Genetics of human longevity within an eco-evolutionary nature-nurture framework

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ABSTRACT

Human longevity is a complex trait, and to disentangle its basis has a great theoretical and practical consequence for biomedicine. The genetics of human longevity is still poorly understood, despite a number of investigations that used different strategies and protocols. Here we argue that such rather disappointing harvest is largely due to the extraordinary complexity of the longevity phenotype in humans. The capability to reach the extreme decades of human lifespan appears to be the result of an intriguing mixture of gene-environment interactions. Accordingly, the genetics of human longevity is here described as a highly context-dependent phenomenon, within a new integrated, ecological and evolutionary perspective, and is presented as a dynamic process, both historically and individually. The available literature has been scrutinized within this perspective, paying particular attention to factors (sex, individual biography, family, population ancestry, social structure, economic status and education, among others) that have been relatively neglected. The strength and limitations of the most powerful and used tools, such as GWAS and whole-genome sequencing, have been discussed, focusing on prominently emerged genes and regions, such as APOE, FOXO3, IL-6, IGF-1, chromosome 9p21, 5q33.3 and somatic mutations among others. The major results of this approach suggest that: i) the genetics of longevity is highly population-specific; ii) small-effect alleles, pleiotropy and the "complex allele timing" likely play a major role; iii) genetic risk factors are age-specific and need to be integrated in the light of the geroscience perspective; iv) a close relationship between genetics of longevity and genetics of age-related diseases (especially cardiovascular diseases) do exist. Finally, the urgent need of a global approach to the largely unexplored interactions between the three genetics of human body, i.e. nuclear, mitochondrial and microbiomes, is stressed. We surmise that the comprehensive approach here presented will help in increasing the above-mentioned harvest.

Key words

Genetics, Human Longevity, Aging, Age-Related Diseases

1. Introduction

It is recognized that long-lived mammalian species (*e.g.* naked mole rat, blind mole rat and elephant, among others) evolved unique traits, highly related to their specific environmental niche, to attain longevity [1]. Many studies have been made on animals models but in humans the capability to reach the extreme decades of lifespan appears to be the result of an intriguing mixture of gene-environment interactions and in this review despite the interesting findings in animals models we will focus only on the genetics of longevity in humans. *Homo sapiens* is a long-lived mammal and likely evolved peculiar longevity mechanisms. Modern humans are characterized by a higher level of complexity due to their biological and cultural capability of adapting to all areas of the planet and changing their environments, by developing an extraordinary variety of cultural adaptive strategies. This specific characteristic had and still has a strong impact on the molecular and cellular mechanisms involved in aging and longevity. Accordingly, the genetics of human longevity is here described as a highly context-dependent phenomenon, within a new integrated, ecological and evolutionary perspective, and it is presented as a dynamic process, both historically and individually. During the entire lifespan new gene-environment interactions emerge as a consequence of the continuous "remodeling" process that the body set up to adapt to the deteriorative changes occurring over time [2], which on the other hand occur concomitantly with the changes of the environment. In this complex scenario, many factors (population genetics, demography, sex, family, immunobiography, physical/geographical and cultural/anthropological environment, social networks, socio-economic status and education) need to be carefully considered and integrated in order to understand the contribution of genetics in attaining healthy aging and extreme longevity. The high throughput technologies used for the analysis of genetic data - as well as the data on the most relevant genes - highlighted the close relationship between the genetics of longevity and population-specific dynamics, as well as the importance of small effect alleles, pleiotropy and the complex "allele timing", supporting the assumption that genetic factors involved in human longevity need to be defined according to a large range of biological and non-biological variables. Moreover, the genetic of longevity is linked with many age-related diseases (especially cardiovascular diseases - CVD), coherently with the new geroscience perspective [3]. Geroscience suggests that the mechanisms underpinning aging are deeply involved in the pathogenesis of age-related non communicable chronic diseases, and has identified seven common pillars (adaptation to stress, epigenetics, inflammation, macromolecular damage, metabolism, proteostasis and stem cells and regeneration). Geroscience emphasizes that aging promotes diseases, being the most important risk factor, and that in turn diseases and/or their treatments accelerate the aging process [4], and at the same time represents the conceptual framework to understand the role of genetic (protective) factors which allow few individuals to reach the extreme limit of human life. This review highlights also the plastic and deep interactions between

autosomal DNA, mtDNA and microbiomes which, together with somatic mutations occurring lifelong, continuously create new and unique interactions among these genomes in each individual.

2. The complexity of human longevity

2.1. The definition of longevity

The definition of “longevity” is highly debated and the lack of a universally recognized definition increases the possibility of misinterpretation and biases in comparing different studies aimed at establishing the genetic contribution on this trait. Moreover, in the literature the terms "lifespan", "oldest old", "aging" and "longevity" are often use interchangeably. Longevity can be defined as the result of mortality selection overall age-classes, where cumulative mortality is historically and context-dependent. The birth cohort and the percentile of survival are thus two parameters crucial for the definition of longevity rather than the chronological age itself. Demographic studies showed that there are differences between members of a birth cohort who live 90, 100, 105 and 110+ years, and thus they may reflect important genetic differences [5]. The study of Sebastiani and colleagues[5] showed that the siblings’ relative probability to survive to older ages increase with age, suggesting that the genetic influence upon survival continuously increases reaching the highest levels around the extreme limits of lifespan.

This observation leads to the important consideration that the power to detect genetic association with longevity is greater for centenarians versus nonagenarians subjects of the same birth cohort [6]. Thus, a recent paper - in order to avoid problems of inconsistency in the definition - suggests that longevity has to be defined according to percentile survival based upon the reference birth cohort for each population, indicating that the environmental context play a critical role on this specific phenotype. One percentile survival is the threshold suggested to maximize the probability to identify genetic association [7]. Moreover familial longevity is also a criteria used in the study of the genetics of human longevity: the GEHA (Genetics of Healthy Ageing) Study includes nonagenarian sibling pairs to select families enriched for genetic influences on longevity [8].

2.1.1. Heritability and missing heritability in longevity

The definition of longevity opens new consideration on the estimation of heritability that is often considered around 25 ± 5% on the basis of twin studies that, however, includes a limited (or null) number of 90+ couples . In fact these studies likely reflect the heritability for age at death in modern society rather than the variation of the trait (longevity) explained by genetic determinants.

An example of the problems and bias inherent in the definition of longevity in human is exemplified by the study from Kaplanis et al. [9] where the authors estimated heritability of longevity around 16%. The results suggested a robust additive genetic component, a small impact of dominance, and no detectable epistasis

on longevity heritability. The study is interesting for the digital approach to genealogical data and for the high number of individuals considered (around 3 million from online dataset of genealogy profiles of worldwide population). However the estimation of heritability is built around a very high range of birth cohorts (1600-1910), from different populations, and no long-living individuals are present (individuals > 100 years old were filtered out) [9]. These contrasting and biased results take us to the emerging concept of missing heritability in the study of complex traits, including longevity. To date genomics identified only a small proportion of the expected heritability of longevity. In a recent study it was proposed that the missing heritability is strictly related to the methods used for the heritability calculation itself [10]. In particular the evaluation of heritability is based on an additive model for complex traits that does not consider other inheritance patterns, such as epistasis among others [10]. In literature example of epistatic interactions in longevity exists [11–13]. Tan and colleagues [14] analyzing centenarians identified a significant association between the interaction REN gene allele/mitochondrial haplotype H and longevity while the two gene analysed separately showed no significant association.

Moreover heritability may be biased by unaccounted environmental effects whose relation with the genetics of human longevity will be extensively described in section 3. Variation between phenotypes in a population arises because of the average differences between the genotypes or because each genotype exhibits phenotypic variance because of environmental variation. A detailed analysis of variants affecting behaviour and mating will be relevant in order to understand another potential bias in heritability, i.e. the covariance between genes and culture. The polygenic nature of longevity promoted the idea that larger cohorts are needed to identify the variants, but this approach inevitably causes an increase of the heterogeneity of the cohorts and of environmental confounding factors at the expense of the biology underpinning the trait. Lastly some authors suggested that part of the missing heritability should be searched in non-DNA factors that may be transmitted between generations [15] as important data that connect epigenetic inheritance and longevity from non-human models are emerging [16–19]. Interestingly analysis and integration of DNA methylation data and RNAseq will be useful tool [20], but a big effort is still needed to collect data on the same pool of samples [21].

2.1.2. The phenotype of human extreme longevity

From the previously mentioned studies emerged that the genetic contribution to longevity increases with age, thus centenarians (100 years of age), semi-super centenarians (105 years of age and more) and super centenarians (110 years of age and more) are the more informative subjects for investigating the genetics of human longevity. One of the emerging problems in the genetic studies is the characterization of the phenotype to include in the analysis. "Phenomics" is a term introduced by the evolutionary biologist Michael Soulè, and it includes all the possible way to characterize populations and individuals [22].

Centenarians and semi-supercentenarians have been deeply characterized and the main results are briefly summarized (and reported in Figure 1):

1) Biochemical parameters: centenarians have a phenotype similar to that of calorie-restricted individuals and they have a good metabolic profile, characterized by preserved glucose tolerance and insulin sensitivity, low serum level of IGF-I and IGF-II [23,24].

2) Prevalence of age-related diseases: centenarians markedly compress the period of their lives spent with disability [25] and they avoided or largely postponed all major age-related disease [4,26–30]. Consistent with the compression of morbidity scenario, people surviving beyond age 105 years are also phenotypically much more similar to one another than younger individuals dying in their 1980s and 1990s [5].

3) Epigenetics: according to the epigenetic clock, centenarians showed a decreased in DNA-methylation ages [31] indicating that they are biologically younger than their chronological age.

4) Metabolomics**:** an analysis conducted on plasma and urine of Italian centenarians and their offspring has identified the first metabolomic signature of healthy aging. Specific glycerophospholipids and sphingolipids and a decreased in tryptophan concentration have been described in centenarians from North Italy [32]. This centenarian metabolomic signature showed peculiar changes in pro- and anti-inflammatory compounds and detoxification through specific modulation of the arachidonic acid metabolic cascade and enhanced cytochrome P450 (CYP) enzyme activity.

5) Biology of circadian rhythms: this is an emerging aspect linked to familial longevity, likely influenced by genetic background and by social and environmental dynamics[33]. Circadian rhythms, including central (located in the suprachiasmatic nucleus) and peripheral clocks, deteriorate during aging [34] but they appear to be well preserved in centenarians, including sleep[39], even if the data are still scarce and often related to a limited number of subjects [23]. Studies on offspring of long lived subjects showed preserved rhythmicity of serum non-HDL-C concentration when compared to general population, and centenarians present good sleep quality. Genetic of circadian rhythms is highly population-specific in humans [35,36]. Study in mice showed that SIRT1 is the gene at the intersection between longevity and circadian rhythms and increasing SIRT1 levels in the brain of genetically altered mice seems to reduce the age-related decay of circadian functions[37].

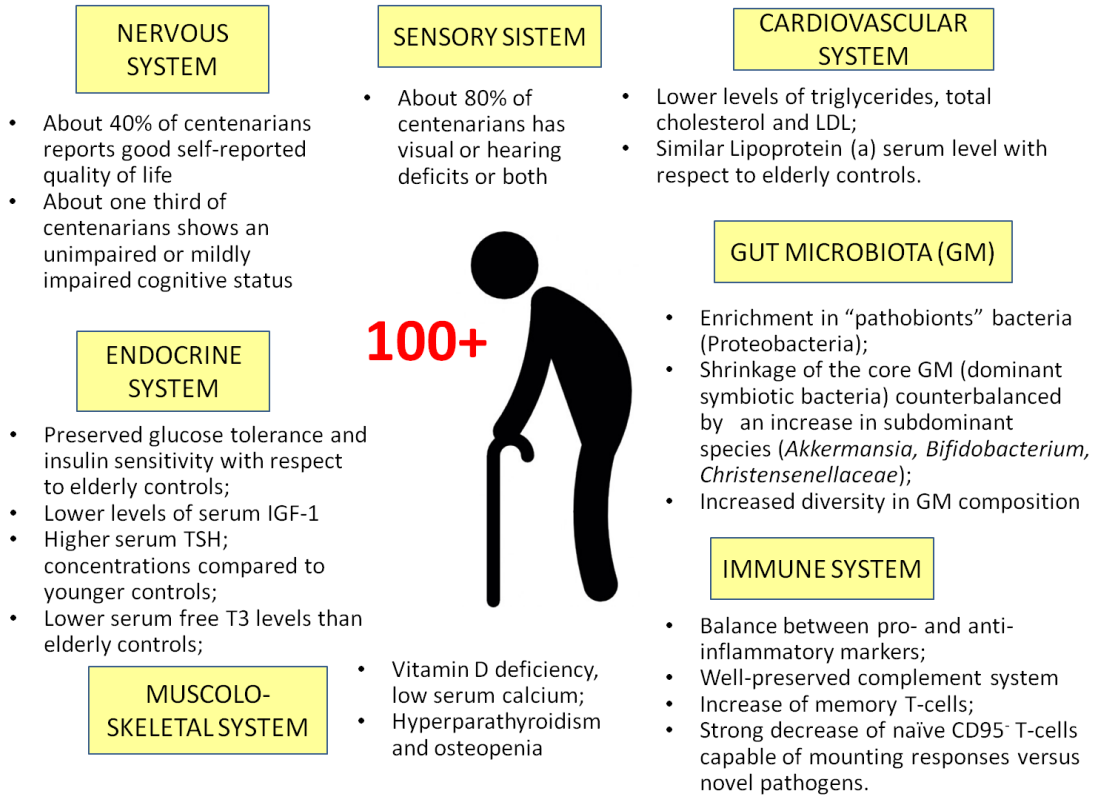

Figure 1. Major characteristics of the centenarians' phenotype. *This figure is inspired by Franceschi et al., Annu. Rev. Nutrition, 2018 and is based on data collected on Italian centenarians.*

2.2. The definition of controls in the genetics of longevity

If the definition of longevity is cumbersome the definition of controls in the study of the genetic of longevity is even more complex, and has constituted a major challenging issue. Noteworthy, the studies can be divided into two main groups: 1) studies including younger healthy controls from the same family or from the general population; 2) studies including patients affected by age-related diseases assuming that such diseases constitute a condition of accelerated aging, as recently argued [4]. In addition, a new definition of controls is emerging thanks to the new era of molecular biomarkers of aging that introduce the concept of "biological age" (described in the section "perspectives").

2.2.1 Controls as healthy individuals younger than cases (e.g. centenarians)

For the study of sporadic longevity the choice of controls constituted a challenging and debated issue. Once the threshold for longevity is defined, many studies randomly selected unrelated subjects from the general population as controls, assuming that the prevalence of possible centenarians in the control group is small and negligible owing to the rarity of the trait [38]. A second strategy is to select as a group of control individuals who did not survive over the average life expectancy of their demographic cohort, thus reducing

in the control group the possible enrichment of longevity variants and increasing the power of a study. Both strategies have pros and cons, as illustrated in a recent paper [7], but in both cases the results may be biased by the different birth cohorts of centenarians and controls.

2.2.2. Controls as individuals affected by age-related diseases

Recently, the idea emerged that patients affected by age-related diseases could be an effective control group for the study of the genetics of longevity[39–41], for two main reasons : 1) long living subjects such as centenarians and patients affected by age-related diseases constitute "extreme phenotypes", and the comparison between most divergent phenotypes can increase the probability to identify disease-specific genetic protective factors linked to longevity; 2) a group of individuals with a specific age-related disease help to disentangle the genetic heterogeneity of the control group that inevitably is composed of individuals most of whom will likely develop one or more age-related diseases that eventually reduce their chances to reach longevity (Figure 2). The concept that aging and chronic pathologies share common mechanisms and constitute a continuum where centenarians and patients affected by age-related diseases represent the opposite side of the spectrum is at the basis of the inclusion of age-related patients in studies on the genetics of human longevity [41].

As far as we know, the first study within this framework was performed in 2013 by Garagnani and colleagues [39] on the most important genetic risk factor for type 2 diabetes (T2D), i.e. the TCF7L2 rs7903146 genetic variant. The authors investigated the TCF7L2 risk allele distribution in a phenotypic *continuum* spanning from diabetic patients with micro- and macro-vascular complications (i.e. the most severe age-related phenotype) to centenarians (that never had T2D) on a total of 1,349 individuals. The frequency of the risk allele genotypes (TT) and the OR value increased gradually according to the decreasing of health/longevity and the increasing of T2D severity.

A second study [40] applied a cross-diseases analysis for the study of longevity. The authors calculated for each SNP an "age-related disease score" (weight) by evaluating a high number of different genome wide analysis on age-related diseases. The score was able to distinguish between disease-specific SNPs and "aging SNPs" that are involved in multiple diseases. Then, these weights were used for the p-values calculated from longevity association analysis in order to integrate in the longevity assessment the age-related disease genetic knowledge. With this method they identified eight genes involved in extreme longevity likely protecting against specific age-related diseases such as APOE/TOMM40 (associated with Alzheimer's disease), CDKN2B/ANRIL (CVDCVD), ABO (tags the O blood group), SH2B3/ATXN2 (neurological disease), HLA, LPA, FADS1 and KCNT2. The latter study we consider compared 53 healthy centenarians and 45 patients with Alzheimer's disease (AD) both of Ashkenazi Jewish ancestry [42]. The authors found that a burden for rare protein truncating mutations is absent in centenarians while they characterized AD

patients. This extreme phenotype approach is twice powerful since it provides information both on risk and protective factors for major age-related diseases[43–45].

Figure 2 summarized the genetic heterogeneity of the groups considered for the genetic study of human longevity.

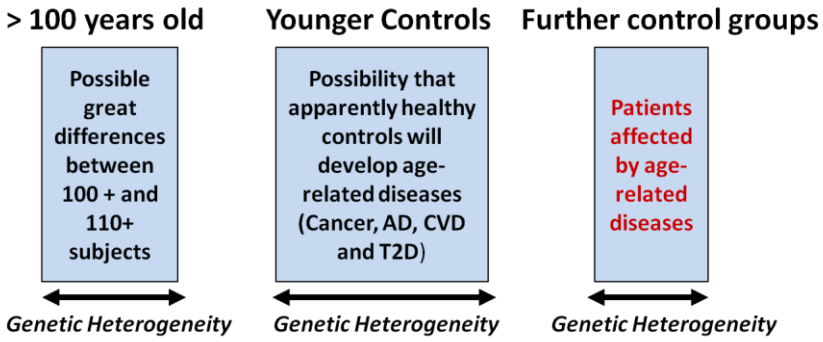

Figure 2. *Definition of longevity and control groups for the study of genetics of human longevity. Genetic heterogeneity of the groups is reported. Lengths of the arrows is proportional to the level of genetic heterogeneity of each group.*

3. The genetic of longevity: insight from ecology

It is well established that longevity is influenced by a complex relation between environment, genetics and stochastic factors. Such complexity is appropriately taken into account by ecological models rarely considered in the study of human longevity [46]. The evolutionary concept of "niche construction" [47–50] suggests that individuals' genetics interacts with the external world and the environment that each species creates. For humans, niche construction is influenced by socially transmitted behaviour that constitutes in fact a triple inheritance system including 1) genetic, 2) culture and 3) ecological dimension. Accordingly, we will assume the relationship with the environment as the *fil rouge* of the analysis of the genetics of longevity presented in this section. To understand this complex ecosystem we assume that the interactions with genetics in each individual may vary according to her/his specific characteristics. Thus we start first from the description of the biological identity of each individual that is determined by innate characteristics (such as sex and rare variants) and by features acquired during lifetime (such as somatic mutations and immunological stimuli that constitutes immunobiography). Then we describe three dimensions capable of encompassing the different timescale of interactions between genes, culture and ecology as reported in Figure 3 (ecological-space):

1) the genetics of families (assortative mating or indirect effect of parents' genotypes);

2) the genetic of human populations (population genetics and population specific gene-environment (GxE) interactions);

3) the genetic of longevity and socio-economic factors.

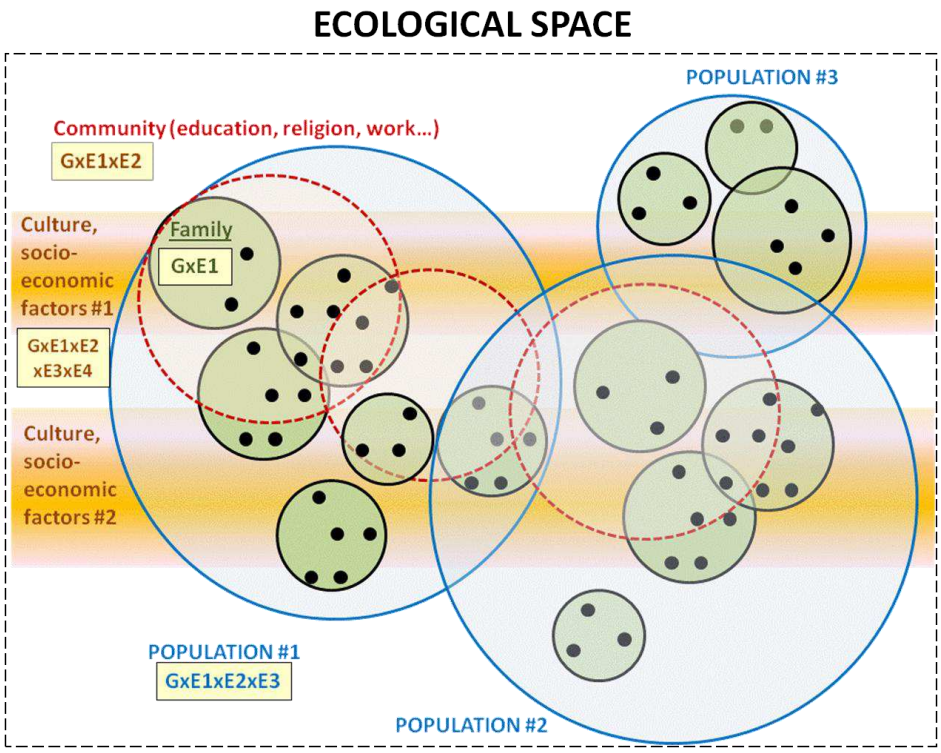

Figure 3. The ecological space is responsible for the peculiar gene-environment interactions of each individual. *Individuals, families, and communities (such as school, work, religion, among others) are represented by black dots, green and red circles, respectively, while the blue circles represent populations in term of genetic ancestry. Cultural processes are represented as horizontal orange shadows. Migration and mobility increase the possibility to identify different human populations in the same communities, which can share, at least in part, the same ecological space.*

3.1. The genetics of longevity: individual variability

3.1.1 Biological biography: the example of immunobiography

The ecological perspective indicates that longevity is the result of dynamic interactions between genetics and environment. In particular gene-environment interaction in centenarians are peculiar of each individual because the combination of stimuli experienced during life interacting with the unique genetic background results in a unique signature. This concept has been conceptualized from an immunological perspective and dubbed "immunological biography" [51,52]. Owing to its memory and plasticity, the immune system (IS), including both the innate and the adaptive ones, is capable of recording all the immunological experiences and stimuli it was exposed to. Immune responses are influenced and shaped by, at least, two dimensions: i) space, composed by the relations between human being and their specific peculiar environment, according to the geography and history of the population to which she/he belongs to (pathogens, nutrition, climate, lifestyle, among others); ii) time, since each individual is the result of time-dependent adaptive processes (such as immunosenescence and inflammaging).

These considerations can be applied to other organs and systems of the body (such as the brain, the endocrine systems, the gut microbota among others) which also have the capability to make records of

previous lifelong stimuli, contributing to create the individual biological biography which impacts on the aging process and trajectory, starting from the first stage of life.

3.1.2. Individual genetic identity: germline (rare variants) and acquired (somatic) mutations

The genetic identity of each individual is a dynamic process because each person has a unique genetic composition with a peculiar combination of common and rare or even private de novo variants (germline variations) but at the same time accumulation of somatic mutations is observed during aging. The combinations of these genetic variants constitute the individual genetic identity that continuously evolves with age.

Genetic variants can be categorized according to their frequency in the general population an from this point of view each individual carries a number of private variants not shared by other individuals. The study of rare variants in aging and longevity is challenging since longevity is by definition a rare phenotype. To this regard two main approaches can be envisaged: i) to study the effect of these variants combining large-scale DNA sequencing and their functional testing in targeted molecular experiments[53,54];ii) to study population isolates. This approach has been successful in detecting rare variants associated with complex phenotypes [55] as these populations are characterized by genetic homogeneity and rare variants may have drifted up in frequency, in fact the effect of random genetic drift on increasing allele frequencies is higher for rare compared with common variants [56,57].

Another element contributing to genetic identity is somatic mosaicism that occurs lifelong in all human tissues: from the fertilization until death individuals accumulate somatic mutations. This accumulation is the result of exposure to external agents (such as UV and pollutants) but also a consequence of errors in DNA duplication process, DNA double-strand breaks, inefficient DNA repair, unbalanced chromosomal segregation [58,59]. The accumulation of unrepaired DNA damages, the decline in DNA repair efficiency and replicative senescence are well established characteristics of aging [60–63], as confirmed in a study on Italian centenarians[64]. Somatic mutations in autosomal DNA and in particular clonal hematopoiesis are an emerging link between longevity and cardiovascular diseases and a detail description is reported in section 5.3.

Somatic mosaicism of mitochondrial DNA, referred as "heteroplasmy", was reported in many aging studies showing an accumulation of mtDNA mutations during aging in healthy individuals. Moreover heteroplasmy inheritance was confirmed in a study that applied an ultra-deep DNA sequencing approach, capable of detecting very low frequency alleles, on female centenarians and their offspring [65]. The analysis showed a heteroplasmy profile (in term of total heteroplasmies levels and loci) peculiar for each individual (private component) but also the presence of heteroplasmies shared between mother and daughter within each family, thus indicating a familial specific contribution of low level heteroplasmies to the genetics of human longevity .

3.1.3. The genetics of human longevity and sexual dimorphism

It is well known that human longevity - with some exception - is strongly correlated with sex, and that females generally live longer than male, likely because of the combination of biological factors (anatomy, reproductive functions, sex hormones, expression of genes on the X or Y chromosome) and social factors related to behaviour, population and peculiar life experiences [66–68] as showed in Figure 4.

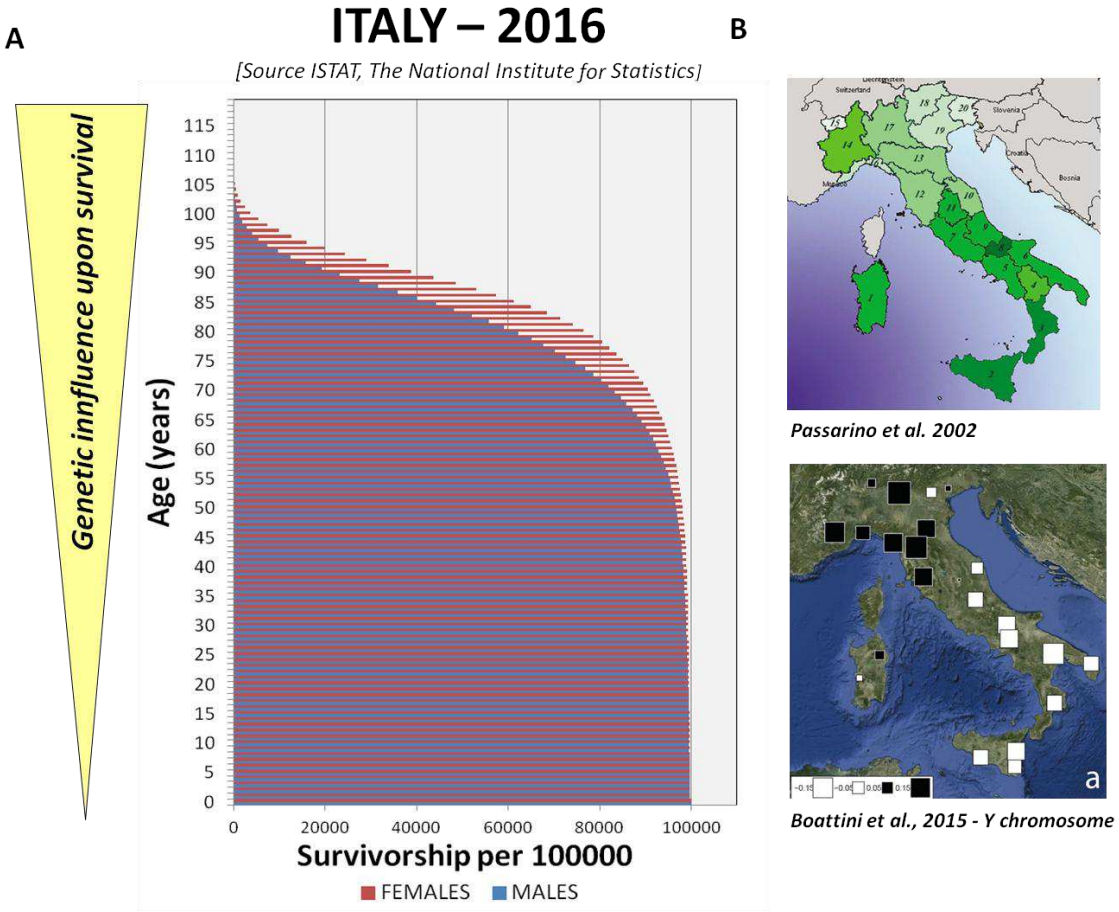

Figure 4. Female/male distribution in the Italian population and centenarians. *A) The survivorship in Italy per 100000 individuals at different ages is reported. Females and males are indicated in red and blue, respectively. The triangle on the left indicates the influence of genetics on the longevity trait. B) The upper heatmap shows that the Italian female/male centenarians' ratio increases from North to South (Passarino et al. 2002). The lower map shows the Y chromosome's genetic variability in Italy (Boattini et al. 2015). Black squares represent positive values of the spatial principal component analysis (sPCA), while white squares represent the negative ones; the size of the squares is proportional to the absolute value of sPCA scores. On the whole, autosomic and uniparental genetic variability, cultural and social structure and sex present a well defined distribution across Italian population*

Gender influences also the inheritance of exceptional longevity [69]. The analysis of parental age of a cohort of Ashkenazi Jews with exceptional longevity revealed that in males both maternal and paternal inheritance are likely contributors to exceptional longevity, whereas among females maternal inheritance appears to be more influential [69]. Evolutionary studies provide clues for the interpretation of these sex specific influences [70].

Sex hormones are the first example and in particular the removal of testosterone at young ages has been demonstrated to increase lifespan in mammals [71] and in humans, as supported by the Korean population of eunuchs [72]. In male, there is a trade-off between immune investment and traits that improve competitive success, which has driven genetic variation in genes involved in testosterone levels during human evolution. Since immune responses are energy demanding processes, testosterone plays a major role in male to increase muscle growth but at the same time reduces immune responses (testosterone is considered an immunosuppressant) [73,74].

A second example is linked to cultural factors that could also contribute to the observed sex differences. The "grandmother hypothesis", spread around 1980 when researchers found that among Hadza hunter-gatherers (Tanzania) mothers faced a trade-off between foraging for food for themselves and caring for new babies. This cultural process - observed only in humans - may have shaped genetic variants conferring sex-specific advantage for survival after menopause because women who remained healthy beyond their fertile years may have enhanced their reproductive success by providing care for their grandchildren [75].

Third, mitochondrial genome is a major player for the differences in longevity between sexes. This is because mtDNA is maternally inherited, and this genome can only make a direct and adaptive response to selection through females, as experimentally demonstrated in Drosophila by cutting edge studies [76,77]. This means that mutations with a negative effect in males can accumulate in the population if they are neutral or beneficial for females (phenomenon called *Mother Curse*). The study of Camus and colleagues [77] suggested that mitochondrial genome is a hotspot for mutations that affect sex-specific patterns of aging, and this phenomenon contributes to sexual dimorphism in aging [78]. The interaction between nuclear DNA and mitochondrial genome (hereafter mitonuclear interactions) plays a major role in explaining variance in penetrance and expression of mitochondrial diseases with different trend in different sexes [79]. The accumulation of mtDNA mutations, which impact on the OXPHOS functionality, constitutes an intense selective pressure on the nuclear genome for counter-adaptations that restore the compromised function.

Data in *Drosophila melanogaster* suggested that this process is strongly influenced by sex. Experiments on isonuclear fly lines whereby distinct mitochondrial haplotypes have been placed into a standardized foreign nuclear background indicate that a disruption of the coevolved mitonuclear genotype lead to males, but not females, sterility [76,80].

More in general, the evolutionary dynamics maximizes the number of offspring leading to different fitness in males and females (Figure 5). Females can have a limited number of offspring for physiological reasons (number of gametes and energetic costs of each pregnancy), while males tend to be limited by the number of matings. This leads to a different selection dynamics in female that it is not observed in male, as supported by a recent study that showed an accumulation of deleterious mutations in genes exclusively

expressed in male [81] (Figure 5). Sex-limited selection can moderate the elimination of deleterious mutation from the general population and leaving deleterious mutations expressed specifically in men.

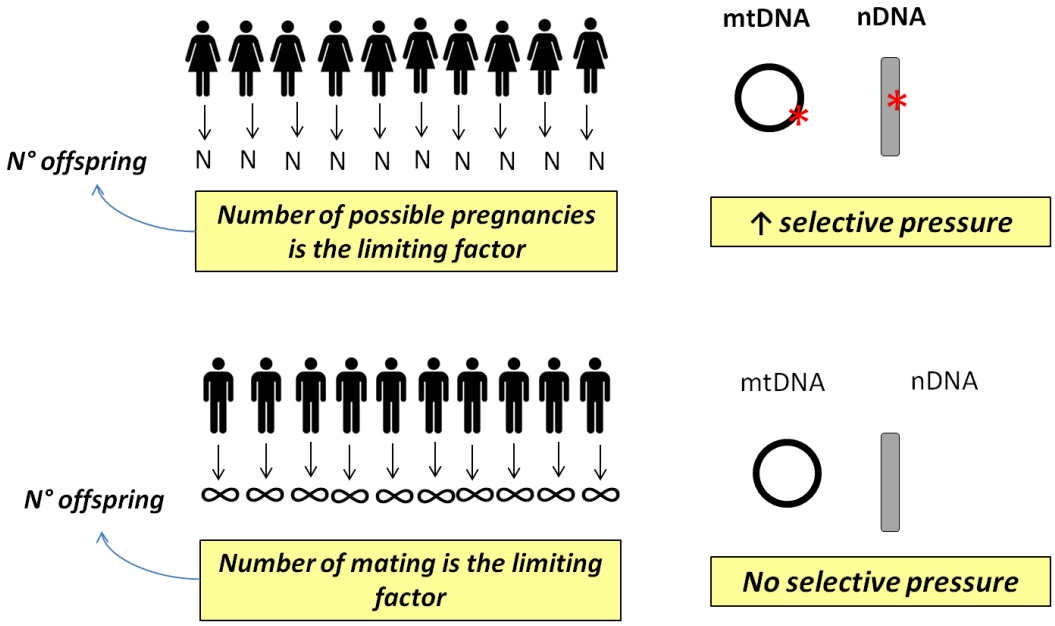

Figure 5. Evolutionary dynamics maximizes fitness according to reproductive effectiveness*. Females can have a limited number of offspring for physiological reasons (number of gametes and energetic cost of pregnancy), while males are limited by the number of matings (down part of the figure). Evolution strongly favoured female fitness since the number of reproductive active females is the limiting factor determining the newborn number for each generation. This difference leads inevitably to different sex-specific dynamics of selective pressures on both mitochondrial and nuclear genomes that are strong for female than for males .*

The dimorphism in longevity has been only recently introduced in the study of the genetics of human longevity. In the GEHA study, a linkage analysis was performed in 90+ male (N pairs=263) and female (N pairs 1145) sibpairs and three loci that linked to longevity in a sex-specific manner were identified: 8p11.21-q13.1 (men), 15q12-q14 (women), and 19q13.33-q13.41 (women) [8]. A very recent GWAS study [82] investigated the association with longevity separately in men and women in a cohort of more than 2200 Chinese centenarians. The authors did not find any locus associated with longevity above the formal 10^{-8} GWAS threshold, but a series of interesting sex-specific longevity alleles were identified. In the study the *polygenic risk score* was successfully applied to investigate associations' signals below the GWAS significance threshold. The score results indicate that different pathways contribute to longevity in men and women. In particular, paths involved in inflammation and immunity emerged as male-specific while those involved in PGC-1a function and tryptophan metabolism emerged as female-specific. It is worth noting that the GWAS approach likely limited the sex-specific analysis of longevity due to the obvious reduction of the dimension of the cohorts when splitted into females and males.

3.2. Familial ecology and the genetic of longevity

"Longevity runs in families" is a statement that introduces the vast majority of papers about the genetics of human longevity and families constitute a niche in ecological term. One of the first evidence came from studies in the Sardinian population (Italy), based on a geographical-genealogical approach. These studies showed that: i) a non-random distribution of centenarians by place of birth and peculiar area (in the province of Nuoro) of exceptional male longevity were identified and called "blue zone" [83]; ii) longevity clusters in families and occurs among the ascendants of a particular branch of the family [84]. A peculiar characteristic of Sardinian familial longevity is that children born from mothers who later became centenarians had significant lower infant mortality when compared to children born to those women belonging to the same cohorts but who did not became centenarians [85]. Thus, history of familial longevity contributes to increasing the odds for an individual's longevity, as reported in a study of 1,700 sibships from families of centenarians in the New England Centenarian Study [86]. Moreover, centenarians offspring are characterized by a better health status when compared with age-matched controls born from parents who died before reaching the expected lifespan for their cohort [87].

However, not only genes but also cultural and ecological dynamics cluster in families, especially in small populations that maintain local tradition and where marriages occurs within the same (or in any case very close) small communities (such as in Nuoro) [84]. One clear example of the effect of the social and family structure on the genetics of longevity come from two studies in different populations such as Dutch and Calabria (South Italy) [88,89]. The first found that spouses of long-lived partner, even sharing most of their adult life with their partner, did not present any advantage in term of survival, suggesting that the effect observed were mainly linked to genetic factors. On the contrary in South of Italy (Calabria) female spouses of long-lived siblings also live longer than members of the corresponding birth cohort indicating that females may benefit more than males of a favourable environment to become centenarian.

These observations constitute the strength of the study, as they clarified to what extent the study of genetic of longevity may depend on familial, social structure and anthropological habits. Also in the Long Life Family Study (LLFS) – a longitudinal family-based study of longevity and healthy aging – spouses of members of long-living families tend to be healthier than a sex- and age-matched members of the general population [90]. The underlying mechanism at the basis of these observations could be cultural, environmental and social but also genetic. Assortative mating based on characteristics such as anthropometric measures, behaviour, educational level, cognitive abilities which all have substantial genetic components may be a mechanism. A high degree of genetic similarities of spouse pairs, particularly for older generations, have been identified, and provide evidences for ancestry-related assortative mating also in families selected for longevity [91]. The first studies and hypothesis of assortative mating in human longevity date back to 1914 and are based on the fact that familial longevity was a trait that families could be proud of. Assortative mating, differently from high level of inbreeding that affects the frequency of all

loci independently, is a phenotype-based assortative mating (driven by different factors such as educational level, socioeconomic status, language, behaviour, culture etc.) and can change LD and the distribution of the genotype frequencies of the loci involved in the assortment to an excess of homozigosity [92]. Accordingly a recent study showed that genetic variants linked to education predict longevity, suggesting that individuals with more education-linked genetic variants had longer-living parents [93]. In addition also the parental genomes (usually ignored in genetic studies) seems to play a role as non-transmitted alleles can affect a child through their impacts on the parents and other relatives, a phenomenon recently called "genetic nurture" [94].

3.3. The genetics of longevity: a population approach

Longevity is highly context-dependent as demonstrated by many studies. What is quite new is that also genes and pathways that impact on longevity are also population-specific. The study about longevity in the Chinese population identified peculiar pathways linked to longevity that are context-dependent [95]. It is likely that longevity could be achieved in a population-specific way, involving both public and private mechanisms [65,96] and could be conceptualized as a sort of "convergent phenotype" [41], reached by context-specific mechanisms (genetic and non-genetic) that are in part public (at least for biological functions) and in part private of each population.

Within this perspective:

1) gene-environment interactions can be strongly influenced by the fast changing and population-specific environmental conditions, and thus the same alleles can exert different effects in different populations;

2) allele frequency can reach high frequency in certain populations because of evolutionary dynamics such as migration, bottleneck, drift or positive selection that acted in the past.

An example of the first scenario is TCF7L2 rs7903146-TT, the most significant genetic marker of Type 2 diabetes associated with cardiovascular events. A study of Corella and colleagues showed that the Mediterranean diet is able to counteract the effect (in term of stroke) of the risk genotype, suggesting that a genetic association can be influenced by population-specific environmental factors [97].

An example of the second scenario come from a genetic study on the Italian population where the frequency of variants involved in different diseases showed different pattern according to past environmental selective pressures. The study published by Sazzini and colleagues [98] considered more than 500,000 SNPs in 780 individuals recruited in the Italian population and demonstrated that past local adaptations and different admixture events with continental and Mediterranean populations shape the frequency of risk variants for complex pathologies - type 2 diabetes and cardiovascular diseases among others - considering North to South cline. Variability between populations is not the only source of variation to consider in the study on the genetics of longevity. Subtle but concordant changes in allele frequencies across population that share geographic area but which differ for ecoregion, dietary components and mode

of subsistence have been reported [99]. Thus the same adaptive allele may increase in frequency in certain populations in different geographic areas that share the same environment. The case of APOE is paradigmatic and described below in a dedicated paragraph. The same considerations apply to mtDNA and Y chromosome. Population variability, past expansion or bottleneck and demographic history (often different for male and female individuals of the same population) is at the basis of the distribution of mtDNA and Y chromosome genetic markers. This consideration is crucial because it is known that the pool of genetic variation is created through de novo mutations in each genome, and through the standing genetic variation. This scenario will differ across populations, and since each population is exposed to different selective pressures as a result of inhabiting distinct spatial and temporal environments, mtDNA variation, Y chromosome variability and co-evolutionary trajectories of mitonuclear co-adaptation are predicted to be population-specific [79].

3.4. The genetics of longevity in different socio-economic settings

The social and ecological dimension is a further niche constructed by humans that plays a major role in the genetics of complex traits. It is well known that socio-economic factors may impact on human longevity. Longevity and the aging rate can change according to social factors, and one of the most striking example for longevity come from the city of Glasgow were higher level of mortality have been registered in persistently deprived areas [100]. Another example is the case of Korea: South Korean population experienced a rapid increase in economic condition becoming one of the most developed nations; on the contrary the North Korean population remains one of the poorest countries. North Korean had 0.7 centenarians /1 million persons in 1925 and 2.7 centenarians/1 million persons in 2010 and South Korean population showed 2.7 centenarians/1 million persons in 1925 and 38.2/1 million persons in 2010 [101]. These demographic data open new insight for the study of the genetics of human longevity as genetic influence in the same population may change according to social pressures as also demonstrated in a study by Rimfeld and colleagues [102]. They considered 12,500 individuals from Estonia and demonstrated that during the Soviet era 2%-of the variance seen in educational and success was due to differences in genetic factors and that this number increased to 6% after independence [102]. Two recent studies investigated the effect that the socio-economic status at birth exerts in whole life mortality and showed that illegitimacy of the birth and parental occupation (in particular paternal one) were associated with an increase in mortality at all ages [103,104]. The most obvious explanation of these differences is that socio-economic status impacts on nutritional profile, stress and exposure to infections, but recent data showed that epigenetic modifications (and in particular DNA methylation) may "recode" information of the father's environmental exposure and then be transmitted to the offspring [105–107], supporting the crucial role of evaluating these factors for the missing heritability. Moreover, it has been suggested that - even if social interactions tend to reduce during aging - the maintenance of social connections help to live longer [108] and is associated with lower mortality.

In fact individuals with social relationships have an higher probability of survival (50% greater) compared to those individuals characterized by few and poor social interactions, as demonstrated by a meta-analysis of 308 849 individuals, followed for an average of 7.5 years [109]. On the contrary, the absence of interactions with the families and in particular with grandchildren can be associated to a higher susceptibility for depression in the elderly [110]. Data on the role played by the behavioural genetics in determining these social interactions has not been analysed.

These studies highlight the importance of social interactions when addressing the factors which contribute directly or indirectly to healthy survival and longevity, including the genetic ones. Accordingly, we propose the following considerations regarding the genetic of longevity in humans: 1) the genetic of longevity is highly dependent from the socio-economic status, as the process of remodelling and the genes favouring longevity appear to be different in different socio-economic backgrounds. As an example the results of the Health and Retirement Survey (HRS) in USA showed by an analysis of 9,317 adults (>65 years old) that the genetic predisposition to major chronic health conditions and the definition of "genetic risk variants" are strongly related to socio-economic status [111]; 2) new socio-economic conditions have emerged recently, such as the obesogenic environment. Thus, genes that favoured longevity a century ago (for example during the First World War or when antibiotics did not exist) are likely different from the genes that favour healthy aging today; 3) social interactions may shape genetic variability. Genes may evolve through positive selection according to their effects on social behaviour (called also indirect effect, because they confer a characteristic crucial to boost social interactions). The most striking example in the study of human evolution is the language gene FOXP2. This gene, even if does not encode for social behaviour traits in any mechanistic sense, indirectly increases the possibility of social interactions between individuals and likely its frequency increased owing to the advantage in social behaviour. Likewise genes involved in testosterone profoundly changed during evolution under social selective pressure when humans changed the way of subsistence, e.g. from nomadic to settled societies and thus force to more numerous social interactions.

4. THE GENETICS OF HUMAN LONGEVITY: THE MAIN FINDINGS

4.1. From whole genome sequencing (WGS)

To date whole genome sequencing is the most informative approach for genetic analysis. However, this technology is also the most recent in terms of availability and the reduction in term of costs/sample occurred in the last few years have radically changed the approach to genetic studies.

While the first studies on the genetics on human longevity were based on candidate gene analysis, to date the approach is based on the use of genome-wide techniques to identify the most informative loci, and functional studies both *in vitro* and *in vivo* constitute the last step of the analysis. The main advantage of whole genome sequencing is that it allows the study of the existing genomic variability of each individual

(both in coding and non-coding regions) without using imputation process, that may include some bias due to the reference population. To date the only few whole genome sequencing studies on the genetics of human longevity are available: (i) the first studies were performed by Sebastiani and colleagues [112] and by Ye and colleagues [113] , and analysed few individuals reaching the extreme decades of life. The Sebastiani's study published in 2011 sequenced two supercentenarians (>110 years old) but the low number of subjects does not allow any statistics. However, an interesting data emerged*:* the two subjects likely reflected two different strategies to reach the last decades of life. The female was characterized by significant stretches of homozygosity across many chromosomes, consistent with inbreeding amongst her ancestors, and she carried only 5 out of 16 common variants selected for their role in metabolism that resulted associated to longevity in GWAS studies. The same analysis in male showed no similar stretches of homozygosity, and 11 out of the 16 common variants associated to longevity. Overall, these results suggested that the woman was enriched for private mutations that promote exceptional longevity (due to her familial genetic background).

(ii) The second study [113] considered a monozygotyic twin pair reaching 100 years and owing to the peculiarity of the samples the study also investigated the prevalence of somatic mutations. (iii) The third study published in 2014 by Gierman [114] and colleagues considered 17 supercentenarians (110 years or older) of European ancestry, but the use of public available genomic data as control reduces the efficacy of the analysis (reducing the number of variants to analyse). The main findings can be summarized as follows: i) no significant evidence of enrichment for a single rare protein-altering variant or for genes harboring rare protein altering variants in supercentenarian compared to controls was found; ii) the gene most enriched for rare protein-altering variants in this cohort of supercentenarians was TSHZ3, but the replication on a second cohort of 99 long-lived individuals failed. iii) The most recent paper - and also the largest in term of sample size - was published in 2016 by Erickson and colleagues [115], but the authors studied an "healthy ageing" phenotype, based on self-reported data (called by the author *Wellderly*, in subjects aged 84.2 ±9.3 years). The age range of the study indicates that very limited information regarding longevity have been investigated [116].The main result of this study is the association with healthy aging in loci in the COL25A1 gene encoding for a protein secreted in the brain and associated to amyloid plaques.

Thus, sequencing study on longevity are emerging, but available data did not allow a real "discovery" phase in comparison with pre-existing data. Few samples and the lack of controls impair the interpretation of the sequencing data.

4.2 From genome wide association studies (GWAS)

The literature regarding exome sequencing in human longevity is not so rich, and only few studies used this type of protocol [117–119]. The vast majority of data regarding GWAS are based on high-dense microarrays analysis (usually Affymetrix or Illumina) that thanks to imputation process can be compared between

different cohorts and populations for millions of SNPs. Even if the purpose of this review is not to elucidate the limitation of imputations process a detailed description on this topic can be found in [120,121]. GWAS can be ideally divided into two major types according to the considered phenotype: the first is focused on aging and longevity evaluates the genotype of each individual correlated with the phenotype of the individual itself; the second includes *parental* longevity and evaluates the genotype of each individual correlated with the age phenotype of the parents. In Table 1 an overview of all (as far as we know) the GWAS studies are reported. The purpose of this section is not to purely describe the genes emerged from each study as other excellent reviews have been produced on this topic, but to let clarify the main conceptual assumptions, results and methodological strength/limitation of the available studies which in a sense have revolutionized the field.

4.2.1 The link between longevity variants and age-related diseases emerges from GWAS studies

The relationship between aging and chronic age-related diseases is based on epidemiological evidence and experimental data that aging is the major risk factor for such pathologies and assumes that aging and chronic age-related diseases/geriatric syndromes share a common set of basic biological mechanisms. Age-related diseases can be conceptualized as accelerated aging and that aging and age-related diseases are part of a continuum, the two extremes being represented by centenarians who largely avoided or postponed most age-related diseases, and patients who suffered one or more severe age-related disease in their 60s, 70s, and 80s [4].

Longevity GWAS provided important evidences supporting the tight interconnection between aging and the age-related diseases. Many "longevity variants" have been identified, but function analysis showed that most of them are involved in age-related diseases. APOE/TOMM40 - that emerged from the vast majority of studies [8,38,122–127] - is a remarkable example as it is involved in Alzheimer's disease onset, in CVD and cancer (see Table 2). Deelen and colleagues [124] in their GWAS study on European longevity in the framework of the GEHA project identified a novel locus on chromosome 5q33.3 associated with survival beyond 90 years, and replicated the locus TOMM40/APOE/APOC1. In particular the SNP, rs2149954-T, in chromosome 5q33.3 is associated with low blood pressure, and they showed that minor allele (T) carriers present lower mortality risk for CVD, eventually linking longevity with a lower risk for stroke. The authors identified for the first time a real "longevity locus" as the association observed is *positive*, meaning that centenarians are characterized by an high frequency of the alleles associated with low blood pressures [128]. The study of Fortney et al [40] described for the first time the overlap at genome-wide level between longevity and age-related diseases. The approach developed by Fortney and colleagues provides to each SNP an age-related risk weight (the approach was called iGWAS, informed GWAS). In particular, it revealed that in longevity there is a reduction of the genetic risk for artery disease, Alzheimer's diseases, diastolic and systolic blood pressure, three blood lipid traits (triglycerides, total cholesterol, LDL), chronic kidney disease and bone mineral density. Beekman et al.[129], and Sebastiani et al. [38] used a different approach to

investigate disease risk alleles in longevity and utilized sets of disease SNPs to construct genetic risk scores (or polygenic risk scores [111]). They found no significant differences between centenarians and controls, concluding that disease risk alleles do not compromise human longevity. These contrasting results are likely related to the different statistical tools applied on the same question. However to move a step forward on this hot topic it is necessary to carefully study each genetic risk alleles in centenarians and patients matched for geographical origin.

In any case, as expected, from available data the enrichment of age-related disease variants has never been observed in centenarians, in accord the prediction of geroscience that aging is the greatest risk factor for a majority of chronic diseases, driving both morbidity and mortality. Thus, we surmise that the geroscience architecture [3] has missed the eighth pillar: genetics. This consideration is supported by a recent review on progeroid models that highlighted the notion that genetics has profound effects in determining the aging process: in particular, looking at lamin-linked progeroid disorders, it is clear that determinants of aging may originate from an altered genetic background [130]. Accordingly, a GWAS study [131] identified a genetic correlation with longevity in the WRN gene, responsible for the Werner syndrome, a progeroid disorder characterized by premature aging [132,133] .

4.2.2 Longevity depends on small-effect alleles

The GWAS study of Yashin and colleagues [134] revealed the importance of including in the analysis also signals with $p \leq 10^{-6}$ in the study of genetics of longevity, suggesting that the traditional $p \leq 5*10^{-8}$ is likely not appropriate for this trait. This cut-off widely used in GWAS data in order to reduce the number of false positive may be difficult to apply to studies on longevity that are characterized by a small sample size owing to the fact that the recruitment of high number of centenarians, particularly from the same population, is often difficult and challenging. In our opinion the use of statistics need to be contextualize into the biological problem (in this case longevity) in order to identify the right trade-off between the risk of identifying false positive signals and the possibility to filter out biologically relevant signals that fall under this cut-off. To assess this problem Yashin and colleagues [134] tested the hypothesis that longevity depends on a number of small-effect alleles using 550k SNPs data in 1,173 genotyped participants of the Framingham Heart Study (FHS). They demonstrated that the joint influence of small-effect alleles on life span is both significant and substantial, explaining in part the numerous GWAS that did not find any $p \leq 5*10^{-8}$ result [122,126,131,135]. This study let emerge the importance to evaluate non-additive (non-linear) joint genetic influence (epistasis) to investigate the "genetic dose – phenotypic response" relationship in longevity. Moreover, this result impacts on genetic calculations such as heritability, whose main assumption is the additive nature of genetic component of phenotypic variations.

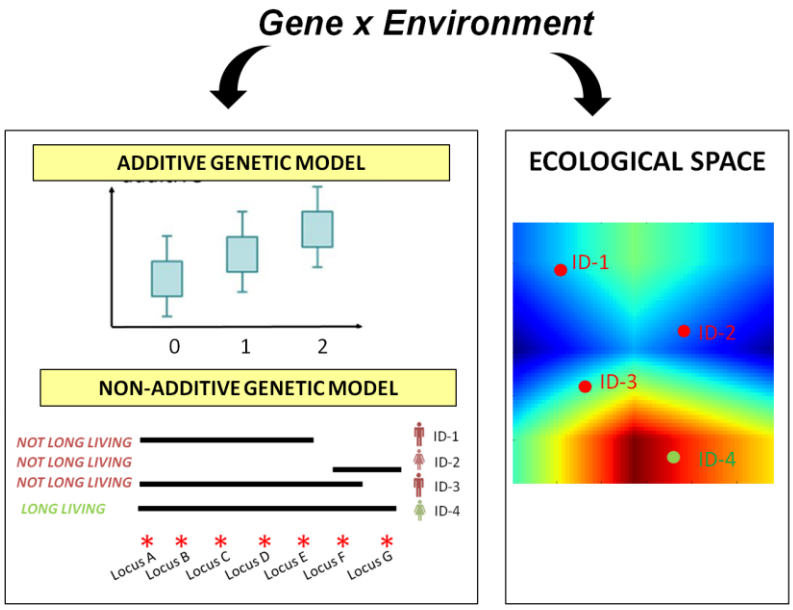

Figure 6. Longevity depends on small-effect alleles, and new type of analysis is required to analyze this trait and to extract the information from available genetic data*. Missing heritability evaluations indicate that the additive model alone is not sufficient to cover all the genetic contribution to "genetic dose – phenotypic response" in complex traits. Non-additive interactions and epistasis should provide part of the human longevity missed heritability. Ecological space concept (right), proposes that genetics of longevity results from individual, time- and space-dependent GxE interactions. ID indicates different non-centenarians individuals and a centenarian, in red and green, respectively.*

New mathematical model to calculate epistasis and effective tools to reduce the high dimensionality of the data, and the use of both additive e non-additive interactions are needed (Figure 6).

A new method for the analysis of small-effect alleles is discussed in the paper of Boyle and colleagues [136] . They proposed an "omnigenic model" based on the definition of core and peripheral genes. According to the authors, the first constitute a network of genes whose mutation lead to the strongest effects on the trait under study, while the second class of genes includes all the genes with no apparently relevant effects on the trait. They observed that a large proportion of the genetic contribution to a complex trait comes from peripheral genes that are only a few steps (in term of distance between the nodes of the network) from the nearest core gene and thus may have an effect on the trait in specific tissues. Boyle et al. propose that complex traits, such as longevity and age-related diseases, are the results of many processes that involve different cell types and tissues, and the role of the genetic variants has to be contextualized in such locations [136].

4.2.3 GWAS analysis showed monotonic as well as non-monotonic age patterns of allele frequencies

The study of different allele/genotype frequencies in distinct age classes has been used as evidence of the presence of genetic influence on survival [137]. With this method, called "gene frequency methods" (GF), it was possible to divide variants in "frail", "neutral" or "robust" according to the trends showed with aging (as reported in Figure 7). GF is based on two main assumptions: i) the allele frequencies in all the considered birth cohorts are the same; ii) mortality for genotypes does not change between birth cohorts.

This approach was adopted in two studies [138,139]. The change of allele frequency with age may follow linear trends (monotonic) or non-monotonic patterns (usually U-shaped patterns or constant until a certain age and then linear) in which allele frequency decreases to a given age but then increases, reflecting trade-offs in their effect at young and old ages.

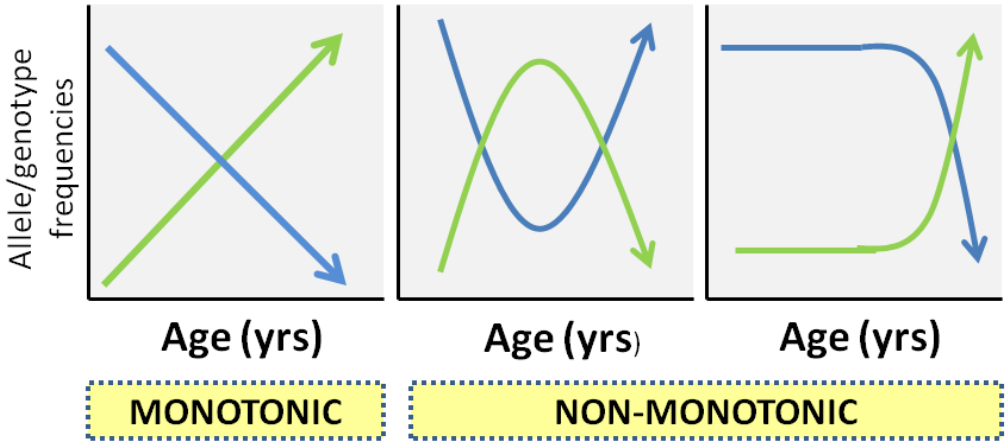

Figure 7. Gene frequency methods. *Different allele/genotype frequencies in distinct ages. If initial alleles frequencies in all birth cohorts are identical and if mortalities for genotypes do not depend on the birth cohorts this approach identified linear trends (monotonic) or non-monotonic patterns (usually U-shaped patterns or constant until a certain age and then linear) of allele frequencies. These trajectories may reflect trade-off in the effects at young and old ages.*

Non monotonic trends have been suggested to correspond to genetic variants whose effect on mortality risk change with increasing age, from detrimental to beneficial (or vice versa) [138].

Such trajectories increase the complexity of the study of genetics of longevity because indicate that the effect of a given allele varies according to the changes of the internal environment that occur from young and adults to the oldest old-[140,141]. This feature, called "complex allele timing" by Benedictis and Franceschi (2006) points out the fact that genetic risks or protective factors are likely age-specific. The concept of "allele timing" should not to be confused with that of antagonistic pleiotropy [140]. According to the antagonistic pleiotropy theory [142] the same allele can act not only on the probability of survival at young/adult age but also on antagonistic traits (for example fertility or traits link to disease) later on, in a different period of life. For example an allele may confer high fertility (or protection from infections) early in life and the same allele might be deleterious for survival late in life. The allele timing is instead linked to the adaptive process of remodelling of cellular and molecular mechanisms that occurs lifelong. According to the remodelling theory of aging [2,143] the same allele can exert different effects on survival because its function change (e.g. its gene expression) according to the internal microenvironment that in turn changes with age). In this case there is no pleiotropy as the effect is on the same traits.

An example of genes whose variants follow a U-shape trend can be found in the paper of Bergman and colleagues, regarding KLOTHO (an aging gene associated with low HDL and reduced CVD risk) and LPA (lipoprotein A) genes [144]. Other studies highlighted the importance of identifying allele frequency changes

during aging as a method of distinguishing between longevity and buffered-deleterious genotypes [145]. Some risk alleles with age-related U-shaped frequency may be buffered by other "pro-longevity" variants, as in the case of CETP (favourable) genotype that was demonstrated to neutralize the deleterious effects of the LPA gene [144,145]. To this regard it is interesting to note that the product of LPA gene, *i.e.* lipoprotein(a) [Lp(a)], a widely accepted risk factors for atherosclerotic vascular diseases, did not change with age until the extreme ages, and a number of atherosclerosis free centenarians had high Lp(a) serum levels, together with low HDL-CT and relatively high TG [146]. These data support the hypothesis that a continuous and complex reshaping of lipid metabolism occurs in physiological aging, likely contributing to longevity.

4.2.4. GWAS remarks the population specificity of longevity

Other GWAS studies [95,122,147] let clearly emerge the importance to take into account population stratification in genetic studies on longevity, in accord with the above-described ecological perspective. The study of Zeng and colleagues on Chinese centenarians is particularly interesting, as the demographic history of the country reduces, at least in part, population stratification and concomitantly allowed the inclusion of an unprecedented number of centenarians. The authors identified both shared and population-specific genes and pathways involved in longevity. They confirmed the loci in APOE and chr5q33.3 and identified new loci in IL6, the well-known "gene of gerontologist", and in ANKRD20A9P. It is to note that the *p-values* in the cohort of Northern Chinese (1115 centenarians) and in Southern Chinese (1063 centenarians) did not reach 10^{-8}, while the combined analysis of the two reached the GWAS threshold. The relative homogeneity of the population and the extreme phenotype (all cases are within the one percentile of survival) support - as mentioned above - that suggestive, nominally significant signals are fundamental in the study of extreme longevity. The study highlighted also longevity pathways peculiar of the Chinese context (xenobiotic, starch and sucrose metabolism), as well as diet-mediated mechanisms, which can differ according to culture and ethnicity. A second study of Malovini and colleagues considered a population of South Italy and identified the association between longevity (defined here with an age range between 90 and 109) and a gene never described by any other study, *i.e.* CAMKIV gene. They also demonstrated by a functional study that CAMKIV is important in the activation of proteins involved in survival such as AKT, SIRT1 and FOXO3A.In general, these examples show that longevity is the result of "phenotypic convergence" [41] reached by context-specific mechanisms (genetic and non-genetic) that in part are “public” and shared among individuals and in part are “private” in terms of population, family and individual specificity. In a 2006 paper De Benedictis and Franceschi suggested to conceptualize the Italian and European centenarians as "phenocopies", according to the classical definition of subjects who share the same phenotype (i.e. longevity) reached by different gene/environment combinations [148].

4.2.5. Results from Genome wide linkage analysis (GWLS)

GWLS searches for chromosomal segments that co-segregate with the phenotype through families. The characteristic of linkage studies is that this approach is highly informative when highly penetrant variants cause the disease/phenotype as they rely on the presence of a single mutation of very strong effect, while genetic heterogeneity, incomplete penetrance and environmental phenocopies reduce the power of this approach. GWAS and GWLS are complementary and not concurrent [149]. Accordingly, even in the same population loci identified through GWLS might not overlap with those identified by GWAS. In the field of longevity one of the first study based on linkage analysis was performed in 2001 by Puca and colleagues in a limited number of samples, and they detected linkage at 4q25 (LOD =3.65) but subsequent studies failed to replicate this signal [150–152]. The first GWLS on a large cohort of European ancestry was performed in the GEHA Study on 2118 European 90+ sibships (couples of brothers and sisters both older than 90 years of age) [8]. The study identified four regions linked to human longevity, and one of them was the APOE region. The authors suggested that the absence of APOE-e4 haplotype in nonagenarians is quite expected, while the APOE-e2 haplotype had to be considered a longevity gene as the linkage they observed was mainly explained by its high frequency.

4.2.6. Parental lifespan: a different approach in GWAS studies

The availability of GWAS data in large population cohorts with thousand and hundreds of thousands of probands ,supplied with the age of death of their parents, opens to the possibility to correlate their longevity genetics with parental longevity phenotype, following the assumption that the "age-of-death for the parents is a measure of longevity" [153–156]. This starting assumption can be misleading for the following reasons: i) these studies investigate the association between offspring genotype with parent phenotype (*i.e.* parent's lifespan), thus the genetic association is indirect and based on the heritability estimation of the life expectancy trait; ii) these studies address the life expectancy phenotype and draw conclusion regarding longevity that is a different phenotype as discussed in a previous paragraph.[157] Moreover, this approach underestimates the relationship between genes and the environmental changes that occur generations after generations, so that genes that impact on longevity may be different in different birth cohort (Figure 8). Industrial progress, improvements in living conditions, changes in nutrition, and other transformations in the human environment (including the emerging obesogenic environment) may have different survival effects on individuals with similar or different genotypes, as previously hypothesized [137] and recently supported [137,158].This scenario is further complicated by the fact that for certain genes GxE interactions change with age[159].

APOE and CHRNA3/5 (nicotinic acetylcholine receptor) are the genes identified and replicated in the highest number of study on parental lifespan. APOE will be thoroughly discussed in one of the following paragraph, while CHRNA3/5 was never identified in other studies on human longevity and it has been associated with traits linked to smoking behavior (nicotine dependence, lung cancer, chronic obstructive

pulmonary disease) [156]. Moreover, some authors suggested that CHRNA3/5 exhibits sexually dimorphic effects on parental mortality, driven mostly by younger male deaths [160].

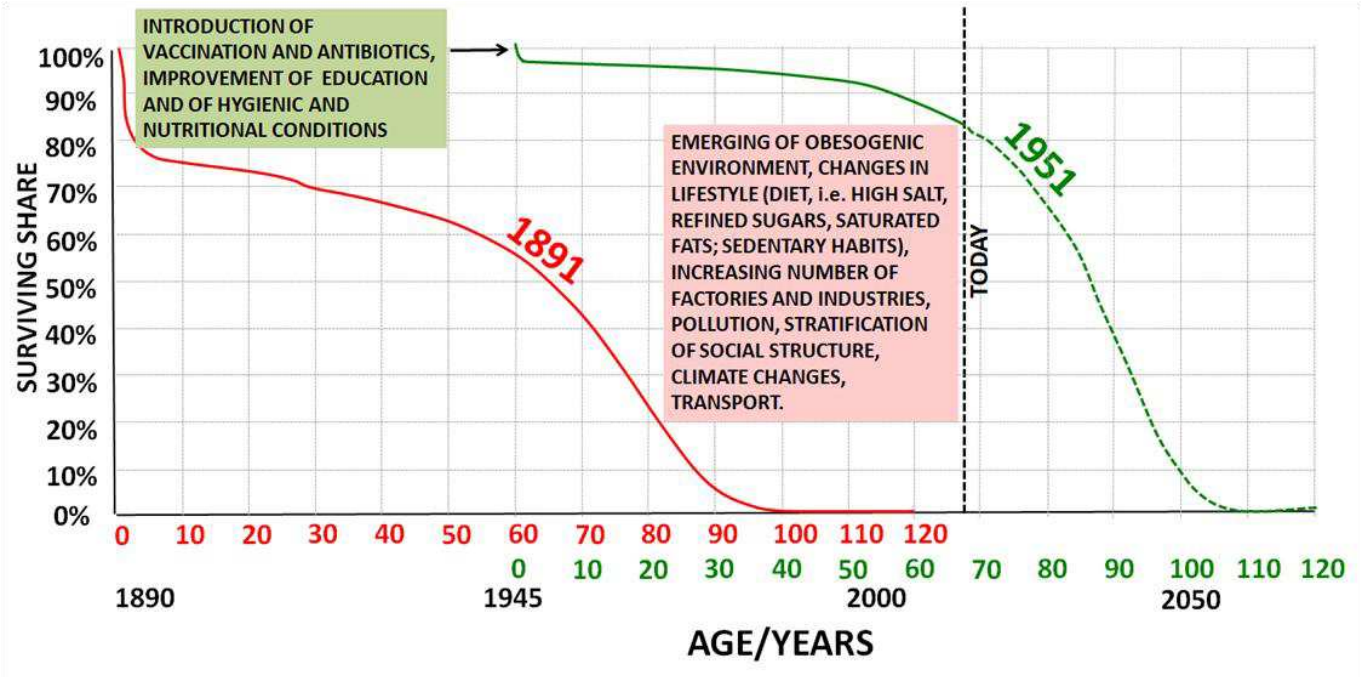

Figure 8. Mortality curve of two distinct birth cohorts, 1891 and 1951, are represented*. The curves reproduce general mortality dynamics of Western countries. These two tendencies are paradigmatic of the dramatic changes in the GxE occurred in the last century. Vaccination campaigns, improvement of hygiene and nutrition, education and antibiotics treatments set infancy mortality to zero around the end of the Second World War. These changes have concrete effects in the genetic pool of Western populations andand selective pressure is dramatically reduced, if not disappeared. Other factors further influenced the Western populations gene pool i.e. the reduction of fertility and the onset of vast migratory events. Concomitantly we experienced the gradual onset of obesogenic environment that is at the basis of the obesity pandemic that many Western countries are facing. Such new detrimental conditions are relatively recent, and since the clinical manifestations of dismetabolic conditions have and adult-elder onset, we have not experienced yet the health load of such changes. Overall this representation shows clearly that to study the genetic of longevity GxE interactions and the birth cohort and population effects have to be considered.*

4.3 LONGEVITY GENES IN CARDIOVASCULAR DISEASES

In this section a subset of the most studied and relevant genes identified in the study of the genetics of longevity are described focusing on the link with CVD. Then, a study using the novel approach of Mendelian randomization is reported in order to investigate the causal effect between lipid profile, genetic variation and longevity. Common genes identified in longevity studies and in studies on CVD are reported in Figure 9

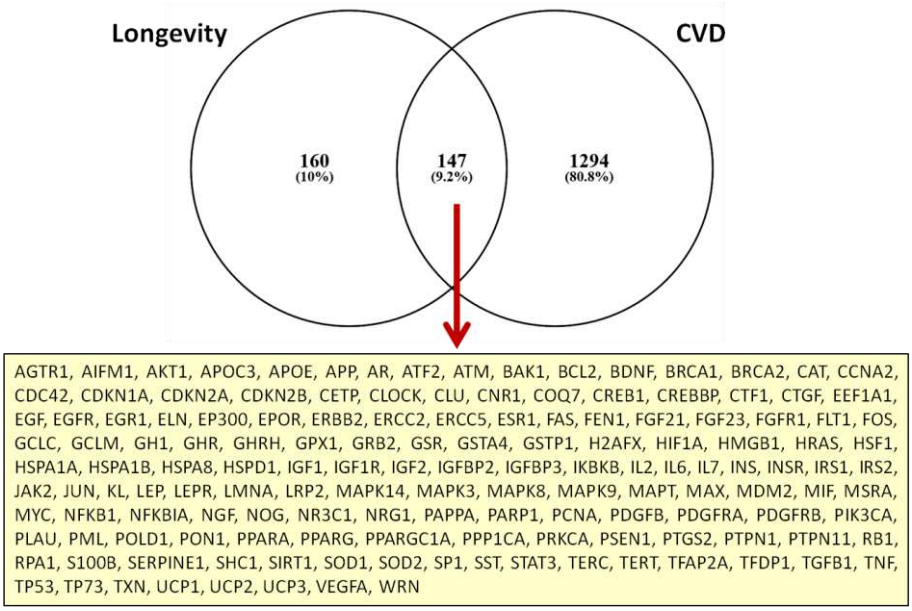

Figure 9*. The genes involved in longevity according to GenAge database and the one involved in CVD according to CardioGenBase database (http://www.cardiogenbase.com/) have been compared and reported through a Venn diagram. Genes in the intersection (N=147) have been listed.*

4.3.1. Apolipoproteins and APOE/TOMM40

Apolipoproteins are soluble molecules present in plasma and many genetic studies showed their role in longevity and in age-related diseases [25]. The idea that they are druggable explains the number of studies devoted to the different apolipoproteins. Some associations with longevity were described for APOB-VNTR in Italian centenarians [161,162], for APOC1 in the Bama populations [163], for APOC3 (-641C allele) in Ashkenazi Jewish [164] . These genetic variants likely influence lipoprotein profile, cardiovascular health and insulin sensitivity. Among all these genes the most replicated is the region of APOE/TOMM40 [8,38,122–127], that is considered the "master" longevity gene [165]. The data show a negative association with APOE-e4 allele suggesting that it has to be considered ‘‘frailty’’ rather than a true longevity allele [166,167]. On the contrary an enrichment of APOE-e2 allele was observed in long-lived individuals [165,168,169] and this suggests a protective role of this allele supported by a positive association between APOE-e2 and healthy cognitive status [169,170].

APOE is involved in lipid metabolism, by regulating production, conversion and clearance of lipoproteins, and is expressed in different tissues (adipose tissue, nervous system and liver) and cells (macrophages) [171].. APOE-e4 is associated to Alzheimer's and CVD [123,124,126,172–177].TOMM40 encodes for a protein that forms a channel in the outer mitochondrial membrane and is crucial for mitochondrial health. Under this scenario three main questions emerge:

1) why APOE-e4 has not been replaced by natural selection with the health-beneficial APOE-e2 allele?

APOE-e4 is the ancestral allele typical of modern humans while APOE-e2 and APOE-e3 evolved around 200,000-300,000 years ago [178]. An hypothesis proposed by Finch and Stanford [179,180] suggests that APOE-e4 confers resistance to diseases associated with meat-eating. APOE-e4 is also associated with improved

cognitive function in Amazonian forager-horticulturalists with a high parasite burden [181], thus conferring an advantage in a specific environment. This and other results led to the hypothesis that the high frequency of APOE-e4 was maintained since it confers protection in environments where infectious diseases are a relevant cause of mortality [182]. APOE gene also influences fertility and APOE-e4 positive women ~~had~~ have significantly higher levels of mean luteal progesterone, suggesting increased fertility, that can contribute to explain the conservation among different populations of this "deleterious" allele [183].

2) Why the association of APO-e4 with longevity seems to vary across different populations? APOE-e4 show a cline in Europe (from about 20% in North Europe to about 6/7% in Southern Europe), and a high level of frequency diversity among populations [184]. Today, the frequency of the APOE-e4 allele in human populations follows a sort of U-shaped latitudinal trajectory, where the highest frequencies (up to approximately 40–50% of the population) are observed at equatorial and high latitudes and lower ones at middle latitudes [185]. This evolutionary-determined allele frequency distribution makes difficult to assess longevity association in population where APOE-e4 is present at very low frequency (around 6-7%) such as in Southern Italy as shown in the study of Deelen et al [124]. Moreover, APO-e4 is a pleiotropic gene, showing many interactions with the environment and in particular with diet, thus creating population-specific and context-dependent gene-environment interactions (for a detailed review see [186]).

4.3.1.1. Cardiovascular risk - APOE has been associated to cardiovascular health in a recent meta-analysis. The study identified a single-nucleotide polymorphism (rs445925) in the APOC1/APOE region that was associated with clinical ideal cardiovascular health (defined according to AHA 2020 goals [187]) at genome-wide level of significance ($P<2.0 \times 10^{-9}$) [188] . Moreover APOE (rs7412) has been identified in a study on the genetic architecture of coronary artery disease [189]. A possible mechanisms for the link was proposed by Li and colleagues, who suggested that ApoE regulates the expression of anti-inflammatory microRNA (miR-146a) in macrophages and intravascular delivery of miR-146a mimetics can inhibit atherogenesis in mouse models [190]. The data about APOE are summarized in Table 3.

4.3.2. IGF-1 and FOXO3

Genetic variance in the insulin-like growth factor -1 (IGF-1) pathway has been associated with longevity in several studies. IGF-1 and related genes is one of the most conserved genetic determinants of longevity across a variety of model organisms [191]. IGF-1 pathway is involved in a broad spectra of functions related to metabolic regulation and energy management[192].There is evidence that in centenarians IGF-1 circulating levels are significantly lower [193], and a positive association between higher circulating IGF1 levels and all-cause mortality, but not CVD events, has been reported [194]. In 2003 Bonafè et al [195] in a study on Italian centenarians reported an association between a SNP in the IGF-1R gene and low plasma levels of IGF-1, and the allele responsible of the low IGF-1 levels was found over-represented in centenarians [196]. Suh et al [197] in 2008 sequenced the IGF-1R gene in a cohort of Ashkenazy Jewish centenarians and they found that centenarians were enriched in genetic variants capable to reduce the efficiency of IGF-1R, generating a sort

of IGF1 resistance**.** According to a 2011 study on an Italian cohort of subjects older than 85 years of age, the IGF-1R rs2229765 polymorphism favour longevity in male carriers of the homozygous A allele [196].

Forkhead box O3 (FOXO3) is a transcription factor that exerts a down-regulation activity on IGF-1 pathway, and its genetic variability is one of the most consistent longevity heritable factors in humans. Sequencing studies on this gene showed that the alleles linked to longevity reside in the 3' region [198–200].

In a study by Flachsbart et al. [201] the authors applied a combined candidate-gene sequencing – SNPs genotyping approach and identified variants linked with longevity in three North European populations., *i.e.* France, Germany and Denmark. In particular, two variants, rs4946935 and rs12206094, were identified, one of them (rs4946935) having been previously identified as longevity gene [198]. A functional validation of these two non-coding variants was performed, and the results show that the longevity-associated allele promotes the expression of the gene. In a broad meta-analysis on 30884 individuals of European ancestry [202] the authors studied the genetic association with circulating levels of IGF-1 and IGF binding protein–3. This original approach allowed the authors to establish a significant association with the circulating levels of IGF-1 and the rs2153960 in FOXO3 gene. A number of loci involved in growth hormone (GH) path also emerged, in particular showing significant associations for the IGF-1, IGFBP3, IGFALS, GHSRASXL2 and CESLSR2 genes, and many of the identified variants were also associated with elderly health traits and with longevity. Functional studies suggested the existence of an HSF1-FOXO3 axis in human cells that could be involved in stress response [203]. FOXO3 became physically connected, through chromatin looping, with 46 other genes on chromosome 6 thus forming an aging hub [199].

4.3.2.1. Cardiovascular risk. IGF1 is implicated in CVD and evidences suggest that IGF-1 has a vascular protective role for the treatment of chronic heart failure and its deficiency may cause CVD [204]. Moreover, IGF-1 has a pleiotropic action in hearts and cardiac activation of IGF-1 receptor (IGF-1R) protects from the detrimental effect of a high fat diet and myocardial infarction [205] . As far as we know, no evidence has been reported for FOXO3 and cardiovascular implications.

4.3.3. IL6

IL6, known as the cytokine of gerontologists [206], is a multifunctional cytokine that presumably plays its major role as a mediator of the acute phase of inflammatory responses and becomes detectable in advanced age and in conditions characterized by the presence of chronic inflammation. Elevated serum IL-6 levels are associated with significantly increased risk of morbidity and mortality in the elderly [207]. The previously-mentioned GWAS study on Chinese centenarians [95] showed that the minor allele of rs2069837 located in IL6 (chromosome 7p15.3) is the variant (intronic) most significantly (negatively) associated with longevity, being significantly less frequent among centenarians than middle-age individuals in Han Chinese (odds ratio = 0.61; P = 1.80×10^{-9}), and alone explaining about 1% of the variance of the variance in surviving to ages 100 + from middle-age. A study on 323 Italian centenarians identified a significant

association between rs1800795-IL-6 (-174 C/G at promoter locus) and longevity, and showed that GG homozygotes decreased in centenarians males, suggesting a sex-specific detrimental effect [208,209]. Other numerous studies showed the implication of SNPs located in IL6 in longevity [209–213].

The case of IL6 is peculiar as it constitutes a link between genetic susceptibility to inflammation, age-related diseases and longevity. However, it is to note that the above-mentioned SNPs are not closely linked to IL6 plasma levels as the SNPs that is better associated to circulating IL-6 levels are located in IL6 receptor (IL6R rs7529229, rs8192284, rs2228145), as demonstrated by a Mendelian randomization analysis in a study that showed that IL6R signalling seems to have a causal role in the development of coronary heart disease [214,215].

4.3.3.1. Cardiovascular risk Long-term increase of IL6 levels is associated with a high risk of coronary heart disease [216]. A recent study identified a genetic variant (s7529229) associated with increased IL6 levels circulating in the blood but decreased IL6R signaling. The authors investigated the effect of the variant comparing it with the effect of anti-IL6R drug (tocilizumab). The results showed that the variant was associated with the same biological changes as the inhibiting drug. The authors suggested that genetic studies in populations could be used more widely to help to validate and prioritize novel drug targets, and IL6R blockade could provide a novel therapeutic approach for coronary heart disease [215]. Also the G allele of -174 C/G polymorphism was found to be associated with increased risk of CVD and other age-related diseases such as Alzheimer's dementia and T2D [217–219].

4.3.4. SIRTUINS

The link between sirtuins and longevity can be attributed to discoveries in animal models (in particular in *C.elegans* and in Drosophila), and the sirtuin story dominated the agenda of several meetings on ageing. However in 2011 some researchers claimed that there was a technical problem: the study published in Nature demonstrated the lack of effects of sirtuin overexpression on aging after proper standardization for genetic background in both *C. elegans* and Drosophila [220].

In humans 7 sirtuin genes (SIRT 1-7) exist, but only few of them are involved in longevity, and studies on the effects of genetic variants in human sirtuins on lifespan are limited, and can be summarized as follows: (i) SIRT1 did not showed any genetic association with human longevity [221,222] but it influenced survival in T2D patients in interaction with dietary niacin and smoking [223]; (ii) SIRT3 has been associated with adaptations to nutrient stresses, such as fasting and calorie restriction. A study by Rose and colleagues in 2003 [224] found that in males the TT genotype (G477T) increased survival (p=0.0272), while the GT genotype decreases (p=0.0391) survival in the elderly. The second study investigated the frequency of VNTR polymorphism (72-bp repeat core) in intron 5 in 945 individuals (20-106 years) and found that the allele lacking enhancer activity was absent in males older than 90 years [225]. The third study is a meta-analysis of SIRT3 SNPs among Italian, French and German centenarians, and no robust positive association was found; (iii) SIRT6 is a chromatin-associated protein promoting heterochromatin silencing at repetitive DNA

sequences, repressing the activity of L1 retrotransposons (that become more active in somatic tissue during aging) thus increasing genome stability [226]. A study performed on the Iowa population found association of the SIRT6 SNP rs107251 minor allele homozygotes (TT) with a decreased lifespan of 5.5 and 5.9 years [227], confirming a previous study by Sorensen et al. about Danes longevity [228]. Negative results emerged in a study on longevity in Han Chinese centenarians[229]. Donlon and colleagues [230] tested 459 SNPs in 58 candidate genes, of which 47 genes were selected as the most differentially expressed in mice under calorie restriction, in a cohort of 440 subjects aged 95 years and over in comparison with controls. Among these genes SIRT7 and SIRT5 showed for the first time a positive association with longevity.

4.3.4.1 Cardiovascular risk- SIRT1 has a protective effect against CVD and through its deacetylase activity regulate exerts many functions, promotion of angiogenesis being one of them [231]. Different polymorphisms in SIRT1 gene have been associated to susceptibility to CVD [232] , blood pressure in Japanese [233]and carotid plaque [234]. Moreover, a study in the Iranian population found that the SIRT1 rs3758391-CC genotype was a risk factor for CVD and that carriers of this genotype were at 3- or 3.7-fold increased risk of CVD than subjects with the TT genotype, respectively [235], but further studies are needed to validate the role of SIRT1 genetic variations in cardiovascular impairment.

4.3.5 Somatic mutations and clonal hematopoiesis

All the above-mentioned genes reported the variability observed in the germline, however a further example of genetic variants affecting longevity and cardiovascular risk has been described. The paper of Jaiswal and colleagues [236] demonstrated that there are recurrent somatic mutations in genes involved in hematological malignancies. This condition is called “clonal haematopoiesis”, or “clonal haematopoiesis of indeterminate potential” (CHIP). Clonal haematopoiesis is a functional phenomenon in which a hematopoietic stem cell has acquired a survival and proliferative advantage. By whole-exome sequencing a common, age-related expansion of hematopoietic clones carrying recurrent somatic mutations, most frequently loss-of-function alleles in the genes DNMT3A, TET2, and ASXL1, was found. This condition has been shown to be associated with increased cardiovascular risk and reduced overall survival among persons with expanded clones, as compared with those without clones, with an effect much larger than that which can be explained by hematologic cancers alone. This somatic condition seems to play a crucial role in aging and such clones rarely accumulate in persons younger than 40 years of age, but are present in more than 10% of persons older than 70 years of age. Persons with such mutations in the absence of any other hematologic abnormalities have been defined as having CHIP. It is interesting to note that in centenarians the prevalence of CHIP is very low (around 2,5% in semisupercentenarians) [237]. These results shows that centenarians seems spared from the exponential increase of CHIP observed after the age of 70

years in previous studies, suggesting that the capability to avoid the clonal expansion of such mutations might have contributed in protecting centenarians from CVD.

4.4. Mendelian randomization in the study of the genetics of longevity

Mendelian randomization (MR) is a recent analytical method applied to observational study that aims to infer and examine causal effects when possible confounding factor may cause indirect association. However, it is to note that this approach is based on the assumption that the gene(s) under study is(are) not pleiotropic and thus it is likely not suitable for many genes like APOE [238]. An example is the recent study aimed at verify the association between genetically-predicted tryglyceride levels (rs662799 in APOA5 gene) and longevity in a Han-Chinese population [239]. The study started from the following observations: i) the rs662799-C allele was found to be associated with higher triglyceride levels; ii) triglyceride levels were also associated with longevity. Thus MR was applied to assess the possible causal link between genetically predicted (on the basis of rs662799) triglyceride levels and longevity. MR analysis revealed that genetically-predicted triglycerides were not associated with probability of longevity while serum triglycerides were observationally strongly associated with longevity [239]. A second study [240] analyzed three Dutch cohorts starting from observational studies in older subjects that did not show (or show inverse) correlation between cholesterol levels and mortality. In this study MR was applied starting from a genetic risk score (GRS) including 51 SNPs associated with LDL-C levels. Interestingly, the authors showed that individuals with a genetic predisposition for longevity are characterized by a lower LDL GRS, and concluded that a beneficial LDL GRS is associated with familial longevity.

5. HOST GENOME AND ITS INTERACTIONS

Longevity is not only the outcome of GxE interactions but it is the results of the interaction(s) between “genomes” (mtDNA, microbiome and nuclear DNA), environment and somatic mutations occurring during aging in all the genomes that reside in the human organism itself (referred as holobiont/metaorganism). Thus, the phenotype emerges from multiple levels of selection that operate simultaneously on the holobiont/metaorganisms [241], and the study of such complex interactions in longevity is still in its infancy. Somatic mutations occurring in the different genomes (Figure 10) are inherited from cell to cell and their frequency change during development, following evolutionary dynamics similar to those applied to population genetics (migration, drift, selection etc...), so that the term "somatic evolutionary genomics" has been recently proposed to indicate the study of such events during lifetime [242]. These mutations may cause variable genetic mosaicisms that could hide important implications for the study of all age-related modification (pathological and physiological) [242,243].

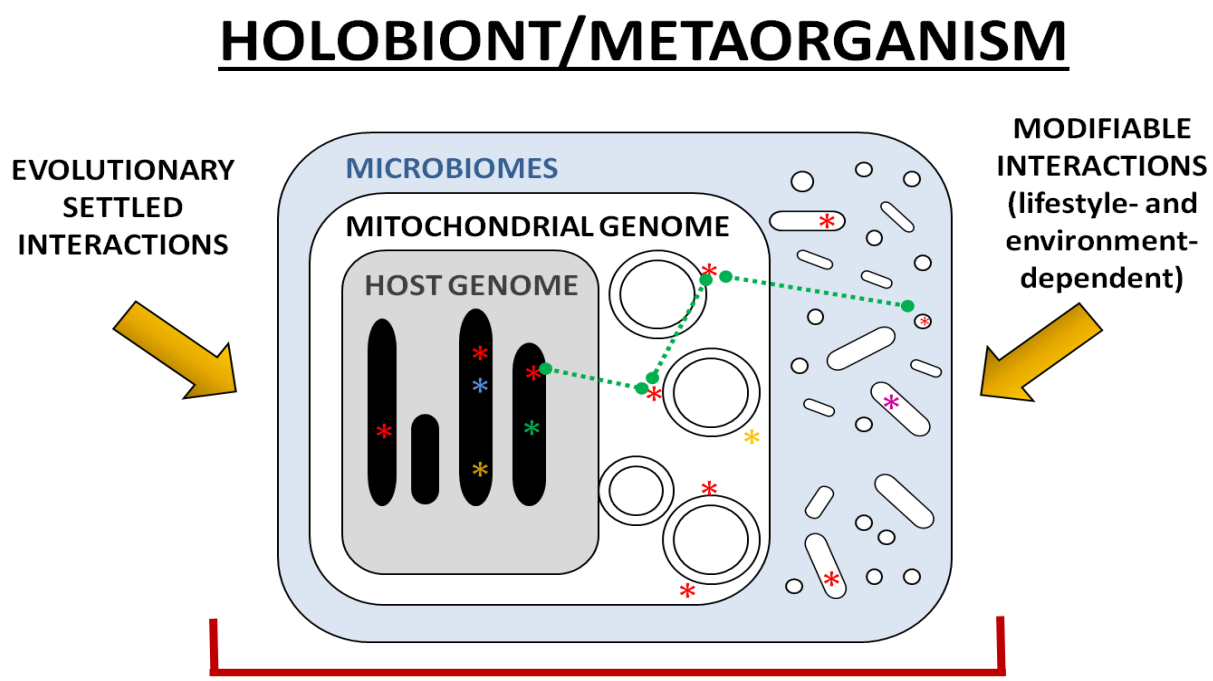

Figure 10. Holobiont and metaorganism are terms coined within the framework of ecology, evolution and zoology. *The genetic of holobionts is highly dynamic, as well as the interactions between the three genetics, i.e. the nuclear DNA (indicated in the figure as "host genome"), the mitochondrial genome and the microbiomes. These interactions are the results of evolutionary dynamics, and co-evolved (red asterisk and green line) to adapt to past environment or as a consequence of migration processes. Co-evolution is a complex process; some variants may affect the holobiont phenotype without a co-evolution with other genomes (indicated with asterisks with different colors). The temporal dimension of these interactions is reported (bottom). Somatic mutations may arise in the genomes during lifespan (from birth to death) in a tissue-specific way. Thus, with aging the phenotype of the holobiont is the result of a high number of interactions, some of them tissue-specific.*

5.1 mtDNA in longevity and its nuclear co-evolution

Mitochondria are the result of an ancient symbiotic event where a bacterium specialized in energy transformation has been integrated in a eukaryotic cell. As remnants of such event mitochondria have their own circular genome. In each mitochondria several copies of a small (16,569 base pairs) genome (mtDNA) is present. mtDNA contains genes involved in the functions of mitochondria, even if the vast majority of the genes necessary for mitochondria physiological operations are sparse in the nuclear autosomal chromosomes. This dislocation of information generates epistatic effects that are responsible for different mitochondrial and energy phenotypes that are supposed to be important also for the aging process [244].

mtDNA has been correlated to longevity in different studies. The first studies investigated the association between haplogroup classification and longevity. Using this approach the haplogroup J has been associated to longevity in Europe, Near East, Northern Italy, Ireland and Finland [245–247]. Functional studies showed that

mtDNA molecules belonging to haplogroup J produce less ATP and ROS [248], and these associations seems to be population specific. In Japanese centenarians, the haplogroup D (characterized by variations in the complex I, such as haplogroup J) is at high frequency [249,250]. However, these associations seem to be highly population-specific, and this could be the cause of the absence of association described in many studies [251–253]. New approach integrating genome-wide data on autosomic DNA to identify admixture events may help in the interpretation of mtDNA variability in centenarians. However, the studies on haplogroups are only part of the possible role of mtDNA in longevity. For this reason mtDNA studies started to focus not only on the analysis of haplogroups but on the whole mtDNA sequence. The above-mentioned GEHA study analyzed 2200 nonagenarians belonging to 90+ sibpairs and the same number of younger controls, and of these samples 650 ultra-nonagenarians (and an equal number of controls) were completely sequenced. The study revealed that mutations in subunits of the OXPHOS (Oxidative phosphorylation) complex I had a beneficial effect on longevity, while the simultaneous presence of mutations in complex I and III (which also occurs in J subhaplogroups involved in LHON) and in complex I and V seemed to be detrimental. The authors also showed differences among the different populations and suggested that, owing to since the very small effect of these mutations a larger sample size is likely needed to identify more significant associations [254] . One interesting study regarding mitonuclear interaction focused on TOMM complex [255]. rs2075650 located in TOMM40 was found to be associated to longevity [123], likely affecting the relative configuration of the partners of TOMM complex, resulting in a more efficient clearance of damaged mitochondria. Mitonuclear interactions between HV haplogroup of mtDNA and nuclear DNA have been observed associated to susceptibility to T2D in Ashkenazi Jews, indicating also a potential relation with age-related diseases [256].

5.2. Gut microbiota in longevity and its nuclear co-evolution

In the last decades the numbers of study on gut microbiota increased exponentially owing to the possibility to influence its composition following external intervention. Gut microbiota plays an important role in human health and diseases, including age-related diseases such as CVD [257].

5.2.1 Microbiotas within an evolutionary perspective

The gut microbiota is a sophisticated ecosystem sensible to environmental stimuli, host genetics and physiological state (e.g. diet, age, among others), in which the different species are in a flux of continuous, ecological reshaping. It is now widely accepted that it is necessary to apply models derived from theoretical ecology to understand and predict microbiota dynamics [258,259]. An interesting view regarding the host-microbiotas coevolution come from a perspective by Foster et al [260]. In this paper the authors started from the idea that the far-reaching holobiont concept (Figure 10), might be misleading if applied simplistically to the host-microbiota coevolution analysis. The authors suggest that the holobiont concept bias the evolutionary thinking toward a simplified conceptualization where host and microbiotas would act in view

of common interests. To obtain effective evolutionary models the authors proposed that host and each microbial species has to be considered as an independent evolutionary object that reacts and evolves according to a Darwinian dynamics, thus undergoing potentially divergent selective pressures. This perspective introduces evolutionary tension in the holobiont system that can be useful to understand the capability of the microbiota ecosystem to respond to a variety of stimuli. A major issue in the study of host-microbiotas coevolution in humans is related to the profound changes introduced in the last century with the sanitation of the anthropological environment. According to the "hygiene hypothesis" the new "clean" and rather sterilized human microenvironment, together with the introduction of antibiotics, caesarean delivery and changes in nutritional habits, involved a drastic reduction of microbiota's diversity [261] . Such changes are likely too recent to allow the host genetics to adapt, thus leading to a sort of mismatch, likely representing a common source of dysbiosis, with far reaching consequences for aging, age-related diseases and longevity. Among the microbiotas of the human body the one present in the gut (gut microbiota, GM) is the largest and more complex, as well as the one that has been more thoroughly studied. GM plays a pivotal role in host metabolism homeostasis and immune system maturation, functioning, renewal and stability. Altered GM composition has been found in several pathological conditions such as obesity, T2D and autism, among others [262]. In a milestone study from Xie H et al. [263], the authors analysed the GM composition of 250 monozygotic and dizygotic twin pairs and showed that host genetic background actively influences GM ecosystem. The authors also reported that with age the GM concordance tend to decrease within couples. Overall, it emerged that a significant portion of the phylotypes associated to diseases are not under genetic control, but are environmentally-determined, indicating that clinical interventions to restore the physiological GM ecology are likely not impaired by host genetic background.

5.2.2 GM and aging

There is a wide consensus on the fact that a lower GM biodiversity is observed during aging together with a concomitant increase of pro-inflammatory bacteria called "pathobionts" that usually are present at low concentration in healthy individuals [264]. However, population-specific dynamics shape this process and for example the study of Biagi and colleagues [264] demonstrated that the Bacteroidetes proportion remained unchanged in elderly Italians, whereas Bacteroidetes proportion strikingly increased during aging considering Irish people [265]. Extreme longevity has been characterized in term of gut microbiota, and loss of genes for short chain fatty acid production (considered anti-inflammatory GM metabolites) was observed. Recently Biagi et al., provided for the first time the phylogenetic microbiota analysis of semi-supercentenarians, in comparison to adults, elderly, and centenarians, thus reconstructing the longest available human microbiota trajectory along aging[266]. A continuous remodelling of GM lifelong emerged, and GM of centenarians and semi-supercentenarians showed changes that accommodate opportunistic and allochthonous bacteria as well as an enrichment and/or higher prevalence of health-associated groups

(e.g., Akkermansia, Bifidobacterium, and Christensenellaceae)[266]. It is interesting to note that Christensenellaceae are among the most heritable microbes [267] . As reviewed by Goodrich et al. [267], the heritability of gut microbiotas revealed a subset of microbes whose abundances are partly genetically determined by the host, but the identification of human genetic variants associated with microbiota composition has proven challenging, but the study of the interactions among nuclear genotype, environment, and the microbiotas is urgently needed owing to its capability to identify possible new contributors to healthy aging, longevity and its genetics, as also suggested by the evolutionary biology field. Microbiota in fact is at the intersection between metabolic, immunological and neuronal processes as recently described [268,269]. Interestingly, this interkingdom communication is supported by the fact that genes involved in innate and adaptive immunity shape the gut microbial community. It has been demonstrated that the different classes of dietary lipids (experiments in mice fed lard and mice fed fish oil) affect the GM composition that, in turn, contributes to white adipose tissue (WAT) inflammation, insulin sensitivity and Toll-like receptor (TLR) activation. These interactions between gut microbiota and saturated lipids promotes WAT inflammation and it is independent of adiposity [270]. Moreover, the interaction between nuclear DNA and gut microbiota is also evident in a study on *C. elegans* daf-2 mutants that live longer due to a slower rate of aging (leading to extension of healthspan) and due to an increased ability to resist death due to bacterial colonization (leading to extension of decrepitude) [271].

6. PERSPECTIVES

6.1. Genome editing in aging research

A major contribution to evaluate the effect of specific putative longevity genetic variants is provided by the recent development of genome editing techniques based on the molecular machineries constituted by zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN) and clustered regulatory interspaced short palindromic repeat (CRISPR)/Cas9-based RNA-guided DNA endonuclease. Such techniques allow printing with high precision specific mutations in animal and in vitro pre-clinical models to test the effect of genetic variants in iso-genic cells or organisms. The in depth description of such protocols and techniques is out of the scope of the present paper and a broad and detailed description of both genome and epigenome editing opportunities can be found in Keung et al., Gaj et al and Suh et al [272–274]. Here we like to stress some aspects: i) such techniques will allow to assess the biological effect of rare mutations and of epistatic effects of combined genetic variants; ii) even more interesting could be the combined analysis of genetic and epigenetic genome editing. A combined approach of these two techniques could provide innovative information on: a) allele timing, b) neutralizing or modifying effects of epigenetic modification; c) organ- and tissue-specific epigenetic aging rate effects on the phenotypic expression of candidate mutations.

6.2. Inclusion of environmental, social and cultural variable in the study of the genetics of longevity

Culture normally evolves more rapidly than genes, creating novel environments that expose genes to new selective pressures or amplify the pre-existing ones. Counteractive niche construction may help also to explain the difficulties of genetics studies to identify large portions of the genetic basis of common, complex traits in humans. Population genetics models and anthropologists demonstrated that cultural processes profoundly shape human evolution, and that the genetic background records all the major cultural changes occurred during human evolution (*i.e.* milk usage, domestication of plants, agriculture, changing climates, social intelligence, language, cooking *etc*.). Cultural practices modified the environmental conditions that in turn led to changes in allele frequencies, as demonstrated by recent acceleration observed in mutation accumulation of certain regions, and some traits have an high level of correlation with cultural history rather than ecology. Thus, an ecological approach of the study of the genetics of human longevity needs to incorporate an integrated view of possible cultural selective pressure [275]. This process is challenging because two main issues exist in the field: 1) there are still analytical challenges in scanning for selection on genetic data; 2) lack of the interdisciplinary expertise that makes possible to integrate cultural data in the genetic analysis. Especially in observational study it is almost impossible to collect accurate data on the past. The use of new omics such as epigenetics may represent a molecular mechanism to recode important information on environmental pressure of external and internal environment.

6.3. The use of biological age phenotype in the genetics of longevity

Recently biomarkers of biological *versus* chronological age have been identified [276–278] and an increasing number of investigations showed that such tools are effectively capable to catch the aging rate at the individual level, which in turn correlates with relevant health parameters in the elderly. These studies show that people who are or will be affected in coming years by age-related diseases tend to present a DNA methylation signature of *accelerated* aging, and studies on human model of accelerated aging such as Down's Syndrome persons showed a clear signature of accelerated aging in circulating cells and in brain [31,279]. Conversely, when these markers were applied to centenarians and their offspring a signature of *decelerated* aging emerged [280,281]. Besides whole genome DNA methylation changes, among the most promising markers of biological age we like to mention, N-glycans profiling [282–285] as well as markers derived from biochemical and anthropometric measurements such as hand grip, chair stand and lung capacity [286]. Particularly promising for the assessment of biological age in individuals is the combination the above-mentioned markers, possibly with the integration of classical biochemical and haematological markers (HDL, LDL, TG, glucose, insulin, albumin, urea, number of leukocytes, presence of anemia, among others) and longitudinal medical data (drugs assumption, hospitalizations). Within this frame it is of particular

interest the growing idea to generate a pro-inflammatory biological age marker capable to assess the inflammaging status of elderly subjects [287].

Thus, biological age represents an innovative phenotypic character that should be integrated in the study of the genetic of longevity and of age-related diseases. In particular, biological age should complement chronological age data in the control cohorts, which will become progressively more heterogeneous in terms of health status with increasing age. In other words, the concept of healthy control becomes blurred with age and likely misleading, and biological age will integrate the rate of age phenotype that is known to be accelerated in age-related diseases and decelerated in longevity. Moreover the identification of genetic variants linked to accelerated/decelerated aging may be crucial to translate the results of study on extreme longevity in the general population.

6.4. Interventions

Many studies on pharmacological interventions have been performed in animal models to extend/improve lifespan but the possible role exerted by the genetic background has been relatively neglected. Until now the only intervention that is spreading also in humans is calorie restriction. Data from animal models (invertebrates and rodents) demonstrated that both chronic dietary restriction (DR) and genetic mutations in nutrient and growth signaling pathways can extend longevity by 30–50% and reduce many age-related diseases including CVD [288]. A meeting held in 2013 [289] contributed by the major experts in the field supported the idea that a strategy to translate these results to humans is to intervene with drugs targeting nutrient-response pathways and mimicking the effects of DR, that are more practical, realistic, and safe than drastic dietary interventions. The most promising strategies taken into account were the following: pharmacological inhibition of the GH/IGF-1 axis, protein restriction and Fasting Mimicking Diets, pharmacological inhibition of the TOR -S6K pathway, pharmacological regulation of certain sirtuin proteins and the use of spermidine and other epigenetic modulators, pharmacological inhibition of inflammation and chronic metformin use. More details are reviewed by Longo and colleagues [289]. We surmise that it is urgent to set up studies capable of assessing the role that the three genetics (nuclear, mitochondrial and GM) can play regarding responsiveness to these and other intervention strategies at the individual level.

7. Conclusions

An innovative road map in the field of the genetics of longevity has been described in this review through the lens of ecological and evolutionary dynamics in order to better understand the individual aging trajectories assumed as highly context-dependent. First of all, a reappraisal of the definition of longevity, based on stringent demographic criteria and potentially applicable to all populations, and of the definition

of the control group was pursued. The genetics of human longevity has been presented on the basis of strong evidence that both human body and environment are not static, but underwent in the past and still undergo a complex reciprocal remodelling [2]. Such dynamics has a strong impact on aging rate and quality and length of life (longevity). The available literature on the genetics of human longevity has been critically scrutinized assuming that all factors contributing to the individual's ecological space - such as sex, individual (immuno) biography, family, population ancestry, social structure, economic status and education) need to be taken into account as major variables for disentangling the complex GxE interactions responsible for aging and longevity in humans. Within this framework, the strength and limitations of the most powerful tools used in the genetics of longevity, such as GWAS and whole-genome sequencing - have been discussed, paying particular attention to genes and regions emerged from such studies, such as APOE, FOXO3, IL-6, IGF-1, Chromosome 9p21 and their role in CVD. Moreover, available data on the interactions between the three genetics of human body (nuclear, mitochondrial and GM) have been reviewed, taking into account the recent data on the fundamental role of the mitochondria and the emerging pervasive role of microbiotas - and particularly of GM in the aging process.. We surmise that such a global approach should also include somatic mutations that occur lifelong in the above-mentioned genomes, thus creating always new interactions. In conclusion, we support that the integration of the above-mentioned data are crucial, and that evolutionary and ecological considerations should guide this process for a better understanding of the genetics of longevity, particularly in humans. This approach should also contribute to a better integration of the data collected in different research fields (such as epidemiology, genetics, anthropology sociology, environmental studies among others) as supported by recent and relevant papers in the field of evolutionary medicine published in Lancet in 2017 [290–292].

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Competing interests

The authors declare no competing interests.

TABLES

Table 1. GWAS on longevity and aging. PUBMED ID, name of the first author, year of publication, journal, type of trait analyzed, number of sample and population and platform is reported.

PUBMED ID	FIRST AUTHOR	DATE	JOURNAL	STUDY	DISEASE/ TRAIT	INITIAL SAMPLE SIZE	PLATFORM
NA	Yi Zeng	2018	JAMA	Sex Differences in Genetic Associations with Longevity	Longevity	Han Chinese 564/1,614 male/female centenarians and 773/1,526 80 male/female middle-aged controls.	5.6 million SNPs (900,015 genotyped SNPs and 4.8 million imputed SNPs) (Illumina HumanOmniZhongHua-8 BeadChips)
29227965	Pilling	2017	Aging US	Human longevity: 25 genetic loci associated in 389,166 UK biobank participants	Parental longevity [mother's age ≥90 years, father's ≥87 years),]	UK Biobank: 451,447 participants Health and Retirement Study (HRS): 15,708 Wisconsin Longitudinal Study (WLS): 9012	Illumina, Affymetrix, Imputation of 39,235,157 genetic variants
29030599	Joshi PK	2017	Nat Commun	Genome-wide meta-analysis associates HLA-DQA1/DRB1 and LPA and lifestyle factors with human longevity.	Parental lifespan	up to 586,626 European ancestry individuals, up to 19,433 African ancestry individuals	Affymetrix, Illumina [at least 13,643,373] (imputed)
28748955	Mc Daid	2017	Nat Commun	Bayesian association scan reveals loci associated with human lifespan and linked biomarkers	Parental lifespan	116,279 individuals in the UK Biobank	
27816938	Tanaka T	2017	J Gerontol A Biol Sci Med Sci	Genome-wide Association Study of Parental Life Span.	Parental longevity (at least one long-lived parent)	1,140 European American individuals with at least one long-lived parent, 3,894 European American individuals, 137 African American individuals with at least one long-lived parent, 545 African American individuals	Illumina [~ 2,500,000]
27029810	Joshi PK	2016	Nat Commun	Variants near CHRNA3/5 and APOE have age- and sex-related effects on human lifespan.	Parental Lifespan	138,536 British ancestry mothers, 133,545 British ancestry fathers	Affymetrix [73,355,667] (imputed)
27015805	Pilling LC	2016	Aging (Albany NY)	Human longevity is influenced by many genetic variants: evidence from 75,000 UK Biobank participants.	Parental longevity (mother's age at death)	52,776 middle-aged British individuals	Affymetrix [9,658,292] (imputed)
26912274	Zeng Y	2016	Sci Rep	Novel loci and pathways significantly	Longevity (100 years and older)	2,178 Han Chinese ancestry centenarian cases, 2,299 Han Chinese	Illumina [5,595,657] (imputed)

				associated with longevity.		ancestry middle-age controls	
26677855	Fortney C	2015	Plos Gen	Genome-Wide Scan Informed by Age-Related Diseases Identifies Loci for Exceptional Human Longevity	Longevity	Discovery: New England Centenarians study (801 centenarians) and 914 controls and 90PLUS (7330 individuals 90 or older and 16121 young controls (age 65 or less)	Illumina [243,980]
25918517	Yashin A	2015	Front Genet	Genetics of aging, health, and survival: dynamic regulation of human longevity related traits.	Lifespan	After applying the QC procedure 1111individuals	Affymetrix [429,783]
25199915	Broer L	2014	J Gerontol A Biol Sci Med Sci	GWAS of Longevity in CHARGE Consortium Confirms APOE and FOXO3 Candidacy.	Longevity (90 years and older)	6,036 European ancestry cases, 3,757 European ancestry controls	Affymetrix, Illumina [2,500,000] (imputed)
24688116	Deelen J	2014	Hum Mol Genet	Genome-wide association meta-analysis of human longevity identifies a novel locus conferring survival beyond 90 years of age.	Longevity (90 years and older)	5,406 European ancestry cases, 15,112 European ancestry controls	Illumina [2,470,825] (imputed)
23286790	Beekman M	2013	Aging Cell	Genome-wide linkage analysis for human longevity: Genetics of Healthy Aging Study	Longevity (90 years and older)	2118 nonagenarian Caucasian sibling pairs	Illumina HumanLinkage-12 Genotyping BeadChip [6,090]
22279548	Sebastiani P	2012	Plos One	Genetic Signatures of Exceptional Longevity in Humans		Discovery 801 subjects enrolled in the New England Centenarian Study (NECS) 95 to 119 years (median age 104 years and 914 genetically matched controls.	
21782286	Walter S	2011	Neurobiol Aging	A genome-wide association study of aging.	Aging (time to death)	25,007 European ancestry individuals	Affymetrix, Illumina [~ 2,500,000] (imputed)
21740922	Nebel A	2011	Mech Ageing Dev	A genome-wide association study confirms APOE as the major gene influencing survival in long-lived individuals.	Longevity	763 European ancestry individuals (mean age=99,7), 1,058 European ancestry individuals (mean age 60,2 years)	Affymetrix [664,472]
21612516	Malovini A	2011	Rejuvenation Res	Association study on long-living individuals from Southern Italy identifies rs10491334 in	Longevity	Southern Italy 582 individuals (age range 90–109 years) and 784 young control individuals (age range 18–45	Illumina [298,715]

				the CAMKIV gene that regulates survival proteins.		years)	
20834067	Yashin AI	2010	Aging (Albany NY)	Joint influence of small-effect genetic variants on human longevity.	Longevity	1,173 individuals (Framingham Heart Study)	NR [~ 550,000]
20304771	Newman AB	2010	J Gerontol A Biol Sci Med Sci	A meta-analysis of four genome-wide association studies of survival to age 90 years or older: the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium.	Longevity	1,836 European ancestry long-lived individuals (survival to age 90 years or older), 1,955 European ancestry controls (range 55-80 years)	Affymetrix, Illumina [2,287,520] (imputed)
17903295	Lunetta KL	2007	BMC Med Genet	Genetic correlates of longevity and selected age-related phenotypes: a genome-wide association study in the Framingham Study.	Aging (age at death)	1,345 individuals from 330 families (Framingham Heart Study)	Affymetrix [70,897]

Table 2. Overview of the genetics of human longevity. The first column reports the data obtained from the published studies and the second column describes possible guidelines for future researches.

Lessons from the past	Guidelines for the future
THE PROPER DEFINITION OF PHENOTYPES TO INCLUDE IN GENETICS STUDIES	
1. Longevity is defined according to demographic criteria (one percentile survival) 2. Controls can include healthy individuals, familial members and also individuals with age-related diseases as a paradigm of accelerated aging 3. Longevity is different between males and females	1. Avoid definition of longevity on the basis of self reported data 2. Biological age need to be included in the definition of controls 3. Genetics analysis need to performed separately for male and females when sample size is high enough
THE ECOLOGICAL PERSPECTIVE	
1. (Immuno)biography and somatic mutations are dynamic processes that change during aging 2. The familial history shapes (directly or indirectly) different gene-environment interactions 3. Geography, cultural aspect, birth cohort and socio-economic dynamics change gene-environment interactions 4. Evolutionary dynamics (migration and natural selection) shape the genetic background of different populations (such as APOE) --> the genetic of longevity is population specific	1. The genetic of longevity is not a static concept and a protective/risk allele need to be integrated with other data that characterized the individual in a given time 2. Identify statistical model to evaluate the direct effect of inherited variants but also the indirect effect of variants carried by parents 3. Define risk/protective allele according to the birth cohort, geography and socio-economic status 4. An evolutionary medicine and the study of genetic structure of each population will help in identifying population specific genes and pathways correlated with longevity
STATE OF THE ART	
1. Variants involved in age-related diseases were identified in many GWAS/gene candidate studies of longevity (such as APOE, chr9p21) 2. Genetic risk factors are age-specific and play a major role in the process of remodeling 3. GWAS highlighted that common and population specific genes involved in longevity. 4. Longevity depends on small-effect alleles (and association signals tend to be spread across most of the genome).The GWAS threshold of 10^{-8} may not be valid for longevity. 5. Gene candidate studies showed the role many genes involved in longevity: APOE/TOMM40, chr9p21, chr15q33.3 FOXO, IGF1, IL6, SIRTUINS among others	1. Patients with age-related diseases have to be included in the study of human longevity (following the geroscience perspective) and centenarians need to be included in the study of age-related diseases as a group of super-control 2. Complex allele timing and antagonistic pleiotropy dynamics help in identifying the role of the genetic variants in different environment 3. To perform study where centenarians and controls are homogeneous in term of population genetic structure 4. Include new methods of analysis such as pathway analysis or as suggested by Boyle and colleague using an "omnigenic approach" mapping cell-specific regulatory networks. 5. Evaluate the effect of these genes by genome editing
HUMAN IS A METAORGANISM	
1. Host genome interacts and coevolved with mtDNA 2. Host genome interacts and coevolved with gut microbiota	1. Characterize mtDNA variability and heteroplasmy and the nuclear-mitocondria interactions 2. Focus on the interaction between the "three genetics" (host, mtDNA, microbiome) usually considered separately

Table 3. The complexity of APOE gene variants

MACRO AND MICRO-EVOLUTIONARY BACKGROUND
• APOE-e4 is the ancestral allele typical of modern humans; • APOE-e2 and APOE-e3 evolved around 200,000-300,000 years ago; • APOE-e4 is associated with improved cognitive function in Amazonian forager-horticulturalists with a high parasite burden; • High frequency of *APOE*-e4 was maintained in the population because it confers a beneficial effect during infections and in the environment where pathogens were more prevalent and the first cause of mortality (data on a rural Ghanaian population);
TODAY GEOGRAPHIC DISTRIBUTION
• APOE-4 showed a cline in Europe (from 20% in North Europe to 6/7% in Southern Europe) • e4 allele in human populations follows a sort of U-shaped latitudinal trajectory --> high frequencies (up to approximately 40–50% of the population) in equatorial and high latitudes and low frequencies in middle latitudes
INVOLVEMENT IN PHYSIOLOGICAL TRAITS
• Lipid metabolism, regulating production, conversion • APOE-e4 is associate to high cholesterol • clearance of lipoproteins • it is expressed in different tissues, macrophage, adipose tissue, nervous system and liver • APOE-e4 allele had significantly higher levels of mean luteal progesterone than women with genotypes without ApoE4, which indicates higher potential fertility • In mice APOE-4 carriers are more vulnerable to a dietary deficiency in omega-3 fatty acids and cognitive decline • High levels of physical activity reduce disease risks in ε4 carriers • Spatial memory of transgenic mice carrying human forms of these proteins and find that it is impaired in mice with apoE4 but not those with apoE3
INVOLVEMENT IN PATHOLOGICAL TRAITS
• APOE-e4 is associated to Alzheimer's disease (AD) and cardiovascular diseases (APOE-e4) • People with APOE-e3 o APOE-e2 have later AD onset than APOE-e4
LONGEVITY
• APOE-e4 is negatively associated to longevity in many studies and meta-analysis on human longevity. • The gene variants identified in these regions are located also in TOMM40 and APOC1.

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