Inflammation and Genetics: An Insight in the Centenarian Model

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Abstract The number of centenarians is growing worldwide. This specific cohort has aroused the attention of scientists worldwide and is considered one of the most valuable models to study the mechanisms involved in the aging process. In fact, they have reached the extreme limits of human life span and, most important of all, they show relatively good health being able to perform their routine daily life. Because they have escaped the common lethal diseases, the role of their genetic background has been brought into focus. In fact, sequence variations, in a variety of pro- or anti-inflammatory cytokine genes, have been found to influence successful ageing and longevity. The key role played by cytokines has been also confirmed in centenarians as we know that inflammation has been related to several pathological burdens (e.g., obesity, atherosclerosis, and diabetes). Successful ageing seems to be related to an optimal functioning of the immune system, 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. This review provides an update in the field of ageing related to inflammation and genetics.

Statistics. The number of centenarians is growing worldwide. According to the report "World Population Ageing 2009," by the United Nations Department of Economic and Social Affairs/Population Division, there were an estimated 455,000 centenarians in the world in 2009 and, by 2050, their number is projected to rise to 4.1 million, a ninefold increase (Figure 1). The same report stresses how the majority of centenarians (69%) live in the more developed regions and, in particular, Japan will experience a remarkable increase in the number of centenarians, from less than 76,000 in 2009 to almost 800,000 in 2050 and, by mid-century, it is expected to have the world's largest number and proportion of centenarians (nearly 1% of Japan's population will be aged 100 yrs or over).

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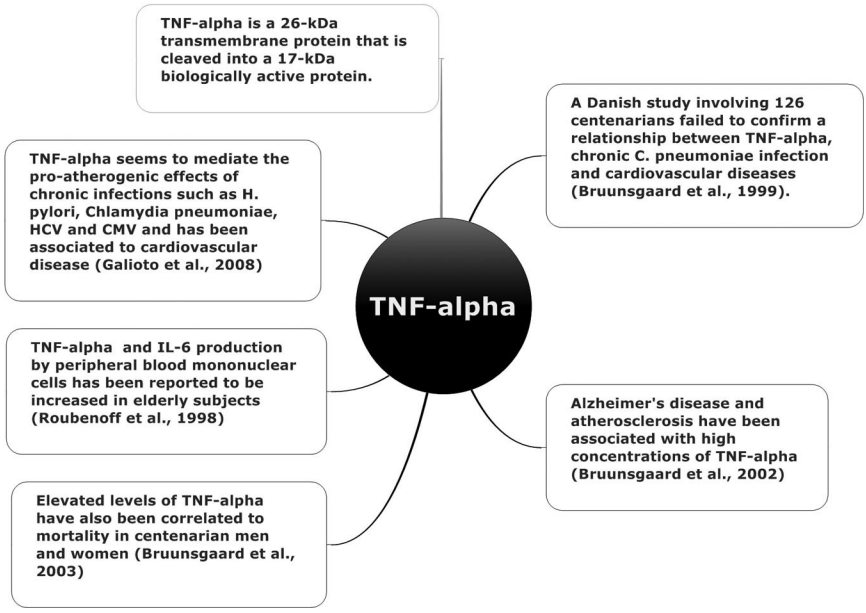

Figure 1. TNF-α role in the elderly and in age-related diseases (Galioto et al. 2008; Bruunsgaard et al. 1999, 2002, 2003; Roubenoff et al. 1998).

Centenarians as a Model of Successful Aging. Improving the quality of life of elderly people is going to become a priority because of the continuous increase in the number of centenarians which underlines the importance of studying the processes involved in ageing.

Centenarians are a widely accepted model of successful ageing, a complex process influenced by several environmental, biological, and lifestyle factors because they have reached the extreme limit of the human life span. In the centenarian model, several aspects have been studied (e.g., immunity, inflammation, metabolism, genetics, and oxidative stress) to understand what is the key for the centenarians' long survival. The importance of the centenarians' model is due to the centenarians' ability to overcome fatal cardiovascular events and several other diseases. Centenarian samples have been widely used in the research fields of ageing and survival, and it has been proposed that centenarians are characterized by more efficient protective molecules or biochemical pathways to activate them (Chondrogianni et al. 2004). A study performed on 95 centenarians from the island of Okinawa showed that their functional autonomy was directly correlated to the albumin levels and inversely correlated to γ-globulins (Nozaki et al. 1998).

It is widely accepted that centenarian women outnumber centenarian men, although centenarian men are in better health status than centenarian women, making the gender factor important to be taken into account (Franceschi et al. 2003). An example of the importance of the gender can be found in the evidence

that interferon-γ (IFN-γ) has been reported to be an inflammaging marker typical of women as showed by a study in Italian centenarians (Lio et al. 2002). The same study showed that the possession of the +874A allele, which is associated with low IFN-γ production, significantly increases the possibility to achieve extended longevity.

Sir Thomas Perls and co-workers have shown that human longevity is an inherited trait. They have demonstrated that at old age, the survival probability for sisters and brothers of centenarians was four times higher than usual (Perls et al. 1998).

It is very important to underline that, although an important familial component has been observed in the ability to survive to extreme old age, it is possible to improve the quality of life and delay cardiovascular aging of older people by following some lifestyle-related rules regarding diet, physical activity, and smoking (Perls et al. 2003).

A review written by Imhof and co-workers report how centenarians display lower scores for anxiety and depression and better coping abilities compared to less elderly individuals, underlining that centenarians may represent a particular group of individuals with relatively slow rates of aging and increased resistance to biological and psychological stress and age-related diseases such as cancer, stroke, and heart disease (Imhof et al. 2007).

Moreover, because most centenarians avoid dementia, they will be able to provide informed consent to donate their stem cells. It has been proposed that these stem cells should be studied to determine their developmental potential, mutational load, telomere lengths, and markers of "stemness" (Lewis et al. 2009).

Review Criteria. We conducted a Medline/PubMed search to identify articles related to inflammation and genetic factors in centenarians between January and August 2010.The key words used were "genetics," "inflammation," and "disease," combined with the key word "centenarians."

Review

Centenarians and Inflammation. Inflammation, triggered by harmful stimuli and agents like infection and tissue injury, is defined as a wide variety of adaptive physiological and pathological processes to avoid infection, repair damage, and restore the organism to the usual state of homeostasis (Majno et al. 2004). Inflammation is involved in the pathogenesis of several age-related disorders (e.g., atherosclerosis, Alzheimer's disease [AD], osteoporosis) and in the increase of mortality risk (Franceschi et al. 2000; Bruunsgaard et al. 2001; Lio et al. 2003a). There is evidence that cytokines are key players in maintaining lymphocytes homeostasis (Sanjabi et al. 2009). Not only do they determine whether a response occurs after an immune insult, but they can also determine the nature of the response (cytotoxic, humoral, cell mediated, or allergic) or, in contrast, they may cause nonresponsiveness and active immune suppression (Commins et al. 2008). Moreover, sequence variations in several cytokines, such

as interleukin-6 (IL-6), interleukin-10 (IL-10), and interferon-γ (IFN-γ) have been shown to be associated with successful aging and longevity (Lio et al. 2002a; Bonafè et al. 2001a; Lio et al. 2002b).

In 2000 Franceschi et al. (2000) used for the first time the word inflammaging to "complete" the "network theory" (1989) which asserts that aging is indirectly controlled by a network of cellular and molecular defense mechanisms, including heat shock proteins, DNA repair mechanisms, apoptosis, and, at a more integrated level, the immune and the neuroendocrine systems (Paolisso et al. 2000). All these mechanisms define the progressive increase in the proinflammatory status which, added to the reduction in the capability to cope with a variety of stressors, i.e. physiological (such as UV and γ radiation, heat), chemical (components of the body and products of metabolism such as oxygen-free radicals and reducing sugars), and biological agents (viruses, bacteria), constitute a major characteristic of the aging process (Franceschi 1989; Kirkwood 1992). According to Franceschi, "a strong inflammatory response is an advantage before the age of 50, but it is detrimental in later life when the reproduction driven force of evolution decreases" and genetics has a greater role in protecting men from inflammatory diseases better than women because the latter have more protective hormones (Louët 2003).

It has been observed that despite the human natural progressive age-related functional autonomy decay, centenarians have proved to maintain a good level of autosufficiency for the basic activities of everyday life (Antonini et al. 2008). Moreover, sequence variations in a variety of pro- or anti-inflammatory cytokine genes have been found to influence successful aging and longevity (Carrieri et al. 2004).

Franceschi (Franceschi et al. 2007), an expert in the field of aging research, has discovered that centenarians are characterized by low levels of IL-6, a pro-inflammatory cytokine, whereas they show high levels of the anti-inflammatory cytokine IL-10. It has been found that centenarians display high levels of anti-inflammatory agents such as IL-10, IL1-Ra, and TGF-β, which likely counteract the effect of the pro-inflammatory ones (Lio et al. 2003b; Cavallone et al. 2003; Carrieri et al. 2004).

Several studies have addressed the expression of inflammatory markers and polymorphisms, and many of them have also correlated cytokines expression with surgery. The main findings involving IL-1, IL-10, TNF-α, and IL-6 are reported in Figures 1–4.

Shi et al. (2010) have described that Chinese centenarians had lower chronic disease risks, higher anti-oxidants activity compared with other age groups, and had a high level of nutritional elements compared with those aged 90 and over. Moreover, it has been shown that the CCR5Delta32 anti-inflammatory variant might be a resistance factor for the development of pancreatic cancer (Pca) (Balistreri et al. 2009). There is also evidence indicating a role played by the metalloproteinases (MMPs) in the weakening of atherosclerotic plaque which predisposes to lesion disruption. The distribution of this −1562°C/T MMP-9

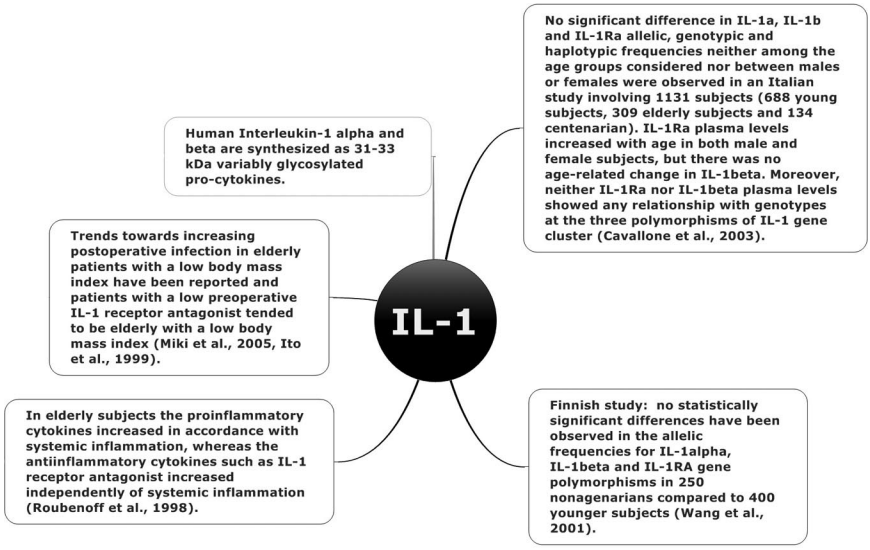

Figure 2. IL-1 role in the elderly and in age-related diseases (Wang et al. 2001; Cavallone et al. 2003; Miki et al. 2005; Ito et al. 1999; Roubenoff et al. 1998).

SNP in 115 MI patients, 123 controls, and 34 centenarians from Sicily has been analyzed, and no significant differences in the genetic distribution and allelic frequency of −1562°C/T MMP-9 SNP, between the studied groups, were observed (Nuzzo et al. 2006).

It has been asserted that the beneficial effects of inflammation, devoted to the neutralization of dangerous/harmful agents early in life and in adulthood, become detrimental late in life in a period largely not foreseen by evolution, according to the antagonistic pleiotropy theory of aging (Franceschi et al. 2000).

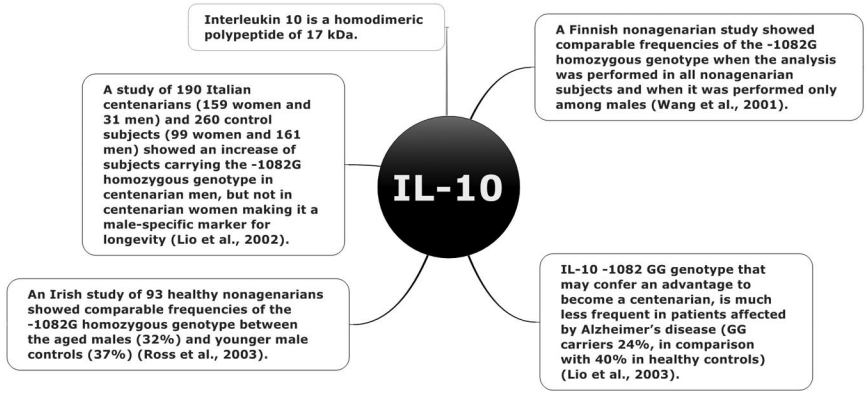

Figure 3. IL-10 role in the elderly and in age-related diseases (Lio et al. 2002b; Ross et al. 2003; Wang et al. 2001; Lio et al. 2003a; Candore et al. 2006).

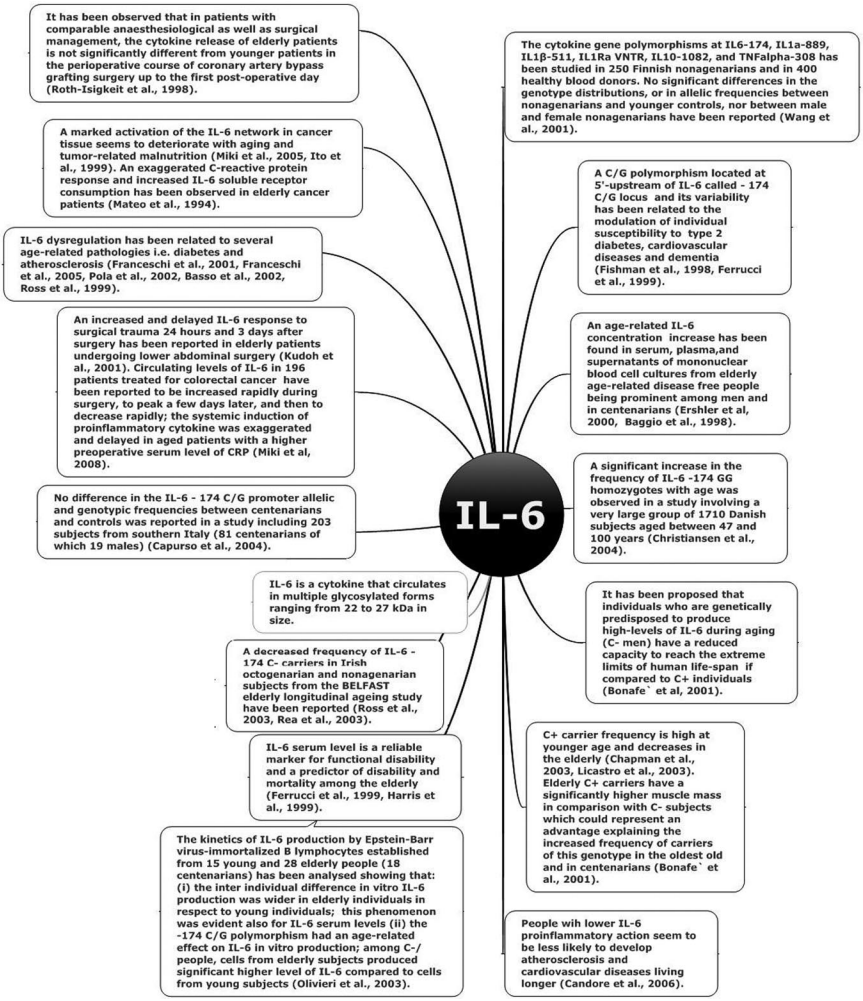

Figure 4. IL-6 role in the elderly and in age-related diseases (Fagiolo et al. 1993; Young et al. 1999; Ershler et al. 2000; Baggio et al. 1998; Ferrucci et al. 1999; Harris et al. 1999; Fishman et al. 1998; Bonafè et al. 2001b; Franceschi et al. 2005; Pola et al. 2002; Basso et al. 2002; Ross 1999; Franceschi et al. 2001; Ross et al. 2003; Rea et al. 2003; Christiansen et al. 2004; Capurso et al. 2004; Wang et al. 2001; Chapman et al. 2003; Licastro et al. 2003; Kudoh et al. 2001; Miki et al. 2008; Roth-Isigkeit et al. 1998; Miki et al. 2005; Ito et al. 1999; Wakuda et al. 2001; Mateo et al. 1994; Ogawa et al. 1998; Olivieri et al. 2003).

The −330GG genotype association to longevity has been studied in 168 centenarians and 214 control subjects. The authors underline that a genetic background, favoring an increased IL-2 production might be detrimental for longevity, and they have also observed that an increase of IL-2 and other pro-inflammatory cytokine production characterize AD serum profile. Therefore,

a reduction of −330G allele frequency might be protective for healthy aging limiting cell mediated inflammation implied in age associated diseases (Scola et al. 2005).

The effects of the TGF-β1 genetic variability on plasma levels of the biologically active form (naturally processed) of this cytokine were studied in 143 randomly selected subjects, including 73 centenarians, showing that the variability of the TGF-β1 gene influences longevity and the age-related increase in plasma levels of active TGF-β1 does not seem to be genetically regulated (Carrieri et al. 2004).

A study (Biagi et al. 2010) has been performed in centenarians enrolled in Emilia Romagna showing that, after 100 yrs of symbiotic association with the human host, the microbiota is characterized by a rearrangement in the Firmicutes population and an enrichment in facultative anaerobes, notably pathobionts; this compromised microbiota in the centenarians is associated with an increased inflammatory status, also known as inflammaging, as determined by a range of peripheral blood inflammatory markers.

Genetics

Lipid Profile. APOE is the signal for the hepatic uptake of triglyceride-rich lipoproteins of dietary origin (chylomicron remnants after extensive peripheral triglyceride removal catalyzed via lipoprotein lipase) and of endogenous origin (principally hepatic), the very-low density lipoproteins (VLDLs) and their remnants, the smaller, denser VLDLs, and intermediate-density lipoproteins (IDLs) (Hazzard 2001). APOE has three common isoforms, namely E2, E3, and E4, which are coded by the alleles 12, 13, and 14 at a single locus on chromosome 19; the APOE isoforms interact differently with specific lipoprotein receptors that alter cholesterol circulating levels (Panza et al. 1999). Apo E4 has been associated with old-age onset AD (Rosenberg 2000) and with a moderately increased risk for cardiovascular risk, while APOE2 seems to have a protective role (Lewis et al. 2004). Saunders et al. (1993) have also reported that APOE ε4 allele is associated with an increased risk for development of sporadic and familial late onset Alzheimer's disease (LOAD). It has also been reported that individuals with atherosclerosis, peripheral vascular disease, or diabetes mellitus have a higher risk of cognitive decline if they carry the APOE4 variant (Haan 1999). A lack of association between AD and APOE allele ε 4, a major risk factor for late-onset AD in younger cohorts, has been demonstrated in centenarians (Hestad et al. 2005; Juva et al. 2000; Salo et al. 2001; Spencer et al. 2003).

The apoJ gene has been found overexpressed during senescence in both human and rat cells (Gonos et al. 1996, 1998). It has also been pointed out that *apoJ* may be a survival gene, exerting a cellular protective function (French et al. 1994; Koch-Brandt et al. 1996).

A cross-sectional analysis in 75 centenarians and 73 healthy older volunteers living in the metropolitan area evidenced the following: (1) the

centenarians showed low concentrations of total and low-density lipoprotein cholesterol (LDL-C) and a relative predominance of high-density lipoprotein 2 cholesterol if compared with the older volunteers–in particular a significant association between low levels of LDL-C and apoB was observed and it was possibly attributed to APOE $\varepsilon 2$ alleles and longevity; (2) the centenarians also showed elevated levels of lipoprotein (a) and decreased high density lipoprotein-cholesterol (HDL-C) which seem to be an unfavorable lipoprotein profile; and (3) lower levels of HDL-C in the centenarians were associated with decreased serum albumin, elevated CRP and IL-6 levels, and cognitive impairment, suggesting that HDL-C could be a sensitive marker for frailty and comorbidity in the oldest old (Arai et al. 2001).

Barzilai et al. studied the lipid profile characteristics of Ashkenazi Jewish centenarians' offspring and compared them with control groups. This study showed the following: (1) centenarians' female offspring had significantly higher plasma levels of HDL-C levels compared with controls and (2) centenarians' male offspring had higher plasma levels of HDL-C levels and significantly lower LDL-C levels compared with controls (Barzilai et al. 2001).

In 1994, certain alleles of two genes, *APOE* and *ACE*, were reported to be more frequent in French centenarians, suggesting an association with such a complex polyfactorial process as longevity (Blanché et al. 2001). Moreover, the *APOE* gene is considered to be a frailty gene that associates with moderate differential mortality in elderly people (Gerdes et al. 2000). It has been shown that no significant differences in survival among different apoE allele carriers are present in very old ages; therefore subjects aged 100 and older, with the $\varepsilon 2$ and $\varepsilon 4$ allele, are a very select group of individuals who might have been exposed to a number of environmental factors during their long lives, and the protection/ risk effect associated with the apo E polymorphism might be attenuated (Louhija et al. 2001).

Studies on the genetics of human aging have shown an age-related variation of the 3′APOBVNTR genotypic pool (alleles: Short, S, $<$35 repeats; Medium, M, 35–39 repeats; Long, L, $>$39 repeats) with the homozygous SS genotype showing a convex frequency trajectory in a healthy aging population. Moreover, this genotype was rare in centenarians, indicating that the S alleles are unfavorable to longevity, while it was common in adults indicating a protective role at middle age. The average effects of S (αS), M (αM), and L (αL) alleles on lipidemic parameters in a sample of healthy people (409 subjects aged 20–102 yrs) recruited in Calabria (southern Italy) have been studied showing that the S alleles significantly lower the serum level of both TC and LDL-C. 3′APOB-VNTR alleles in cardiovascular atherosclerotic disease (CD). A specific effect exerted by the S alleles on both TC and LDL-C was observed showing that the S alleles are protective in CD-L while neutral in CD-H. The authors conclude that the S alleles would be advantageous in adults (by protecting from CD-L) while dangerous in the elderly, probably because they lower serum cholesterol below a critical threshold (Garasto et al. 2004).

A pooled statistical analysis to assess any association between the serum araoxonase 1 (PON1) gene polymorphisms PON1 55 (L/M) and 192 (Q/R) in a large combined group of Italian centenarians and octo/nonagenarians from Northern Ireland (NI) has been conducted showing a modest association between the 192R allele and longevity in these populations (Rea et al. 2004).

The Pro12Ala polymorphism of PPAR γ 2 has been studied to understand whether it is related to essential hypertension among Chinese nonagenarians/centenarians. The genotype frequencies of the Pro12Ala polymorphism were 0.2% Ala12Ala, 9.4% Pro12Ala, and 90.4% Pro12Pro in all participants. The authors observed the following: (1) the Ala12 allele of the PPAR γ Pro12Ala polymorphism is associated with lower prevalence of hypertension and (2) the Ala12 allele of the PPAR γ Pro12Ala polymorphism is associated with lower concentration of triglycerides (Lu et al. 2008).

APOE polymorphism has been studied in 79 sporadic LOAD patients, 125 unrelated caregivers or volunteers (19 $\pm$ 80 yrs), and 67 centenarians from Apulia, southern Italy. The following results were reported: (1) the frequency of *APOE* 12 allele was higher in centenarians than in LOAD patients, while the frequency of 14 allele was lower; (2) in middle-aged adults, the 14 allele frequency was higher than in centenarians; (3) the 14 allele frequency was lower in healthy adults than in LOAD patients, while 12 was higher; (4) compared with the allele frequencies of Northern and Central European countries, a geographic trend for 13 and 14 alleles in LOAD and middle-aged adults was observed; (5) the frequency of 13 increased from Northern to Southern Europe, while 14 allele frequency decreased significantly; and (6) in centenarians, 12 showed a North$\pm$South increasing pattern, while 14 allele frequency was in opposite trend. This study provides a novel finding that the *APOE* 14 allele frequency decreases according to a geographic trend from Northern to Southern Europe in two highly selected populations as those ones of LOAD patients and centenarians. However, the *APOE* 13 allele frequency appears to increase in the opposite direction (from South to North) in LOAD patients and middle-aged adults. This decrease in the 14 allele frequency in healthy centenarians and LOAD patients, as well as in normal middle-aged adults, should be associated with a decrease in the prevalence of AD from Northern to Southern Europe (Panza et al. 1999).

Telomere Length and Other Genetic Variants. It is widely accepted that the aging process in humans seems to be associated with genetic instability and with a decline in the efficiency of repair processes and accumulation of mutations attributable to an adverse endo- and exogenous condition result in the increased level of DNA damage leading to, at the cytogenetic level, an increased frequency of chromosomal aberrations (Vijg 2004; Barnett et al. 1995; Bohr 1995; Wojda et al. 2006). It is known that somatic dividing cells progressively lose telomeric DNA at the ends of the chromosome arms as the organism ages. The telomere hypothesis of cellular aging proposes that cells become senescent when progressive telomere shortening during each division produces a threshold telomere

length, thus suggesting that telomeres shortening may be a molecular counting mechanism (Gonos 2000). The "Hayflick replicative senescence model" is considered the only model for studying aging in vitro in cells having a proliferative potential, and it has been widely used for cloning and the subsequent expression of the human telomerase gene. The current hypothesis is that telomere shortening may be a molecular clock that triggers senescence (Gonos 2000). A study in which two telomerase-negative normal human cell types were transfected with vectors encoding the human telomerase catalytic subunit shows that transfected cells, apart from having elongated telomeres, extend their normal life span by at least 200 population doublings (Bodnar et al. 1998; Holt et al. 1999). Telomere length, a tandem repeat of DNA sequence at the ends of eukaryotic chromosomes, has been shown to be a marker of oxidative stress (Saretzki 2009) and has been inversely related to several chronic diseases, such as CAD (Brouilette et al. 2008), as well as obesity (Nordfjall et al. 2008), and insulin resistance (Gardner et al. 2005). Studies have shown that there is a rapid decrease in telomere length in the first four years of life (Rufer et al. 1999), a decline again in early adult life, and gradual progress with advancing age (Frenck et al. 1998). A significant association between obesity and telomere length has been observed in adults but not in children (Zannolli et al. 2008). A study designed to determine the associations of telomere length to markers of obesity, insulin resistance, and inflammation in Saudi children (69 boys and 79 girls, aged 5–12 yrs participated in this cross-sectional study) has shown that mean telomere length is significantly shorter in obese boys compared with their lean counterparts, not in girls. It is not associated with insulin resistance, adipocytokines, and markers of inflammation. In girls, the significant predictor of telomere length was waist circumference, explaining 24% of variance ($p = 0.041$), while in boys systolic BP explains 84% of the variance (Al-Attas et al. 2010b).

Based on the fact that adipocytokines are linked to obesity and to the development of insulin resistance, which in turn may lead to accelerated aging, a cross-sectional study has been designed to determine associations of chromosomal telomere length (TL) to markers of obesity and insulin resistance in middle-aged adult male and female Arabs with and without diabetes mellitus type 2 (DMT2) (193 non-diabetic and DMT2 subjects without complications [97 males and 96 females]). Circulating chromosomal leukocyte TL had significant inverse associations with BMI, systolic BP, fasting insulin, HOMA-IR, LDL- and total cholesterol, Angiotensin II (ANG II), and hsCRP levels. Adiponectin, body mass index (BMI), systolic BP, and LDL-C have predicted 47% of the variance in TL. HOMA-IR is the most significant predictor for TL in males, explaining 35% of the variance. In females adiponectin accounts for 28% of the variance in TL. In conclusion, obesity and insulin resistance are associated with chromosomal TL among adult Arabs. The author has observed that the positive association of adiponectin to TL has clinical implications as to the possible protective effects of this hormone from accelerated aging (Al-Attas et al. 2010a).

A study in a cohort of Ashkenazi Jewish centenarians, their offspring (approximate age of 70 yrs), and offspring-matched controls has shown that centenarians and their offspring maintain longer telomeres compared with controls with advancing age. This study has shown that rare synonymous or intronic variants in hTERT are enriched in centenarians (n =19) compared with controls (n =3) as observed through sequence analysis of *hTERT* and *hTERC* genes. A common *hTERT* haplotype that is associated with both exceptional longevity (EL) and longer telomere length has been found; centenarians and their offspring have significantly longer telomeres than unrelated controls, which is a strongly heritable trait. Telomere length in younger persons (<75 yrs of age) is not significantly different between offspring of centenarians and unrelated controls suggesting that families with EL have superior telomere length maintenance. Longer telomeres are associated with lower prevalence of hypertension, the metabolic syndrome, type 2 diabetes, a better cognitive function, and healthier lipid profiles. Haplotypes of the *hTERT* gene, derived from four common variants (two intronic variants, IVS1–187 T > C and IVS16+99°C > T, and two synonymous variants, 973 G > A [Ala-305 Ala] and 3097°C > T [His-1013 His]) have shown associations with longevity and/or telomere length. These common variants may contribute to the regulation of *hTERT* gene expression (Atzmon et al. 2010).

A population of 394 French centenarians was studied to determine serum activity and kinetic constants of ACE, parallel to an insertion/deletion (I/D) polymorphism in its gene. It was observed that the ACE D allele and ACE D/D genotype were more frequent in centenarians in comparison with controls. It was observed that the plasma ACE activity is subject to a similar genotypic influence in centenarians as in adults 20–70 yrs of age. However, ACE itself appears to be functionally similar for each genotype; the D allele as well as the higher serum ACE activities, associated with the D/D genotype, cannot discriminate individuals at high risk for cardiovascular diseases, major causes of mortality before the age of 100 yrs (Faure-Delanef et al. 1998).

Sixteen known *FOXO3A* SNPs (one of the homologues of daf-16 in *Caenorhabditis elegans*; the human forkhead box O3A [*FOX03A*] gene is known to encode an evolutionarily conserved key regulator of the insulin–IGF1 signaling pathway that is known to influence metabolism and life span in model organisms) have been studied in an extensive collection of 1762 German centenarians/nonagenarians and younger controls showing that polymorphisms in this gene were associated with the ability to attain exceptional old age. The *FOXO3A* association was considerably stronger in centenarians than in nonagenarians, highlighting the importance of centenarians as a model of genetic longevity. This study indicates that both genders were likely to be equally affected by variation in *FOXO3A* (Flachsbart et al. 2009).

A genetic study comparing the I405V polymorphism (a common polymorphism in exon 14 of the cholesteryl ester transfer protein [*CETP*] gene) distribution in 364 Caucasian subjects, including 175 Italian centenarians, did not

show any association between the I405V *CETP* variation and a healthy aging phenotype (Cellini et al. 2005).

The frequency of the simultaneous presence of polymorphisms in phase I and phase II genes and of several p53 germline mutations has been studied in a group of 66 nonagenarians and centenarians in good health, selected from a sample of a multicentre Italian study in northern Italy, and in a sample of 150 young healthy volunteers of the same ethnic group. After the estimation of the genotype frequencies of several genes involved in xenobiotic metabolism (*GSTM1*, *GSTT1* and *CYP1A1*2*) and of various p53 polymorphisms (codon 72, Msp1 and a 16-bp duplication) for more than 95% of the study population, no statistically significant differences in the genotype frequencies were found between the two groups analyzed. *GSTT1* deletion was higher in centenarians compared with young subjects, as previously found, but the difference did not reach statistical significance. In the same study a possible interaction between *GSTT1* deletion and p53 gene polymorphisms have been evaluated calculating the combined genotype frequencies in the two groups. A statistically significant difference in the frequency of the *GSTT1* deletion and certain p53 genotypes was observed. The absence of any p53 polymorphisms and of *GSTT1* deletion, and the simultaneous presence of the three p53 polymorphisms and of the *GSTT1* deletion, were much more frequent in young subjects than in centenarians (41.5% versus 26.9% and 8.8% versus 3.8%, respectively). Combinations of p53 genotypes and *GSTM1* or *CYP1A1*2* were not differently distributed in the two groups; the absence of any p53 polymorphisms and of both *GSTT1* and *GSTM1* deletion was 22.5% in controls and 13.5% in centenarians, while a simultaneous presence of the three p53 polymorphisms and of double deletion of both *GSTT1* and *GSTM1* was 4.8% in controls and was not found in centenarians. The authors propose two hypotheses: (1) subjects with both *GSTT1* deletion and p53 polymorphisms may accumulate carcinogens and may have reduced DNA repair ability, for that reason they are more at risk for cancer and (2) both metabolic genes and p53 act on pathways related to cell aging and death. Therefore, certain composite genetic patterns could represent a generic mechanism of protection against aging, not just against the development of chronic diseases. The authors emphasize that longevity is related to a complex genetic trait as well as to certain environmental exposures (Gaspari et al. 2003).

Gondo et al. compared the polymorphic variation of the *5HTT* gene between 265 Japanese centenarians and control subjects. They evaluated the relationships between the 5HTT genotype and the physical, cognitive, and biologic status of centenarians, as indicated by the Barthel Index, the Mini-Mental State Examination, and serum albumin concentration, respectively. The frequency of the l/l genotype and the l allele was significantly greater in centenarians than in younger control subjects, particularly women. A significant effect of the 5HTT genotype on serum albumin concentration was observed in both sexes. The authors underline that l allele may carry a longevity advantage through behavioral mechanisms (Gondo et al. 2005).

A study has been performed on a total of 1,089 (669 women and 420 men) unrelated individuals from 19 to 109 yrs old, born and residing in northern and central Italy. They were subdivided into three age classes defined on the basis of the survival curve constructed using Italian demographic mortality data, and genotyped for the functional variant of Klotho termed "KL-VS" allele. A significant increase of the heterozygous Klotho genotype in the class of elderly people compared with young controls was observed. No difference was present between centenarians and young controls. These findings are in agreement with the hypothesis that the KL-VS heterozygous genotype is favorable for survival in old people and its beneficial effect decreases thereafter and becomes no more evident at the extreme ages. Such unusual age-related changes in the Klotho KL-VS genotype frequency is compatible with the hypothesis that alleles and genotypes involved in aging and longevity may exert their biological effect at specific time windows (Invidia et al. 2010).

A study tried to elucidate whether the age-related changes in the level of hypoploidy correlate with the occurrence of micronuclei (MN) and chromosome nondisjunction (ND) in men and women. Cytokinesis-blocked (binucleated) lymphocytes treated with cytochalasin B, from 127 donors varying in gender and age, including 53 centenarians, were analyzed; fluorescent in situ hybridization, with probes specific for several autosomes and for the sex chromosomes, was applied to analyze the chromosomal content of MN and to analyze the frequency of reciprocal loss and gain attributable to ND in binucleated interphase cells. The general level of MN in Giemsa-stained preparations was higher in women and, in both genders, it increased with age until ~70 yrs and ranged, depending on the age group, from 0.5 to 1.4% in men and from 0.9 to 1.8% in women. Gender-related differences were mostly observed in the youngest age groups ($\leq$50 yrs), with an almost twofold difference between men and women; frequencies of autosome positive MN in both genders and of sex chromosome-positive MN in men were comparable and remained unchanged in older groups. The frequency of X-positive MN in women was higher than the average frequency of autosome positive MN and continued to increase until the oldest age. The frequency of NDs, involving the analyzed chromosomes, was on average twofold higher in women than in men. In both genders, the frequency of NDs increased with age and was, on average, an order of magnitude higher than that one of cells with MN, consistent with the previous reports asserting that the efficiency of elimination of micronucleated cells is higher than that one of cells presenting chromosome ND. The authors conclude that the individuals surviving until the oldest age most likely represent a fraction accumulating these lesions at a slower speed. The higher frequency of NDs compared with MN involving analyzed chromosomes, in donors of all ages and both genders, suggests that the efficiency of elimination of micronucleated cells is higher than that one of cells presenting chromosome ND. ND could be the most essential contributor to the observed age-related increase in the frequency of spontaneous aneuploidies (Wojda et al. 2007).

A study has been realized to assert the effects of two bifidobacteria strains, isolated from healthy centenarians, on the intestinal function in mice. *Bifidobacterium adolescentis* BBMN23 and *Bifidobacterium longum* BBMN68 were orally administrated to specific pathogen-free BALB/c mice at different doses (2×1011, 2×109, or 2×107 CFU/kg body weight) each day for 4 weeks *B. adolescentis* and *B. longum* strains, isolated from healthy centenarians, can produce changes in the structure of the intestine mucosa in healthy BALB/c mice. The heights and widths of duodenum villi, villus/crypt ratios, and sIgA content had all increased significantly, with inferred benefits for efficiency and mucosal immunity. These specific bifidobacterial strains can enhance intestinal function in mice and may also be of benefit in human subjects (Yang et al. 2009).

The autopsied brains and spinal cords from 23 centenarians were collected to investigate the characteristics of alphasynucleinopathy. Coronal slices were prepared from a section of the cerebral hemisphere, and spinal cord specimens were prepared at each segment from the third cervical to the third sacral segment. In any case standard stainings of hematoxylin–eosin, Kluver–Barrera, and Gallyas–Braak, combined with Luxol fast blue/cresyl violet, and α-synuclein (AS), phosphorylated τ (AT8), and β-amyloid protein immunostainings, were performed. Overall, AS-positive structures were found in 8 (34.8%) of our 23 centenarians, six (35.3%) of 17 demented patients, and four (40%) of 10 AD patients. The frequencies of AS lesions in the brains with senile plaque (SP) stage 0–A, B, and C were 27.7, 33, and 50%, respectively. No statistical differences were found among the frequencies of AS lesions in the subgroups of NFT stages I–II, III–IV, and V–VI. Most cases showed a widespread distribution of AS-positive structures, except for one patient in whose brain only the medulla was involved. The distribution pattern of AS-positive lesions was similar to that in Parkinson's disease or DLB, but the pigmented neurons in substantia nigra were relatively well preserved. These findings indicate that there is a high frequency of α-synucleinopathy in centenarians, and SP-positive and AS-positive lesions may involve a synergistic interaction (Ding et al. 2006).

The amino acid variations in the cytochrome b molecule of 64 Japanese centenarians have been studied to test the hypothesis that centenarians are free from deleterious mitochondrial variations. The authors have observed that the frequencies of some variations, such as N260D and G251S, differed significantly between centenarians and patients with Parkinson's disease, but the most striking feature of centenarian cytochrome b was the rareness of amino acid variations in contrast to the variety of amino acid replacements in patients with Parkinson's disease (Tanaka et al. 2002).

The heat shock response in B-lymphoid cell lines from 44 centenarians and 23 younger subjects has been studied analyzing HSP70 synthesis and cell survival after hyperthermic treatment. It has been shown that in centenarian cells the synthesis of an important stress protein (i.e., HSP70i) does not follow the decline usually occurring with aging. Moreover the (A/C)2110 polymorphism in the *HSP70-1* gene seems to affect the synthesis of HSP70-1, giving a functional

explanation to the finding that this polymorphism may influence longevity. In addition, centenarian cells may be more resistant to apoptosis than those ones from younger subjects, according to the cell subtype and apoptotic stimulus. This study supports the idea that the stress response plays an important role in successful ageing (Marini et al. 2004).

A study has been designed to analyze the mean length of the terminal restriction fragments (TRF) in fibroblast strains from 4 healthy centenarians, that is in cells aged in vivo, and from 11 individuals of different ages. This study reports that no correlation between mean TRF length and the donor's age was found. Telomere shortening was detected during in vitro propagation of centenarian fibroblasts, suggesting that in fibroblasts, aged in vivo, telomeres can be far from reaching a critical length. In blood cells from various individuals, the expected inverse correlation between mean TRF length and donor's age was found. In particular, a substantial difference (about 2 kb) between telomere length in the two cell types was observed in the same centenarians. The expression analysis of three senescence induced genes (i.e., fibronectin, apolipoprotein J, and p21) revealed, for only the fibronectin expression levels, a clear positive correlation with the donor's age. These results suggest that (1) telomere shortening could play a different role in the aging of different cell types and (2) the characteristics of fibroblasts, aged in vitro, might not be representative of what occurs in vivo. The authors conclude that caution should be taken in the use of dermal fibroblasts as a model for in vivo aging, because their in vitro aging might not be representative of the aging of the organism as far as both telomere shortening and gene expression are concerned (Mondello et al. 1999).

The capacity of the thymus to produce new cells at the limit of human life span has been investigated analyzing some basic mechanisms responsible for the renewal and maintenance of peripheral T lymphocytes in 44 centenarians. Thymic functionality was analyzed by the quantification of cells presenting the T-cell receptor rearrangement excision circles (TREC); a new method based upon real-time PCR was used, and it has been found that most centenarians (84%) had undetectable levels of TREC+ cells; six-color cytofluorimetric analysis revealed that centenarians had an extremely low number of naïve T cells; central memory and effector memory T cells had greatly increased, while terminally differentiated cells were as numerous as in young (aged 20–45) or middle-aged (aged 58–62) donors. Interleukin (IL)-7 and IL-7 receptor α-chain (CD127) levels were the same at all ages, as shown by ELISA, flow cytometry, and real-time PCR. However, IL-7 plasma levels were higher in centenarian females than males. The presence of TREC+ cells and of very few naïve T lymphocytes suggests that in centenarians such cells could either derive from residues of thymic lymphopoietic islets or even represent long-living lymphocytes that have not encountered their antigen yet. IL-7 could be one of the components responsible, among others, for the higher probability of reaching extreme ages typical of females (Nasi et al. 2006).

Ninety-two nonsynonymous SNPs in 49 DNA repair genes, for a possible association with longevity in a sample of 395 German centenarians and 411 controls, have been tested. The obtained association signal in exonuclease 1 (*EXO1*) was further investigated by fine mapping and mutation detection, leading to the identification of the functionally relevant SNP rs1776180. It was found that the C allele of this promoter SNP is significantly enriched in female centenarians, and this finding was replicated in 455 female French centenarians and 109 controls. The C allele led to the loss of a binding site for the basic helix-loop-helix transcription factor E47, resulting in higher *EXO1* expression. An undescribed role for E47 as a negative regulator of *EXO1* transcription and a genetic variant in the *EXO1* promoter, which counteracts the E47-mediated repression of the gene, have been detected. The authors underline that, given the survival advantage that is associated with the C allele of rs1776180, *EXO1* can be considered as a candidate for a novel longevity-enabling gene (Nebel et al. 2009).

There is an association between Pro12 Ala polymorphism in peroxisome proliferator-activated receptor-γ (PPARg2) and longevity. A study on Chinese nonagenarians and centenarians has demonstrated an association between the Pro12Ala variant of *PPARg2* gene and depression. This study has also provided evidence that 12Ala carriers have a significantly decreased risk for depression compared to non-12Ala carriers (Ji-Rong et al. 2009).

Amino acid variations in the cytochrome b molecule of 64 Japanese centenarians have been analyzed to test the hypothesis that centenarians are free from deleterious mitochondrial variations. This study involved 96 patients with Parkinson's disease and 96 young obese adults. The frequencies of some variations, such as N260D and G251S, differed significantly between centenarians and patients with Parkinson's disease, but the most important feature of centenarian cytochrome b was the rareness of amino acid variations in contrast to the variety of amino acid replacements in patients with Parkinson's disease. The authors emphasize that these findings have at least three implications: (1) the absence of certain variations in centenarians and their presence in patients with Parkinson's disease indicate that these variations are not beneficial for long-term survival, but do predispose individuals to adult-onset diseases and that centenarians are genetically hitting the golden mean; (2) the multiple variations present in young adults are unlikely to greatly influence either individual survival before maturation or the transmission of the genome to subsequent generations; and (3) a multiplex detection system for mildly deleterious mitochondrial variations, in combination with genetic tests for longevity-associated genotypes, will be useful both for predicting longevity and for assessing the risk of age-related diseases (Tanaka et al. 2002).

Drug metabolizing enzymes are known to be involved in the detoxification of several drugs, environmental substances, carcinogenic compounds. Moreover, their polymorphisms have been associated with the risk for a variety of cancers. Therefore, the frequency of polymorphisms in cytochrome P450-1A1 gene (*CYP1A1*), a phase 1 gene (oxidation, activation), and of two polymorphisms of

glutathione S-transferase enzymes (GSTM1, GSTT1), two phase 2 genes (conjugation, detoxification) have been studied. This study involved two groups, 94 nonagenarians and centenarians and 418 control subjects of younger age. The following results were observed: (1) a significant difference in the proportion of nonagenarians and centenarians homozygotes for a *GSTT1* deletion (28%) in comparison to control subjects (19%) and (2) the distribution of the other gene polymorphisms did not differ in the two groups. The authors underline the lack of association between *GSTM1* and longevity reporting new data for two polymorphisms in drug metabolizing enzymes that have never been tested in centenarians, namely *CYP1A1* and *GSTT1*. The authors propose a mathematical model which assumes that a genetic risk factor can confer an increased risk to die until a certain age, above which its impact is nullified or reversed. This phenomenon may occur because those people, who survived at very advanced ages, have been selected by mortality forces, and thus could benefit from those genetic variants that are detrimental for younger people Accordingly, the lack of *GSTT1* (or other phase 2 genes) could be related to deleterious effects in young age (high risk of cancer), but it could exert long-term beneficial effects on survival, by avoiding the catabolism of important substances, which have chemopreventive effect on cancer and other diseases. *GSTT1* is a candidate longevity gene, and it can open a new perspective on a possible "pharmacogenetics of longevity." These findings on phase 2 drug-metabolizing enzyme gene polymorphisms may help in disentangling gene environmental interactions that can have a role in successful aging and longevity, as well as in cancer incidence in the oldest old (Taioli et al. 2001).

The biochemical, phenotypic, and genetic variations in a cohort of Ashkenazi Jewish centenarians, their offspring, and offspring-matched controls have been studied showing a gender-specific increase in serum IGFI associated with a smaller stature in female offspring of centenarians. Sequence analysis of the IGF1 and IGF1 receptor (*IGF1R*) genes of female centenarians showed overrepresentation of heterozygous mutations in the *IGF1R* gene among centenarians relative to controls that are associated with high serum IGFI levels and reduced activity of the *IGFIR* as measured in transformed lymphocytes. The authors underline that genetic alterations in the human *IGF1R* that result in altered IGF signaling pathway confer an increase in susceptibility to human longevity thus suggesting a role of this pathway in modulation of human life span. This study shows that the *IGF1R* can be a genetic determinant of human longevity and "IGF-related longevity genes" do exist in human populations. This study suggests that centenarians may harbor individually rare, but collectively more common, genetic variations in genes encoding components of the GH/IGF signaling pathway (Suh et al. 2008).

mtDNA and Aging. Mitochondria are organelles responsible for energy production and essential metabolic pathways in the cell, and these organelles have been implicated in processes such as apoptosis, aging, and cancer (Yogev

et al. 2010) Mitochondria provide ATP, but are also central to the metabolism of each of the major energy fuels, biosynthesis of nonessential amino acids, biosynthesis of DNA precursors, signaling of cell death and survival, biosynthesis of hormones and cell signaling molecules. Mitochondria have been linked to acute and chronic disease processes (PD and other neurodegenerative diseases, vascular disease, metabolic syndrome and diabetes, cardiomyopathies, and renal failure; Roede et al. 2010). They possess their small, circular 16 569-bp genome (mitochondrial DNA [mtDNA]), which is present in different copies for each mitochondrion and encodes for 13 polypeptides component of respiratory chain complexes. This mtDNA has a mutation rate which is pretty high with respect to the nuclear DNA because of its vicinity to the main site of reactive oxygen species (ROS) production, the lack of protective histones, and the presence of a less-efficient repair system (Salvioli et al. 2008). It has been proposed that the mitochondrial dysfunction, which occurs with aging and chronic disease, may be explained by a low-level production of free-radical oxidants which cause mtDNA damage and lead to more mitochondrial dysfunction that result in higher levels of free radical oxidants. According to this consideration oxidative damage to mtDNA is the evident key which can result in a protein mutation, a generation of additional free radicals, and an altered energy production (Roede et al. 2010).

Salvioli et al. (2008) extensively reviewed the impact of mtDNA on the aging process reporting that: (1) an accumulation of mtDNA somatic mutations occurs with age, and many studies have reported an association between mtDNA mutations and aging, particularly in post-mitotic neuronal cells; (2) a number of non-pathogenic mutations are fixed in the mtDNA to form a series of population-specific lineages that can be identified by the presence of conserved groups of haplotypes (haplogroups). These haplogroups can be further subdivided into subhaplogroups, and such classification is continuously updated and used for tracing back the origin of populations or for forensic purposes. These germline inherited mtDNA variants (haplogroups and subhaplogroups), considered to be biochemically neutral, are probably able to differently modulate the mitochondrial metabolism; (3) mtDNA germ-line variants might be associated with human longevity or, on the contrary, with age-associated pathologies and unsuccessful ageing.

In Caucasians and in northern Italians, haplogroup J was over-represented in long-living people and centenarians, thus suggesting a role for this mtDNA variant in longevity (haplogroup J was only significantly overrepresented in male centenarians; De Benedictis et al. 1999).

The distribution of the inherited mtDNA variants, according to tyrosine hydroxylase (THO) genotypes, has been analyzed in three sample groups of increasing ages (20–49 yrs; 50–80 yrs; centenarians). It has been observed that the mtDNA haplogroups and the THO genotypes are associated randomly in the first group, while in the second group, and particularly in the centenarians, a non random association is observed between the mtDNA and

nuclear DNA variability (De Benedictis et al. 2000). Moreover, in centenarians the U haplogroup is over-represented in subjects carrying the THO genotype unfavorable to longevity.

Mutated and WT mtDNA molecules can coexist within the same cell, tissue, or organ, a condition called heteroplasmy. Cells containing only one type of mtDNA (mutated or WT) are indicated as homoplasmic (Wallace et al. 1999). Using a large-scale screening of the mtDNA CR in leukocytes obtained from Italian centenarians, three intriguing findings have been observed (Zhang et al. 2003): (1) a homoplasmic C150T transition near an origin of heavy mtDNA-strand synthesis occur in approximately 17% of 52 subjects and in only 3.4% of 117 younger individuals; (2) somatic events, under probable nuclear genetic control seem to contribute to the striking selective accumulation of the mutation in centenarians; and (3) an aging-related somatic expansion of the C150T mutation, up to homoplasmy, seems to occur according to human fibroblast longitudinal studies. In addition, functional studies have revealed a new replication origin at position 149, substituting for that at 151, only in C150T mutation-carrying samples of fibroblasts or immortalized lymphocytes (Zhang et al. 2003). This last finding suggests that the striking accumulation of the C150T mutation in centenarians is not a debris attributable to the extreme old age of these subjects, but a genetically controlled phenomenon favoring longevity. The new replication origin may restore the efficiency of the mitochondrial machinery as the individual ages and stochastic damage accumulates in the mitochondrial genome. These results open a new perspective on the possible role played by mtDNA heteroplasmy in human aging. However, if the occurrence/accumulation of C150T heteroplasmy is a phenotypic consequence of extreme aging or a genetically controlled event favoring longevity remains an open question. In fact, by analyzing C150T heteroplasmy in centenarians and their descendants, it has been demonstrated that the levels of heteroplasmy are similar between relatives, despite the different age, and correlated in parent-offspring pairs (Rose et al. 2007). Studies that correlate mtDNA CR heteroplasmy with biological markers of the quality of aging will help to clarify the significance of the above data in relationship to longevity. In addition, in the same study we have also showed that the heteroplasmy levels are independent of inherited mtDNA variability (haplogroup and sequence analysis) and are probably under control of the nuclear genome. The latter observation is in line with a recent study showing that the age-related accumulation of somatic mtDNA mutations in mouse hematopoietic stem cells is influenced by the nuclear genetic background and that these mutations may not obviously correlate with either cellular ROS content or cell senescence (Yao et al. 2007). In any case, the observation that the nuclear genome contributes to mtDNA heteroplasmy marks the importance of the mitochondrial-nucleus cross-talk in modulating mitochondrial function and cellular homeostasis and, consequently, the quality of aging and life span (Ryan et al. 2007).

The analysis of the C150T transition in leukocytes from three groups of an Ashkenazi Jew population (centenarians and their offspring) has revealed a situation different from that found in Italian centenarians (Rose et al. 2007). In particular, the incidence of the C150T mutation is very low and, by contrast, the homoplasmic T152°C transition and a homoplasmic T195°C transition has shown a fairly high frequency in all three groups of subjects (Iwata et al. 2007). Furthermore, most significantly, an aging-related increase in incidence of the heteroplasmic T152°C transition, not associated with a change in the replication origin and presumably resulting from somatic events, has been demonstrated in the Ashkenazi Jews (Iwata et al. 2007).

Possible longevity-associated polymorphisms/haplogroups in an African population, from Tunisia, have been studied by performing complete mtDNA sequencing because this population has a mixed Eurasian/sub-Saharan mtDNA gene pool, which could potentially facilitate the evaluation of association for sub-Saharan lineages. It has been observed that sub-Saharan haplogroups are significantly less represented in centenarians (9.5%) than in controls (54.5%), but it is not possible to rule out an influence of population structure, which is high in these populations; no recurrent polymorphism has been more frequent in centenarians than in controls, and although the Tunisian centenarians have presented less synonymous and replacement polymorphisms than controls, this difference is not statistically significant. The authors conclude that it does not seem that centenarians have significantly less mildly deleterious substitutions, not only in Tunisia but also in Japanese and UK/US samples not favoring a "golden mean" to longevity (Costa et al. 2009).

The association of a mtDNA mutation with a prolonged life span in humans has been investigated. It has shown that a large-scale screening of the mtDNA main control region in leukocytes, from subjects of an Italian population, revealed a homoplasmic C150T transition near an origin of heavy mtDNA-strand synthesis in ~17% of 52 subjects 99–106 yrs old but, in contrast, in only 3.4% of 117 younger individuals. Another study, among leukocyte mtDNA samples from 20 monozygotic and 18 dizygotic twins, 60–75 yrs old, 30% and 22%, respectively, of the individuals involved exhibited the homoplasmic C150T mutation. In five human fibroblast longitudinal studies, convincing evidence for the aging related somatic expansion of the C150T mutation, up to homoplasmy, was obtained. Most significantly, 5′ end analysis of nascent heavy mtDNA strands consistently revealed a new replication origin at position 149, substituting for that at 151, only in C150T mutation-carrying samples of fibroblasts or immortalized lymphocytes. The authors, considering the aging-related health risks that the centenarians have survived and the developmental risks of twin gestations, propose that selection for a remodeled replication origin, inherited or somatically acquired, provides a survival advantage and underlies the observed high incidence of the C150T mutation in centenarians and twins. These results on the C150T mutation in both leukocytes and fibroblasts indicate that this mutation can be inherited or can arise "de novo" during life (Zhang et al. 2003).

Heteroplasmy in leukocytes of centenarians (100 subjects), their offspring and nieces/nephews, and in leukocytes of 114 control subjects sex- and age-matched with the relatives of centenarians have been studied. It has been observed that the centenarians and their descendants, despite the different ages, show similar levels of heteroplasmy which are significantly higher than levels in controls. In addition, heteroplasmy levels have been significantly correlated in parent-offspring pairs, but they are independent of mtDNA inherited variability (Rose et al. 2003).

The relations between three classes of people (96 Japanese centenarians, 96 Japanese AD patients, and 96 Japanese PD patients) and their mitochondrial mtSNP frequencies at individual mtDNA positions of the entire mt-genome have been analyzed by using the radial basis function (RBF) networks. New findings of mtSNPs representing the characteristics of individual classes have been found. These mtSNPs show distinct differences for three classes of individual classes of people that are characterized by unique mtSNPs. Interestingly, Japanese centenarians are closely associated with haplogroup D4, Japanese AD patients with haplogroup G2a, and Japanese PD patients with haplogroup M7a. These characteristics of mtSNPs are different from those of the previously reported works. As the amino acid replacement mtSNPs have been found at four mtDNA positions, it is indicated that mtSNPs of synonymous nucleotide substitutions, as well as those of nonsynonymous nucleotide substitutions, may play important roles in mitochondrial functions (Takasaki et al. 2008).

It has been investigated whether there was evidence in southern Italy of an association between the *IL6* gene variable number of tandem repeats (VNTR) polymorphism and extreme longevity, and it has been tested for the possible interaction of *APOE* alleles with the *IL6* VNTR alleles. Four alleles, coding for variants of 4 different lengths, have been identified: allele A (760 base pairs [bp]), allele B (680 bp), allele C (640 bp), and allele D (610 bp). *IL6* VNTR and *APOE* allele and genotype frequencies were studied in a total sample of 155 subjects from Apulia (southern Italy). This study included 61 centenarians and 94 middle-aged, unrelated subjects or volunteers. The distribution of the *IL6* VNTR and *APOE* genotypes in this population were in Hardy-Weinberg equilibrium. When the *IL6* VNTR allele distribution was analyzed, a statistically significant overrepresentation of the B allele in middle-aged subjects, compared with centenarians, was found. These results suggest that the presence of the *IL6* VNTR allele B could be detrimental for reaching extreme longevity (Capurso et al. 2007).

A study has been performed to investigate whether there was evidence of an association among IL-6 −174 G/C promoter polymorphism and extreme longevity in a population of 81 centenarians compared with a control group of 122 middle-aged healthy subjects (mean age: 51 ± 18 SD; range: 19–73 yrs), from Apulia (southern Italy). A possible interaction of *APOE* alleles with the IL-6 −174 G/C promoter polymorphism has also been tested. The frequencies of the different IL-6 −174 G/C promoter genotypes in this population were in

Hardy-Weinberg equilibrium. No statistically significant differences have been found in the IL-6 −174 G/C promoter genotype between centenarians and middle-aged subjects in our population; similarly, the frequency of the IL-6*C allele (or vice versa IL-6*G allele) has been comparable between centenarians and middle-aged control subjects. No three-way statistically significant interaction has been found among IL-6 -174 G/C promoter genotype, *APOE* alleles, and longevity. The authors conclude that regional genetic differences in conjunction with differing environmental factors may explain in part previous results suggesting a role of this polymorphism in longevity (Capurso et al. 2010).

Conclusions

This review highlights the complexity of the genetic background, either in nuclear or in mtDNA, affecting different pathways of centenarian physiology and involving metabolism, immune processing response, cardiovascular function, brain integrity, and so on. More recently, environmental interactions basically enclosing gut microbiota and their immunogenic and vitaminic potential have also been emphasized in the centenarian balance. Even the colonic bacteria harboring and balance might be genetically determined by the food quality input and digestive activity of enzymes, biliary salts, mucus, and so on. Changes in the gut microbiota have been associated to several pathological conditions (e.g., atopic eczema inflammatory bowel syndrome and allergic disorders). Moreover, they influence energy balance and, in turn, gut microbiota play a key role in the pathophysiology of obesity. The initial acquisition of intestinal microbiota is also fundamental in the development of immune processes and protection against pathogens (Iannitti et al. 2010). The largest study reported to date (161 subjects aged 65 yrs and older and 9 younger control subjects), aiming at characterizing the fecal microbiota, shows that the core microbiota of elderly subjects is distinct from that one established for younger adults with a greater proportion of *Bacteroides* spp., distinct abundance patterns of Clostridium groups and a lower proportion of phylum Firmicutes. In addition, the fecal microbiota of the elderly shows temporal stability over limited time in the majority of subjects, but it is characterized by unusual phylum proportions and extreme variability (Claesson et al. 2010). Recently, a genome-wide association study of EL in 1,055 centenarians and 1,267 controls has been performed to build a genetic classification model that is based on 150 SNPs. This model, applied to an independent set of centenarians and control individuals of average longevity (AL), correctly classifies individuals into the EL or AL group 77% of the time. According to this study, 90% of centenarians can be grouped into 19 clusters, characterized by different combinations of SNP genotypes or genetic signatures of varying predictive value. The different signatures, which are associated with the genetic of EL, correlated with differences in the prevalence and age of onset of age-associated diseases, may help divide this complex phenotype into subphenotypes of healthy aging (Sebastiani et al. 2010). These very puzzling genetic characteristics also involve the centenarians' offspring and relatives in a complex

background from which the gene selection promotes single or clusters of privileged individuals which escape from most epidemiologic diseases that are supposed to be lethal in the long run. In this centenarian aging process, we also have to take into account the emerging role of preventive clinical pharmacology and geriatric surgery which is, at the moment, not clearly outlined, being preferably addressed to acute disease control and treatment, rather than to a safe and uneventful up to a hundred year survival. In this perspective, a few years ago we conceived the term " Jurassic surgery" as a specific description of a group of surgical procedures that could really help the centenarians or pericentenarians in overcoming some of the old age–onset life threatening diseases, thus allowing the individual genetic background to fulfill their long living role. If we take into account and improve our medical and surgical performances on the very old people, we'll probably take the challenge to expand the cohort of centenarians, offering them the best quality of life as well.

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