showing changes dramatically in women after menopause [83].

Much research has been carried out into the role of sex hormones in determining lifespan [84], and one hypothesis is that sex hormones appear to influence the immune system. It is well known that estrogens, androgens and progesterone affect cells of the innate and adaptive immune system differently during the reproductive phase of life [85]. It is widely recognized that estrogens inhibit natural killer cell cytotoxicity and reduce neutrophil chemotaxis and consequently inflammation [86, 87]. Moreover, macrophages treated in vitro with oestradiol display a reduced production of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α [88]. Estrogens and androgens are responsible for a reduced immature number of T-lymphocytes and thymus involution after puberty [89] and can also influence the adaptive immunity in an opposing way, contributing to an improved humoral response in women but also favoring the appearance of autoreactive clones and the susceptibility to autoimmune diseases [90].

The sudden loss of ovarian estrogen and progesterone production that characterizes menopause induces pathophysiological changes in different organs and systems [91], bone density, breast cellular composition, cardiovascular health, mood/cognitive function and sexual well-being. Menopause reflects the inevitable final hallmark of a woman's fertile lifespan and of the above-described beneficial effects of estrogens on immune responses. Rapid reduction of estrogen levels results in an increased susceptibility and mortality toward a series of infectious diseases caused in old women losing of their immunological privilege toward infection [85, 92].

It is noteworthy that women will soon spend half of their life in postmenopause, if the current trend of increasing human life expectancy should persist.

7. Lifestyle and physical activity

The ageing process is dynamic and characterized by a continuous remodeling, and it appears evident that the main actors are DNA repair, apoptosis, immune response, oxidative stress and inflammation that these mechanisms are necessary to interact in an efficient way. But life expectancy at birth has been increasing in western societies thanks to the continuous improvement of medical assistance with respect to age-related diseases, especially CVD and cancer, environmental factors and lifestyle.

Among lifestyle factors, having a major impact on the whole organism oxidative stress response, there are impaired nutrition, reduced physical activity, alcohol consumption and cigarette smoking, which are major contributors to the failure of systemic homeostasis, especially if persisting for a long part of the individual's life. For these reasons the effects of physical activity and diet in oxidative stress response in humans have been suggested that both are able to tip the balance of oxidative burden/antioxidant response.

In support of this hypothesis, it has been reported that dietary restricted mice, which live much longer and show a very delayed aging phenotype [93, 94]. Thus, dietary restriction can trigger a molecular genetic response which postpones ageing and age-related phenotypes. Specifically, reduced glucose metabolisms result in an increase in ROS accumulation

by stimulating the basal metabolic rate and consequently increase oxidative stress [95]. This has brought to search for drugs or interventions which may act on these mechanisms without the side effects of calorie restriction [96]. Among the most important measures in this context, you may name the protein restriction and the use of drugs involved in the IGF-1 axis and in the FOXO/TOR pathway [97]. As emerged from the analysis of data resulting from studies in geographical areas with exceptional longevity (such as Sardinia and Okinawan), it seems to be important to follow a low-protein diet, such as the Mediterranean diet [98]. In these cases, the traditional diet seems to allow to stimulate molecular mechanisms that increase the lifespan. The Mediterranean diet and red wine consumption, rich in antioxidants like resveratrol, have been shown to have protective effects against oxidative damage. People who consume large amounts of fruits and vegetables have a lower incidence of CVD, stroke and tumors, and it has been proposed that the assumption of micro-nourishments with antioxidant activity could be responsible for the reduction of chronic diseases [99]. Consistently, long-lived individuals seem to prefer a diet rich in vegetables and in natural antioxidants. In addition, lower degree of oxidative stress was found in healthy Okinawan centenarians compared to aged subjects, data obtained by measuring the level of lipid peroxide and tocopherol (Vitamin E) in plasma [100]. Okinawan diet, with its high intake of green leafy and yellow-root vegetables, sweet potatoes as a dietary staple and soy as a principle protein, supplemented by small amounts of fish and meat, may be a significant advantage in achieving their exceptionally long life expectancy, thanks to a particularly high amount of antioxidant vitamins [102]. However, this finding could be due not only to nutritional factors [101] but also to a specific genetic background.

Moderate physical activity attenuates several age-related diseases, reduces blood pressure in hypertensive patients [103] and improves the serum lipid profile with an average reduction of 3.7% in triglyceride and 5% in low-density lipoprotein (LDL)-cholesterol levels and a 4.6% increase of HDL-cholesterol levels [104]. Benefits derived by physical activity in older people, for the maintenance of an optimal health status and the prevention or management of chronic diseases, can derive from exercise-induced adaptations of the cellular antioxidant defence systems [105]. In these subjects indeed, the presence of higher serum levels of antioxidants associated with higher strength and physical performance was demonstrated [106, 107].

8. Epigenetics

In the field of longevity, more recent studies have shown that an important role is also played by epigenetics. In fact, studies in various models have revealed that genetic differences and somatic modulations underlie longevity, but also non-genetic factors play an important role. These considerations have pointed out the importance of epigenetic mechanisms in modulating longevity pathways.

Epigenome, the intermediate layer of genomic information between the genome and transcriptome, is another molecular level that could provide additional insight in the processes of ageing. Epigenetic regulation of transcription is mediated by histone modification, DNA methylation and microRNAs.

Chromatin and epigenetic factors influenced gene expression dynamically by regulating access of transcriptional machinery to DNA. It is clear that, with age, there is a general loss of histones coupled with local and global chromatin remodeling, an imbalance of activating and repressive histone modifications and transcriptional change in all ageing models.

Additionally, particularly in mammalian systems, there is a global and local change in DNA methylation, site-specific loss and gain in heterochromatin and significant nuclear reorganization

Methylation patterns of genes involved in, for example, development and morphogenesis, DNA binding and regulation of transcription [108, 109], seem to change with age; a progressive linear increase in methylation with age at the ELOVL2 gene was found in a cohort of 501 long-lived individuals [110].

The epigenomic field recently became more accessible for the screening of large study populations; however, larger studies, new methodologies and the consistent use of different study designs to follow up results might help to unravel the genomic component of healthy ageing and longevity.

It was proposed that a mathematical model, on the basis of the methylation levels of 353 CpG units, formulated a mathematical model, the so-called epigenetic clock, which was able to predict the chronological age of a subject starting from the methylation level of several cells and tissues of his body [111]. This model also indicates that methylation represents one of the most accurate biomarkers of age and it was able to predict all-cause mortality also after adjusting for traditional risk factors [112]. Finally, when it was used to estimate the biological age of several tissues from supercentenarians, it has been demonstrated that the brain and muscle represent the youngest tissues of these exceptional individuals [113].

However, even if the relationship between methylation process and ageing is still not clear, this discovery has some wide potential applications, ranging from detailed monitoring of changes occurring with age to forensic purposes. It therefore appears clear that future advances in this field could help the understanding of the complex physiology of ageing, lifespan and age-associated diseases.

Starting from the observations, epigenetic modifications affect not only the ageing process but also its quality [114]. Epigenome-wide association studies identified hundreds of sites spread along the entire genome in which methylation levels change between the oldest old and younger subjects.

Although serum microRNAs play roles in the diagnosis of various diseases, little is known about circulating miRNAs in the ageing process. Human serum from healthy individuals contains abundant quantities and species of circulating miRNAs, and they can be readily detected with almost all routine RNA analysis techniques, including qRT-PCR. Zhang et al. evaluated miRNA expression profiles in the sera of healthy individuals at different steps of the ageing process, and they found a different miRNA regulation in the ageing process. In particular, they found that miRNAs changed significantly in the ageing process: miR-29b, miR-106b, miR-130b, miR-142-5p and miR-340 decreased, and miR-92a, miR-222 and miR-375 increased [115].

Evidence provided suggested also that TLR signaling transduction is impaired in cells from aged animals, as suggested by lack of response to the stimulation of lipopolysaccharide (LPS), and the increased pro-inflammatory cytokine release observed in macrophages of aged mice even if no conclusive results can be obtained on the TLR baseline expression level in animal model during ageing [116]. In particular, a trio of miRNA, such as miR-155, miR-21 and miR-146a, has proven to be key TLR signaling modulators, and, importantly, these miRNAs are codified by endotoxin-responsive genes [117].

9. Discussion

Human longevity is an extremely complex trait on which genetic, epigenetic and environmental factors act. It is becoming evident that the genetic differences concur lowly to life expectancy before 60 years, but their impact on survival becomes more prominent at old ages. Unfortunately, the currently available knowledge is not sufficient to explain the secret of longevity, even because available data, reviewed in this work, are sometimes conflicting. The precise reason for these data discrepancy is unknown; however, several factors may be involved, such as ethnic, lifestyle and cultural differences among the population analyzed in the diverse studies.

During the past decade, several longevity gene candidates have been identified; the majority of them concern components of the inflammatory system, stress response or lipid and glucose metabolism.

As discussed above, longevity is characterized by a balance between pro-inflammatory and anti-inflammatory agents, which act as key players. A pro-inflammatory propensity can confer high resistance against infectious diseases, but it also may increase susceptibility to inflammation-based diseases. On the other hand, an anti-inflammatory tendency, instead, may cause an increased susceptibility to infections and might not allow to reach a more advanced age. According to a perspective suggested in recent years, the best candidates to become centenarians are not the strongest, and most robust subjects among their age cohort, but subjects that better adapt to the environment, show more biological plasticity.

Since genetic variants may exert small to moderate effects on human longevity, additional prospective investigation with large sample size is needed to elucidate the role of genetic variation. It may also be worthwhile for future studies to evaluate the genetic basis of ageing separately in male and female, which is especially importantly given the longer lifespan of females.

Therefore, the balance between lifestyle and physiological changes during ageing on the one hand and risk factors for age-associated diseases on the other should be taken into consideration. Several studies suggest that on the oxidative stress response both genetic factors and modifiable lifestyle have major impact. Studies in experimental organisms and in exceptionally long-lived individuals have tried to identify genetic factors modulating the oxidative stress response, but the relative contribution of genes and lifestyle in promoting an efficient stress response in cells is difficult to estimate.

Data deriving from different studies indicate that stress response pathway is an integrated network of molecular activities, ranging from immunity to inflammatory regulation and activation, to glucose homeostasis and mitochondrial metabolism. Therefore, ageing seems to be associated with a loss of complexity in the dynamics of multiple control systems that may reduce the ability to adapt to stress, leading to a state of impaired homeostasis and vulnerability to internal and external stressors. On the contrary, the healthy oldest old seems be able to maintain a higher level of integration among the different physiological pathways operating within the cell, thus interacting more successfully with stressors.

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