Title: The genetics of human longevity: an intricacy of genes, environment, culture and microbiome

Authors: Serena Dato, Giuseppina Rose, Paolina Crocco, Daniela Monti, Paolo Garagnani, Claudio Franceschi, Giuseppe Passarino

PII: S0047-6374(16)30255-X
DOI: http://dx.doi.org/doi:10.1016/j.mad.2017.03.011
Reference: MAD 10945

To appear in: *Mechanisms of Ageing and Development*

Received date: 4-11-2016
Revised date: 4-3-2017
Accepted date: 30-3-2017

Please cite this article as: Dato, Serena, Rose, Giuseppina, Crocco, Paolina, Monti, Daniela, Garagnani, Paolo, Franceschi, Claudio, Passarino, Giuseppe, The genetics of human longevity: an intricacy of genes, environment, culture and microbiome.Mechanisms of Ageing and Development http://dx.doi.org/10.1016/j.mad.2017.03.011

The genetics of human longevity: an intricacy of genes, environment, culture and microbiome

Serena Dato*[a], Giuseppina Rose[a], Paolina Crocco[a], Daniela Monti[b], Paolo Garagnani[c], Claudio Franceschi[c], Giuseppe Passarino[a].

[a]*Department of Biology, Ecology and Earth Science, University of Calabria, 87036, Rende, Italy*

[b]*Department of Biomedical, Experimental and Clinical Sciences Mario Serio, Viale Morgagni, 50, 50134 Florence, Italy*

[c]*Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Institute of Neurological Sciences of Bologna, Bellaria Hospital, Via Altura 3, 40139 Bologna, Italy*

*Corresponding author

Serena Dato
Department of Biology, Ecology and Earth Science,
University of Calabria,
87036, Rende Italy.
Email serena.dato@unical.it

HIGHLIGHTS

- Longevity is a highly plastic trait, a remodelling process comprising modification of internal and external stimuli.
- Genetic contribution to longevity underlies half a hundred of genes associated with long lifespan in different populations
- Lifestyle changes, social and cultural factors can account for survival differences among individuals.
- Microbioma is an example of complex interaction organism-environment
- New approaches analysing gene-environment and gene-gene interactions can help us to understand interplays acting in determining the chance to survive at extreme ages

ABSTRACT

Approximately one-quarter of the variation in lifespan in developed countries can be attributed to genetic factors. However, even large population based studies investigating genetic influence on human lifespan have been disappointing, identifying only a few genes accounting for genetic susceptibility to longevity. Some environmental and lifestyle determinants associated with longevity have been identified, which interplay with genetic factors in an intricate way. The study of gene-environment and gene-gene interactions can significantly improve our chance to disentangle this complex scenario. In this review, we first describe the most recent approaches for genetic studies of longevity, from those enriched with health parameters and frailty measures to pathway-based and SNP-SNP interaction analyses. Then, we go deeper into the concept of "environmental influences" in human aging and longevity, focusing on the contribution of life style changes, social and cultural influences, as important determinants of survival differences among individuals in a population. Finally, we discuss the contribution of the microbiome in human longevity, as an example of complex interaction between organism and environment. In conclusion, evidences collected from the latest studies on human longevity provide a support for the collection of life-long genetic and environmental/lifestyle variables with beneficial or detrimental effects on health, to improve our understanding of the determinants of human lifespan.

Keywords: genetics determinants of longevity, microbiota-host cross talk, genomic instability, miRNA, lncRNA, contribution of lifestyle to longevity

1. Introduction

The last two decades, probably due to the continuous increase of lifespan in western societies and the consequent growing of the elderly population, have witnessed a blooming of studies on the factors determining the quality of aging. In particular, in the biomedical fields, the main aim of researchers has been the search of biological and molecular factors able to promote a healthy aging and reach longevity. In this frame, a great attention has been devoted to the analysis of the role played by the genetic factors in determining healthy aging and longevity. The lack of evolutionary established mechanisms linking genes to age-related traits makes the problem of genetic susceptibility to health span inherently complex (Kulminsky et al., 2015). Within this scenario, the analysis of families of long-lived subjects indicated that healthy aging and longevity have a hereditary component. In particular, the reconstruction of long-lived's sibship (Perls et al, 2002a; Schoenmaker et al, 2006) and the comparison of their survival curves with cohort members born in the same geographical area, showed that brothers and sisters of long-lived subjects had a clear survival advantage (at any age) with respect to the general population. Siblings of centenarians have also lower risk of suffering from major age-related pathologies, such as cardiovascular diseases, diabetes and cancer, when compared to appropriate selected controls from the same population (Terry et al, 2003); as well, offspring of centenarians are healthier than age-matched controls belonging to the same demographic cohorts but not born from long-lived parents (Bucci et al, 2016), suggesting that the biological components of longevity might largely overlap with the biological components of healthy aging.

Further studies compared the survival function of brothers of centenarians with those estimated for their brothers in law, that is with the men who married their sisters: these subjects were supposed to share the same familiar environment, so may help to distinguish the genetic from the "familiar" effect (Montesanto et al, 2011). This approach showed that the survival advantage of siblings of long-lived subjects was not completely shared by their brothers in law, despite they shared the same environment for most of their life. This suggested that beyond the family environment, there are genetic factors influencing survival and, consequently, lifespan. The genetic component of lifespan in humans has

also been analyzed by comparing the age of death of monozygotic and dizygotic twins. This has allowed to estimate that about 25 % of the variation in human longevity can be due to genetic factors and indicated that this component is higher at older ages and more important in males than in females (Hjelmborg et al, 2006; Herskind et al, 1996; Skytthe et al, 2003). The rest of phenotypic variation associated with the trait is influenced by epigenetic and environmental factors, whose characterization is becoming more and more important in association studies of human longevity.

2. The search for genetic determinants of longevity

Although there is compelling evidence for a genetic contribution to longevity, identification of specific genes that robustly associate with longevity has been a challenge (Christensen et al, 2006), and several approaches were carried out to highlight the genetic predisposition to long life. Table 1 summarizes the principal methodologies applied for the analysis of the genetic component of human longevity. The simplest strategy is a candidate approach, where a set of SNPs flanking a gene which is believed to be involved in longevity are tested for their association to longevity in long-lived subjects and in younger geographically matched controls. The underlying hypothesis is that the alleles predisposing to longevity are more frequent in long-lived subjects (such as centenarians) than in the younger subjects, because those carrying alleles predisposing to longevity are likely to survive to the subjects not carrying them and thus, as cohort grows older these alleles become more and more frequent (Perls et al, 2002b). Candidate genes have been selected either on the basis of pathways correlated to age related diseases (such as inflammation, oxidation, lipid metabolism) or on the basis of data gathered from the analysis of model organisms selected for extreme longevity (see Dato et al, 2013 for a list of genes consistently associated with longevity). Among the many genes which have been studied in different populations, only *APOE* and, to some extent but not always, *FOXO3A* were consistently replicated in different populations. *APOE* was initially investigated for the risky ε4 allele for Alzheimer's disease and coronary artery disease (Schächter et al, 1994; Davignon et al, 1988), and it was subsequently replicated in candidate, linkage and genome-wide association studies

(GWAS) (Gerdes et al, 2000; Bathum et al, 2006; Christensen et al, 2006; Deelen et al, 2011; Nebel et al, 2011; Beekman et al., 2013; Deelen et al., 2014); *FOXO3* has been candidated for its homology with the corresponding genes influencing lifespan in experimental organisms, from *C. elegans* to Drosophila and mice (Murphy, 2006; Willcox et al, 2008), but was replicated only in single-SNP studies and not in GWAS (Anselmi et al, 2009; Flachsbart et al, 2009; Soerensen et al, 2010; Deelen et al., 2014).

2.1 The first "good" SNP associated with human longevity

A recent meta-analysis on the genetics of human longevity (Deelen et al., 2014) showed genome-wide significant association with survival to 90 years of age at two loci: i) one on chromosome 19q13.32 (P = 3.40 x 10-36), i.e. the previously identified *TOMM40/APOE/APOC1 l*ocus; ii) a new locus on chromosome 5q33.3 (OR = 0.64, P = 4.09 x 10-21). Both loci associate with survival but at opposite directions. The intergenic region on chromosome 5q33.3 is the first GWAS-identified locus promoting human longevity. In particular, rs2149954 (T) in chr5q33.3 locus is associated with: lower all-cause mortality, lower mortality risk for CVD, a decreased risk for coronary artery disease, lower diastolic and systolic blood pressure. This chromosomal region deserves further attention, particularly for the presence of *EBF1* gene associated with adipogenesis and in diseases related to chronic psychological stress (Singh et al., 2015).

2.2 The problem of control: new approaches to tackle the complexity

The analysis of contrasting results obtained for many genes candidate to have a role in human aging have highlighted the complexity of the genetic component of longevity. The main problem of the case-control approach for studying the genetics of longevity is the age of the control cohort. The allele frequency of SNPs as a function of age may not change in a simple monotonic fashion: as observed for the first time on the *APOB* gene (De Benedictis et al., 1998) and confirmed by several studies (Bergman et al, 2007; Huffman et al, 2012), alleles may show a U-shaped pattern such that young

and very old people have similarly high allele frequencies, but 80 year olds have a lower level. In these cases, the choice of the control cohort would affect whether or not an allele is significantly different in centenarians.

Difficulty to find consistent associations with survival to ages ≥90 years may also be due to the fact that longevity represents the sum of multiple complex traits, with heterogeneous genetic underpinnings, or instead because of the effect of rare variants, not captured by genome-wide genotyping and imputation methods used for common variants. To overcome this obstacle, some new approaches have been recently proposed. One of the genetic mechanisms which could favour extreme longevity involves the lack of certain risk alleles that predispose to common diseases, including coronary artery disease, Alzheimer's, high cholesterol and chronic kidney disease. Among the methods which use disease-related variants to find genetic determinants of extreme longevity is the informed GWAS (iGWAS), proposed by Fortney and collaborators (Fortney et al, 2015). The method takes advantage of prior knowledge from large studies of age-related diseases, in order to narrow the search for SNPs associated with longevity. Several whole genome sequencing or GWAS analyses showed that the genomes of centenarians contain many risk variants for disease (Beekman et al., 2010; Sebastiani et al, 2011; Freudenberg-Hua et al, 2014), and that the overall count of disease-associated variants does not significantly differ for centenarians versus controls (Beekman et al, 2010; Sebastiani et al, 2012). As argued by Fortney, while this implies that many disease variants may not be depleted in centenarians, there may be additional variants enriched in disease and also present in centenarians, probably having pleiotropic functions on different traits (Garagnani et al, 2013; Postmus et al, 2015). In order to discover these longevity variants, it is possible to take advantage of the results of large GWA studies of disease to narrow the search. Whatever is the case, age-related diseases are the other side of medal of successful aging, so their pathological mechanisms are deeply investigated to find genetic variants influencing longevity. iGWAS started from high-ranked variants of 14 large studies of disease and disease-related traits, hypothesizing that a strong association signal in disease can boost a weak association for longevity to statistical significance. The "in silico"

analysis carried out by Fortney and collaborators highlighted four genetic loci associated with both diseases and exceptional human longevity, then replicated in independent centenarian cohorts, including *APOE/TOMM40* (associated with Alzheimer's disease), *CDKN2B/ANRIL* (regulating the cellular senescence), *ABO* (tagging the O blood group), and *SH2B3/ATXN2* (a signalling gene able to extend lifespan in Drosophila and involved in neurological disease). These results imply a genetic overlap between longevity and age-related diseases and traits. Furthermore, many of the SNPs found by iGWAS showed an association for not one, but many diseases which seem to have distinct aetiologies, thus suggesting their implication in some general mechanisms (such as reduced aging rate or cellular impairment) that act across multiple diseases.

In order to find genes associated with healthy aging phenotypes, Minster and collaborators proposed the genome-wide analysis association of families selected for exceptionally healthy aging, stratified for "Healthy Aging Index" (HAI) (Minster et al, 2015). This index is built summing five measures, including systolic blood pressure, forced vital capacity, Mini-Mental State Exam, serum creatinine, and serum fasting glucose, with scores ranging from 0 (healthiest) to 10 (unhealthiest). HAI is sex-specific, strongly predictive of low mortality risk in Cardiovascular Health Study and heritable in families selected for longevity (Sanders et al, 2014). Taking into account the HAI values, Minster found a new locus, *ZNF704* on chromosome 8q21.13, as a potential candidate gene for aging in women. Although the poor information available on this gene, association was found between one of its variant and amyotrophic lateral sclerosis in United States veterans (Kwee et al, 2012) and with muscle quality in pigs (Ponsuksili et al, 2014), thus suggesting an influence of the gene on muscular functionality.

Furthermore, a functional-association approach was very recently proposed by Yerges-Armstrong (2016): with the aim of identifying longevity-enhancing genes, they measured differential gene expression between offspring of long-lived Amish (older than 90 years) and their spouses (controls) and correlated differentially expressed transcripts with locations of longevity-associated variants detected in a prior GWAS of survival to age 90 (Newman et al, 2010). Expression of one of these

transcripts, 3′-phosphoadenosine 5′-phosphosulfate synthase 2 (PAPSS2), was significantly higher in offspring of nonagenarians than in controls. The gene *PAPSS2* codifies for a sulfation enzyme located on chromosome 10, involved in premature onset of joint degeneration and impaired dehydroepiandrosterone (DHEA) sulfation; because DHEA sulfate is believed to be a critical hormone, which levels drop with age, and maintenance of its levels were associated with greater longevity in men, this result deserves future attention (Enomoto et al, 2008).

Finally, particularly interesting is the question about the role of *APOE* gene variants in aging, longevity and age-related diseases. As previously discussed, genome-wide association studies (GWAS), performed on long-lived cohorts, confirmed the association with longevity of variants near *APOE/TOMM40/APOC1* locus at genome wide significance ($P<5x10^{-8}$) in different populations (Nebel et al, 2011; Deelen et al, 2011, 2014; Sebastiani et al, 2012; Broer et al, 2015; Zeng et al, 2016). In all these studies, eplicated loci are considered significant if the False Discovery Rate (FDR)-adjusted P-value (the 95th or 75th percentile) was less than 10% or to a more stringent level of 5% (Nebel et al, 2001). Recently, Kulminski and collaborators pointed out that one complicating factor in understanding the role of *APOE* gene in aging and longevity is genetic trade-off (Kulminski et al., 2015). They analyzed the participants of the Long Life Family Study (LLFS), their offspring and spouses, to highlight the complex role of the e4 allele in genetic susceptibility to health span. In particular, they focused on age-related macular degeneration, bronchitis, asthma, pneumonia, stroke, creatinine, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol, diseases of heart (HD), cancer, and survival. Their results showed favorable associations of the ε2 allele with lower LDL-C levels, lower risks of HD, and better survival, while the e4 allele confers risks of heart diseases in a sex-, age-, and LLFS-population-specific manner. Furthermore, a protective effect of e4 allele against cancer was documented in long-lived men and their sons (>75 year), while the protective effect against heart diseases was limited to the first generation and not potentially transmitted (Kulminski et al., 2016).

2.3 G*ene-gene and pathway-based analyses: the importance of genomic context*

Considering that the genetic component of human longevity is assumed to be determined by many genes (Deelen et al., 2013), interactions between different genetic markers, not due to Linkage Disequilibrium (LD) effect, could explain a part of the missing heritability in human longevity, still unexplained. Interactions between different genes or SNP-SNP interactions at gene level may reasonably exist and have an influence on any human complex trait. From a genetic point of view, epistatic interactions may hide the role of some genetic variant, as well as synergistic relationships may be discovered respect a particular phenotype. Furthermore, the analysis of the co-occurrence of marker pairs with survival or age-related phenotypes, can suggest specific sub-processes more strongly associated with the phenotype than single-SNP analysis does (Cordell, 2009). Recently, the importance of genomic environment for studying the genetic components of human longevity was demonstrated by Raule and co-workers (2014), who found an overrepresentation in ultranonagenarians of mitochondrial DNA (mtDNA) mutations, previously found to be associated with a number of degenerative disorders. However, individuals were not affected by such disorders, thus suggesting that mtDNA mutations can be either positive or negative according to other factors, most likely including environmental factors as well as other mitochondrial and nuclear genomic background (Raule et al, 2014). Previous evidences in accordance with this hypothesis were found by Achilli (2012), who reported as in LHON (Leber's hereditary optic neuropathy), the penetrance of mutations is increased by the concomitant occurrence of mutations on different complexes, like I and III, defining specific J sub-haplogroups (Achilli et al, 2012). The availability of complete sequences allowed Raule and collaborators to evaluate for the first time the cumulative effects of specific, concomitant mtDNA mutations, including those that *per se* have a low, or very low, impact. Studying cumulative effects on genomic DNA is of course much more difficult, unless small fractions (or specific regions harboring genes involved in relevant pathways) are analyzed. Gene-gene interactions can be studied by logistic regression or by multifactor dimensionality reduction (MDR) approaches (Hahn et al, 2009; Gilbert-Diamond and Moore, 2011). MDR is a non-parametric, model-

free data mining strategy, designed to detect interactions with large sample size (Cordell et al, 2009), without requiring an accurate estimate of the model's parameters. This methodology overcomes logistic regression, deeply used in genetic epidemiology when dealing with very large sample size and many independent variables. However, the large number of tests performed when searching for the combined effect of genetic variants requires a strict assessment of the significance. To avoid false positive findings, it is necessary to correct for multiple testing and consider low P-values, very often obtained by permutation approaches by random data shuffling, which requires of course major calculation efforts.

Pathway-based analyses between different genes can reduce the number of different variants tested, by focusing the attention on candidate mechanisms for human longevity. From a biological point of view, the rationale for pathway-based approach is that it considers the joint effect of the gene products on cell metabolism (Wang et al, 2007). From a statistical point of view, a combined analysis of SNPs and genes sets reduces the multiple testing burden and typically increases power. The advantage to apply a gene-set analysis to investigate the joint effect on human longevity was recently demonstrated in large GWAs datasets. To date, different analytic approaches were proposed, from PLINK set-based test, to Global test, GRASS, SNP ratio test and competitive gene-set enrichment analysis (GSEA), all suitable for studying gene-gene interactions in complex traits (Wang et al, 2011; Deelen et al, 2013; Debrabant et al, 2014). Their application confirms the relevance of conserved mechanisms along the evolutionary scale, previously indicated also by univariate analysis, such as DNA repair (Debrabant et al, 2014), telomere maintenance (Atzmon et al, 2010; Soerensen et al, 2012a; Crocco et al, 2015), stress response (Altomare et al, 2003; Ross et al, 2003; Rose et al, 2011), nutrient-sensing signaling, and regulation of gene transcription mechanisms, like IGF-1/insulin axis (Soerensen et al, 2012b), TOR (target of rapamycin) pathway (Johnson et al, 2013), MAPK (Mitogen-Activated Protein Kinase) and calcium signalling pathways; in addition, the analysis of sub-processes allows to discriminate pathways with epistatic effects, responsible for the association with longevity, (like *INS/IGF-1* signalling, found in Deelen et al, 2013) from others where one master gene can concentrate

the effect (as *POT1* in the telomere maintenance pathway) (Deelen et al, 2013). Particularly interesting is the observation that many of these pathways, like immunity, stress response or xenobiotic metabolism, have homeostatic functions and answer to different kind of environmental stimuli (Zeng et al, 2016).

2.4 A step forward the classical genetics: role of non coding, regulatory DNA and transposable elements in the aging process

Many of the phenotypic changes that characterize the aging process are governed by widespread changes in gene expression (Glass et al, 2013). In addition to proteins that bind DNA and RNA, a variety of noncoding (nc) RNA, both short (mainly microRNAs) and long (lncRNAs), are capable of tightly regulating gene expression, acting both transcriptionally and post-transcriptionally (Djebali et al, 2012). miRNAs are the best characterized small ncRNA capable of modulating the expression of hundreds of genes, by interacting with complementary sequences in coding and non-coding regions of their mRNA targets, and finally leading to translational repression and/or degradation. The ability to regulate multiple targets simultaneously makes miRNA crucial pleiotropic regulators of many physiological processes. Data generated from model systems such as C. elegans, Drosophila, and mouse, show that specific miRNAs are able to regulate lifespan by targeting genes belonging to signaling pathways such as insulin/IGF-1, p53-, and SIRT1- mediated pathways, widely implicated in aging and longevity (Smith-Vikos et al, 2014). Furthermore, several studies reported tissue-specific up- or down-regulation of miRNAs in model organisms (Ibáñez-Ventoso et al, 2006; Maes et al, 2008; Li et al 2011; Liu et al, 2012). Similarly, studies in humans have shown that miRNA expression changes with age: age-related changes have been demonstrated in circulating miRNAs (such as miR-19b, miR-21, miR-126 and miR-146a) having an extensive role in complex molecular processes related to inflammation, cellular senescence and cancer. Notably, changes in miRNA expression in centenarians compared to either adults or octogenarians were also observed (Olivieri et al, 2013).

Moreover, there are indications that aging and longevity may be affected by the variability of specific miRNA targeted regions (Crocco et al, 2016).

A potential involvement in aging has been suggested also for lncRNAs, a heterogeneous class of numerous non-protein coding transcripts longer than 200 nucleotides. Although little is known about the molecular and biochemical functions of lncRNAs across the cell, the literature shows many examples of their effects on gene expression regulation, in processes ranging from regulation of chromatin structure to transcriptional, post-transcriptional, and translational regulation (Vance and Ponting, 2014). A significantly altered expression level of lncRNAs was observed in skin aging by Chang and co-workers (Chang et al, 2013), as well as by Abdelmohsen et al. (Abdelmohsen et al, 2013), who identified a set of lncRNAs differentially expressed during replicative senescence. Recently, evidence has started to accumulate indicating that lncRNAs can influence both the molecular and cellular processes that underlie the physiologic decline occurring with aging and age-related pathologies, including neurodegeneration, cardiovascular disease, and cancer (see reviews like Grammatikakis et al, 2016, Kim et al, 2016; Kour and Rath, 2016).

Finally, one of the hypothesis currently of interest regards genome instability as one of the major causes of aging (Vijg et al, 2013). A potentially important mechanism impacting genome stability is the activation of endogenous mobile genetic elements (transposable elements). Retrotransposons, which replicate through an RNA intermediate, constitute a large fraction of mammalian genomes: their activity alters genome architecture and likely influences the expression of many genes by inserting in or very close to their regulatory regions. Transposition in somatic cells is very low, being subject to epigenetic silencing; however, recent evidence suggests that silencing mechanisms acting on retrotransposons may deteriorate with age (De Cecco, 2013). With aging, the age-related loss of heterochromatin leads to an increased expression of otherwise silent retrotransposons, and the consequent translocation of these elements within genomes, finally determining a disruption of cellular homeostasis during aging (Wood and Helfand, 2013). In particular, activation of long interspersed element-1 (LINE-1 or L1) and short interspersed elements (SINEs, essentially Alu

elements), has been implicated in aging and aging-related disorders by several lines of evidence (Hancks et al, 2012; Beck et al, 2011; Mustafina, 2013). Interestingly, interventions that extend lifespan, such as dietary restriction, have been shown to counteract the increased expression of retrotransposons in aged mice (De Cecco et al, 2013). It has been also reported that SIRT6, a key regulator of mammalian lifespan, induces the upregulation of L1 activity during the course of aging and in response to genotoxic stressors (Van Meter et al, 2014).

3. Gene-environment interactions: the role of culture.

The correlation between genetic background and environment in determining the individual chance of a delayed or successful aging is a hot topic in modern gerontology. The precise knowledge of the effects of environment and lifestyle on the basic molecular mechanisms of aging may allow to elaborate preventive measures, in order to increase life expectancy and thus, the chance to attain longevity. The improvement of living conditions, such as increased availability of food resources, exercise attitude, housing, advance in medicine and pharmacology, has contributed to a significantly increased longevity, globally and especially in Western societies (Vaupel, 2010). Environmental factors may have either additive or multiplicative effects on health and longevity, triggering a series of coordinated changes in the physiological and developmental patterns able to produce alternative phenotypes during the life-course of an individual - a process commonly known as developmental plasticity. The existence of "healthy lifestyle and environments" comes from the observation of a geographical clustering in centenarians' distribution around the world: Okinawa, Nicoya, Ikaria, Sardinia, Loma Linda, respectively in Japan, Costa Rica, Greece, Italy and Mexico (Poulain et al, 2013), places which are possibly more conducive to fostering longer lifespan respect to the rest of the world. Called "Blue Zones" (Poulain et al, 2004; 2013), these places are defined as "limited region(s) where the population shares a common lifestyle and environment and whose exceptional longevity has been accurately verified". In these zones, centenarians have been found at higher frequency compared with the rest of the population, and lead a distinct lifestyle, which includes greater intake

of vegetables, stress-free and active lifestyle, with strong community bonds and spirituality (Buettner, 2012). Progeny of Okinawan and Ashkenazi centenarians has been shown to live longer and also has lower levels of cardiovascular diseases and lipid abnormalities, related to their age-matched counterparts (Atzmon et al, 2005; Willcox et al, 2006). Their study confirm that among the modifiable factors, diet has a major impact on healthy aging: nutrients and their metabolites control energy balance, enzymatic activities, and genome stability throughout the life cycle; as a consequence, robust association of dietary restriction with increased lifespan and reduction of age-associated decline were demonstrated across a wide range of species, and trials are going on in humans, with the aim of verifying the effect of intermittent fasting and specific component of food deprivation on health and longevity (Longo et al, 2015 and references therein).

Genetic and epigenetic variability influence diet-mediated mechanisms, and have an influence upon extreme survival through well-known metabolic pathway: i.e. caloric restriction, often mediated by epigenetic process affecting transcriptional levels of genes, involves critical nutrient-sensing factors like *AMPK*, *SIRT1* and *mTOR*, influencing inflammation, cell survival, stress response, autophagy and protein synthesis, well-known as mediators of the aging process (Finch, 2007; Dato et al, 2016).

The cultural context within each individual's life is also known to exert influence on health outcomes. Relevant *social and cultural environments* include not only an individual's immediate personal environment (e.g., his/her family), but also the broader social contexts such as the community in which he/she resides. Their influences on aging and longevity may be so important that different "environments" can account for population-specific associations: different subgroups may have both different genetic backgrounds and different cultures or socioeconomically influenced habits' patterns. Dynamicity of social environment implies that during the life course there are periods, as the prenatal period, that impact on later life health outcomes, as development of coronary heart disease and some cancers at middle age (Barker and Bagby, 2005). In addition, social environmental conditions often cumulate over time, so that for example, persistent poverty has more disadvantageous effects on health with respect to transient difficulties. Finally, such conditions may be reproduced across

generations, because parents, aside from their genes, "pass on" their familiar habits to their children and, consequently, the advantages/disadvantages of healthy or unhealthy lifestyles. According to the concept of developmental plasticity, appropriate lifestyle changes hold promise for increasing longevity (at least to a limited extent) (Govindaraju et al, 2015).

Studying gene-environment (GXE) interactions is a challenging clue in case-control studies of longevity, because environmental factors may not be constant over time, considering that cases could have experienced a different environment when they were at the age of the younger controls. A longitudinal design that investigates survival over time can be more suitable for GXE studies, possibly monitoring the individual changes in diet, physical activities, medications etc. As underlined before, social and cultural variables like socio-economic and occupational status, education level, social networks and social support, as well as psychosocial variables, should be also collected, because studying the dynamic trajectories of social changes can help explaining health and lifespan patterns, and have a deep relapse in terms of socio-economic policy.

4. Maintenance of the microbial counterpart as a key for longevity: an example of complex interaction between organism and environment

Nutrition-related factors and the complex gut-associated microbial ecosystem (gut-microbiota) are known to be important environmental triggers for the development of lifestyle-related chronic diseases and metabolic pathologies, like inflammatory diseases, which ultimately reduce the quality of life and the chance for a successful aging. Age-related factors, such as changes in diet, lifestyle, inflammation and frailty, can deteriorate the mutual microbiota–host interaction, compromising the health status of the individual. The intestinal microbiota (IM) is a complex, diversified and dense population of bacteria (Costello et al, 2009) composed by ~100 trillion of microbial symbionts, which shares an intense metabolism with its host; thus, it is an essential factor for several aspects of human physiology, as the digestion of complex dietary polysaccharides, the functionality of the immune system, the protection from pathogen colonization, the maintenance of the gut epithelial structure and

the regulation of enteric nerve function and brain development (O'Hara and Shanahan, 2006; Neish, 2009). Characterized by a relevant degree of plasticity, IM is able to adapt its functional profile in response to changes in diet, environment or lifestyle, through an optimization of metabolic and immunological performances (Candela et al, 2012).

Besides environmental factors, the composition of gut microbiota is determined by the host genome, whose own genes complement and shape the kind of indigenous microbial communities; the co-evolution of individual and microbic genome (metagenomic) is so important that was found to be phylogenetically conserved (Ley et al, 2008). In humans, the role of genetics was investigated by twins studies, demonstrating a comparable degree of similarity in monozygotic and dizygotic twins for their IM communities (Turnbaugh et al, 2009). Interestingly, several genes of both innate and adaptive immunity have been shown to play a role in shaping the gut microbial community. Examples can be found in *MYD88*, a protein involved in the communications between Toll-like receptors (TLR) and microbial products during inflammatory responses (Asquith et al, 2010), *IgA locus*, activated by commensal flora with the aim to protect both the individual and the herd against gastrointestinal tract's infections (Wijburg et al, 2006), and *APOA1*, the major component of high density lipoprotein in plasma. In mice models, mutations in *APOA1* gene correlate with a different microbiota community structure in comparison with wild-type mice (Zhang et al, 2010). Fine detailed analysis of microbiota composition showed significant re-sculpturing of microbiota community configuration in the elderly (Rampelli et al, 2013). By applying a shotgun sequencing to total fecal bacterial DNA in long-lived samples, researchers demonstrated that with age, besides the high degree of inter-individual variability, a compromised stability of the IM occurs, together with a tendency toward a reduced biodiversity, with age-related modifications both of the genetic (i.e. loss of genes for shortchain fatty acid) and functional profile of the human gut microbiota (Rampelli et al., 2013). The presence of such a compromised microbiota is associated with an increased inflammatory status, also known as inflammaging; furthermore, remodelling of centenarians' microbiota determines a marked decrease in symbiotic species with anti-inflammatory properties (i.e. *Faecalibacterium prauznitzii)* and an up

to ten-fold increase of other facultative anaerobes bacteria, including *Streptococci*, *Staphylococci*, *Enterococci* and *Enterobacteria* (Biagi et al., 2010, 2012; Gavini et al., 2001). Recently, an extensive analysis of the age-related trajectory of microbiome changes, involving subjects within a broad age-range (22-109 years), highlighted a complex adaptive remodelling of gut microbiota with age (Biagi et al., 2016): researchers demonstrated that while a core microbiota of highly occurring bacterial groups (mostly belonging to Ruminococcaceae, Lachnospiraceae and Bacteroidaceae families) accompanies human life, such a community strongly decreases along with aging. At extreme ages, a sort of ‘‘longevity adaptation’’ seems to occur, characterized by the enrichment of health-associated taxa, such as Akkermansia and Bifidobacterium, known to activate immunomodulation, protect against inflammation, and promote a healthy metabolic homeostasis, that might support extreme aging and longevity. On the contrary, individual specific variables accompanying the age-related decline may impact the deterioration of the IM host mutualism: frailty changes in physiological functions, including taste and smell (Weiffenbach and Bartoshuk, 1992), masticatory functions (Newton et al., 1993), reduction of appetite, constipation (Wald, 1990), saliva production, together with reduction in mobility and depression, may severely compromise dietary patterns, resulting in the consumption of a nutritionally unbalanced and less variable regimen, generally poor in fruits and vegetables (Ervin, 2008). As a consequence, the reduction of fibre intake may determine for example the depletion in members of the Clostridium cluster XIVa in the elderly, ultimately leading to a lower bio availability of short chain fatty acids in the gut, which can be detrimental for the wellbeing. There is not a precise threshold at which the IM-host mutual interaction may provide advantages for longevity, but IM can be considered a non-stable and non-homogeneous process in the mosaic model of human aging, which can stress and amplify the different genetic makeup of the host, finally influencing his chance to live a long life (Cevenini et al., 2008).

Conclusions

Traditionally, factors which may influence how long we live have been divided in two general categories: heredity (nature) and environment (nurture). In the last few centuries, as Ridley (2003) has pointed out, nurture appears to have shaped the nature of human longevity in the modern human societies: that is, lifestyle changes at population levels may have contributed disproportionately to world-wide increase in human longevity. The study of genetic counterpart to longevity obtained interesting conclusions, underlying half a hundred of genes associated with long lifespan in different populations (Dato et al, 2013). However, progress in understanding the genetic determinants of human longevity has been disappointing if compared to other complex traits: phenotypic heterogeneity, lack of replication among aged cohorts, significance thresholds, and missing heritability are still drawbacks in longevity association studies. As in other complex traits and diseases, the presence of many genes with small effects, mutually interacting to determine the phenotype (the individual chance to reach an advanced age) can complicate the picture but can be overcome by pathway-based and SNP-SNP interaction analyses, based on multidimensional reduction statistics. In different sections of the present work, we underlined how longevity inherently represents a highly plastic trait, a remodelling process comprising modification of internal and external stimuli, and so, influenced by a wide range of lifestyle changes. Dietary interventions, for instance, provide excellent examples of lifestyle modification having an immediate impact on health and longevity, holding the promise for increasing longevity. In the future, multivariate approaches should focus on the analysis of dynamic interaction of multiple genes with environmental, socio-cultural and individual factors, like microbioma composition, to complete the picture of longevity determinants in human populations. The knowledge of such determinants, genetic and environmental, can increase the chance of a healthy aging, helping to set up correct social policies for responding to increase in life expectancy. In a perspective view, looking at the future of human longevity genetics we argue that, beyond gene-environment interaction (lifestyle, nutrition, education and cultural habits), other basic variables such as chronological age and information about birth-cohort population

will become central, to be carefully considered in studies on the genetics of longevity and major age-related diseases (Franceschi and Garagnani, 2016).

References:

Abdelmohsen K, Panda A, Kang MJ, Xu J, Selimyan R, Yoon JH, Martindale JL, De S, Wood WH 3rd, Becker KG, Gorospe M. Senescence-associated lncRNAs: senescence-associated long noncoding RNAs. Aging Cell. 2013;12:890-900.

Achilli A, Iommarini L, Olivieri A, Pala M, Hooshiar Kashani B, Reynier P, La Morgia C, Valentino ML, Liguori R, Pizza F, Barboni P, Sadun F, De Negri AM, Zeviani M, Dollfus H, Moulignier A, Ducos G, Orssaud C, Bonneau D, Procaccio V, Leo-Kottler B, Fauser S, Wissinger B, Amati-Bonneau P, Torroni A, Carelli V. Rare primary mitochondrial DNA mutations and probable synergistic variants in Leber's hereditary optic neuropathy PLoS ONE 2012; 7: e42242

Altomare K, Greco V, Bellizzi D, Berardelli M, Dato S, DeRango F et al The allele (A) (-110) in the promoter region of the HSP70-1 gene is unfavorable to longevity in women. Biogerontology 2003; 4:215-20

Anselmi CV, Malovini A, Roncarati R, et al Association of the FOXO3A locus with extreme longevity in a southern Italian centenarian study. Rejuvenation Res 2009; 12:95–104.

Asquith MJ, Boulard O, Powrie F, Maloy KJ. Pathogenic and protective roles of MyD88 in leukocytes and epithelial cells in mouse models of inflammatory bowel disease. Gastroenterology. 2010;139: 519-29.

Atzmon G, Rincon M, Rabizadeh P, Barzilai N. Biological evidence for inheritance of exceptional longevity. Mech Aging Dev. 2005; 126:341-5.

Atzmon G, Cho M, Cawthon RM, Budagov T, Katz M, Yang X, Siegel G, Bergman A, Huffman DM, Schechter CB, Wright WE, Shay JW, Barzilai N, Govindaraju DR, Suh Y. Evolution in health and medicine Sackler colloquium: Genetic variation in human telomerase is associated with telomere length in Ashkenazi centenarians. Proc Natl Acad Sci U S A. 2010; 107: 1710–17.

Badi I, Burba I, Ruggeri C, Zeni F, Bertolotti M, Scopece A, Pompilio G, Raucci A. MicroRNA-34a Induces Vascular Smooth Muscle Cells Senescence by SIRT1 Downregulation and Promotes the Expression of Age-Associated Pro-inflammatory Secretory Factors. J Gerontol A Biol Sci Med Sci. 2015;70:1304-11.

Barker DJ, Bagby SP. Developmental antecedents of cardiovascular disease: a historical perspective. J Am Soc Nephrol. 2005;16: 2537-44.

Bathum L, Christiansen L, Jeune B, Vaupel J, McGue M, Christensen K. Apolipoprotein e genotypes: relationship to cognitive functioning, cognitive decline, and survival in nonagenarians. J Am Geriatr Soc 2006; 54:654 –58.

Beck CR, Garcia-Perez JL, Badge RM, Moran JV. LINE-1 elements in structural variation and disease. Annu Rev Genomics Hum Genet. 2011;12:187-215.

Beekman M, Nederstigt C, Suchiman HED, Kremer D, van der Breggen R, Lakenberg N, et al. Genome-wide association study (GWAS)-identified disease risk alleles do not compromise human longevity. Proc Natl Acad Sci. 2010;107: 18046–49.

Beekman M, Blanché H, Perola M, Hervonen A, Bezrukov V, Sikora E, Hahn F, Christiansen L, De Craen AJ, Kirkwood TB, Rea IM, Poulain M, Robine JM, Valensin S, Stazi MA, Passarino G, Deiana L, Gonos ES, Paternoster L, Sørensen TI, Tan Q, Helmer Q, van den Akker EB, Deelen J, Martella F, Cordell HJ, Ayers KL, Vaupel JW, Törnwall O, Johnson TE, Schreiber S, Lathrop M, Skytthe A, Westendorp RG, Christensen K, Gampe J, Nebel A, Houwing-Duistermaat JJ, Slagboom PE,

Franceschi C; GEHA consortium. Genome-wide linkage analysis for human longevity: Genetics of Healthy Aging Study. Aging Cell. 2013;12:184-93.

Bergman A, Atzmon G, Ye K, MacCarthy T, Barzilai N. Buffering Mechanisms in Aging: A Systems Approach Toward Uncovering the Genetic Component of Aging. Cohen P, ed. PLoS Computational Biology. 2007;3: e170.

Biagi E, Nylund L, Candela M, Ostan R, Bucci L, Pini E, Nikkïla J, Monti D, Satokari R, Franceschi C, Brigidi P, De Vos WT. Through AGING, and beyond: gut microbiota and inflammatory status in seniors and centenarians. PLoS One 2010; 5: e10667.

Biagi E, Candela M, Fairweather-Tait S, Franceschi C, Brigidi P. AGING of the human metaorganism: the microbial counterpart. Age. 2012; 34:247-67.

Biagi E, Franceschi C, Rampelli S, Severgnini M, Ostan R, Turroni S, Consolandi C, Quercia S, Scurti M, Monti D, Capri M, Brigidi P, Candela M. Gut Microbiota and Extreme Longevity. Curr Biol. 2016; 26:1480-5.

Broer L, Buchman AS, Deelen J, Evans DS, Faul JD, Lunetta KL,Sebastiani P, Smith JA, Smith AV, Tanaka T, Yu L, Arnold AM, Aspelund T, Benjamin EJ, De Jager PL, Eirkisdottir G, Evans DA, Garcia ME, Hofman A, Kaplan RC, Kardia SL, Kiel DP(,Oostra BA, Orwoll ES, Parimi N, Psaty BM, Rivadeneira F, Rotter JI, Seshadri S, Singleton A, Tiemeier H, Uitterlinden AG, Zhao W, Bandinelli S, Bennett DA, Ferrucci L, Gudnason V,Harris TB, Karasik D, Launer LJ,Perls TT, Slagboom PE, Tranah GJ, Weir DR, Newman AB, van Duijn CM, Murabito JM. GWAS of Longevity in CHARGE Consortium Confirms APOE and FOXO3 Candidacy. J Gerontol A Biol Sci Med Sci 2015; 70:110-8.

Bucci L, Ostan R, Cevenini E, Pini E, Scurti M, Vitale G, Mari D, Caruso C, Sansoni P, Fanelli F, Pasquali R, Gueresi P, Franceschi C, Monti D. Centenarians' offspring as a model of healthy aging: a reappraisal of the data on Italian subjects and a comprehensive overview. Aging (Albany NY). 2016;8: 510-9.

Buettner, Dan. The Blue Zones, Second Edition: 9 Lessons for Living Longer From the People Who've Lived the Longest. 2012. Washington, D.C.: National Geographic.

Candela M, Biagi E, Maccaferri S, Turroni S, Brigidi P. Intestinal microbiota is a plastic factor responding to environmental changes. Trends Microbiol 2012; 20:385-91

Cevenini E, Invidia L, Lescai F, Salvioli S, Tieri P, Castellani G, Franceschi C. Human models of aging and longevity. Expert Opin Biol Ther. 2008; 8:1393-405.

Chang AL, Bitter PH Jr., Qu K, Lin M, Rapicavoli NA, Chang HY. Rejuvenation of gene expression pattern of aged human skin by broadband light treatment: a pilot study. J Invest Dermatol 2013; 133: 394-402.

Christensen K, Johnson TE, Vaupel JW. The quest for genetic determinants of human longevity: challenges and insights. Nat Rev Genet 2006; 7: 436–48

Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, Harris HM, Coakley M, Lakshminarayanan B, O'Sullivan O, Fitzgerald GF, Deane J, O'Connor M, Harnedy N, O'Connor K, O'Mahony D, van Sinderen D, Wallace M, Brennan L, Stanton C, Marchesi JR, Fitzgerald AP, Shanahan F, Hill C, Ross RP, O'Toole PW. Gut microbiota composition correlates with diet and health in the elderly. Nature. 2012; 488:178-84.

Cordell HJ. Detecting gene-gene interactions that underlie human diseases. Nat Rev Genet 2009; 10:392-404

Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI. Knight R. Bacterial community variation in human body habitats across space and time. Science. 2009; 326:1694-7.

Crocco P, Barale R, Rose G, Rizzato C, Santoro A, De Rango F, Carrai M, Fogar P, Monti D, Biondi F, Bucci L, Ostan R, Tallaro F, Montesanto A, Zambon CF, Franceschi C, Canzian F, Passarino G, Campa D. Population-specific association of genes for telomere-associated proteins with longevity in an Italian population. Biogerontology. 2015; 16:353-64.

Crocco P, Montesanto A, Passarino G, Rose G.Polymorphisms Falling Within Putative miRNA Target Sites in the 3'UTR Region of SIRT2 and DRD2 Genes Are Correlated With Human Longevity. J Gerontol A Biol Sci Med Sci. 2016;71(5):586-92.

Dato, S., Crocco, P., D'Aquila, P., dr Rango, F., Bellizzi, D., Rose, G., Passarino, G. Exploring the role of genetic variability and life style on oxidative stress response for healthy aging and longevity. Int. J. Mol. Sci. 2013; 14: 16443–72.

Dato S, Bellizzi D, Rose G, Passarino G. The impact of nutrients on the aging rate: A complex interaction of demographic, environmental and genetic factors. Mech Aging Dev 2016; 154:49-61

Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arterioscler Dallas Tex 1988; 8: 1–21.

De Benedictis G, Carotenuto L, Carrieri G, De Luca M, Falcone E, Rose G, Yashin AI, Bonafè M, Franceschi C. Age-related changes of the 3'APOB-VNTR genotype pool in ageing cohorts. Ann Hum Genet. 1998; 62: 115-22.

Debrabant B, Soerensen M, Flachsbart F, Dato S, Mengel-From J,Stevnsner T, Bohr VA, Kruse TA, Schreiber S, Nebel A Christensen K, Tan Q, Christiansen L. Human longevity and variation in DNA damage response and repair: study of the contribution of sub-processes using competitive gene-set analysis. Eur J Hum Genet. 2014; 22:1131-6.

De Cecco M, Criscione SW, Peterson AL, Neretti N, Sedivy JM, Kreiling JA. Transposab.le elements become active and mobile in the genomes of aging mammalian somatic tissues. Aging *(Albany NY)* 2013;5(12):867-83.

Deelen J, Beekman M, Uh HW, Helmer Q, Kuningas M, Christiansen L, Kremer D, van der Breggen R, Suchiman HE, Lakenberg N, van den Akker EB, Passtoors WM, Tiemeier H, van Heemst D, de Craen AJ, Rivadeneira F, de Geus EJ, Perola M, van der Ouderaa FJ, Gunn DA, Boomsma DI, Uitterlinden AG, Christensen K, van Duijn CM, Heijmans BT, Houwing-Duistermaat JJ, Westendorp

RG, Slagboom PE. Genome-wide association study identifies a single major locus contributing to survival into old age; the APOE locus revisited. Aging Cell 2011; 10: 686–98

Deelen J, Uh HW, Monajemi R, van Heemst D, Thijssen PE, Böhringer S, van den Akker EB, de Craen AJ, Rivadeneira F, Uitterlinden AG, Westendorp RG, Goeman JJ, Slagboom PE, Houwing-Duistermaat JJ, Beekman M. Gene set analysis of GWAS data for human longevity highlights the relevance of the insulin/IGF-1 signaling and telomere maintenance pathways. Age (Dordr) 2013; 35:235-49.

Deelen J, Beekman M, Uh HW, Broer L, Ayers KL, Tan Q, Kamatani Y, Bennet AM, Tamm R, Trompet S, Guðbjartsson DF, Flachsbart F, Rose G, Viktorin A, Fischer K, Nygaard M, Cordell HJ, Crocco P, van den Akker EB, Böhringer S,Helmer Q, Nelson CP,Saunders GI, Alver M, Andersen-Ranberg K, Breen ME, van der Breggen R, Caliebe A, Capri M, Cevenini E, Collerton JC, Dato S, Davies K, Ford I, Gampe J, Garagnani P, de Geus EJ, Harrow J, van Heemst D, Heijmans BT, Heinsen FA, Hottenga JJ, Hofman A, Jeune B, Jonsson PV, Lathrop M, Lechner D,Martin-Ruiz C, Mcnerlan SE, Mihailov E, Montesanto A, Mooijaart SP, Murphy A, Nohr EA, Paternoster L, Postmus I, Rivadeneira F, Ross OA, Salvioli S, Sattar N, Schreiber S, Stefánsson H, Stott DJ, Tiemeier H, Uitterlinden AG, Westendorp RG, Willemsen G, Samani NJ, Galan P, Sørensen TI, Boomsma DI,Jukema JW, Rea IM, Passarino G, de Craen AJ, Christensen K,Nebel A, Stefánsson K, Metspalu A, Magnusson P, Blanché H,Christiansen L, Kirkwood TB, van Duijn CM, Franceschi C,Houwing-Duistermaat JJ, Slagboom PE. Genome-wide association meta-analysis of human longevity identifies a novel locus conferring survival beyond 90 years of age. Hum Mol Genet 2014; 23:4420-32.

Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, et al. Landscape of transcription in human cells. Nature 2012; 489:101–8.

Enomoto M, Adachi H, Fukami A, Furuki K, Satoh A, Otsuka M, Kumagae S, Nanjo Y, Shigetoh Y, Imaizumi T. Serum dehydroepiandrosterone sulfate levels predict longevity in men: 27-year follow-up study in a community-based cohort (Tanushimaru study). J Am Geriatr Soc. 2008; 56: 994-8.

Ervin, R.B. Healthy eating index scores among adults, 60 years of age and over, by sociodemographic and health characteristics: United States, 1999-2002. Advance Data from Vital and Health Statistics. 2008;395: 1-20.

Finch C. The Biology of Human Longevity, 1st Edition. Inflammation, Nutrition, and Aging in the Evolution of Lifespans. 2007. Academic Press.

Flachsbart F, Caliebe A, Kleindorp R, Blanché H, von Eller-Eberstein H, Nikolaus S, Schreiber S, Nebel A. Association of FOXO3A variation with human longevity confirmed in German centenarians. Proc Natl Acad Sci U S A. 2009; 106: 2700-5.

Fortney K, Dobriban E, Garagnani P, Pirazzini C, Monti D, Mari D, Atzmon G, Barzilai N, Franceschi C, Owen AB, Kim SK.Genome-Wide Scan Informed by Age-Related Disease Identifies Loci for Exceptional Human Longevity. PLoS Genet. 2015;11: e1005728.

Franceschi and Garagnani P Suggestions from Geroscience for the Genetics of Age-Related Diseases. Plos Genetics 2016 in press).

Freudenberg-Hua Y, Freudenberg J, Vacic V, Abhyankar A, Emde A-K, Ben-Avraham D, et al. Disease variants in genomes of 44 centenarians. Mol Genet Genomic Med. 2014;2: 438–50.

Garagnani P, Giuliani C, Pirazzini C, Olivieri F, Bacalini MG, Ostan R, Mari D, Passarino G, Monti D, Bonfigli AR, Boemi M, Ceriello A, Genovese S, Sevini F, Luiselli D, Tieri P, Capri M, Salvioli S, Vijg J, Suh Y, Delledonne M, Testa R, Franceschi C. Centenarians as super-controls to assess the biological relevance of genetic risk factors for common age-related diseases: a proof of principle on type 2 diabetes. Aging (Albany NY). 2013; 5:373-85

Gavini F, Cayuela C, Antoine JM, Lecoq C, Fabure B, Membré JM, Neut C. Differences in distribution of bifidobacterial and enterobacterial species in human fecal microflora of three different (children, adults, elderly) age groups. Microb Ecol Health Dis. 2001; 13:40–5.

Gerdes LU, Jeune B, Ranberg KA, Nybo H, Vaupel JW. Estimation of apolipoprotein E genotype-specific relative mortality risks from the distribution of genotypes in centenarians and middle-aged men: apolipoprotein E gene is a "frailty gene," not a "longevity gene". Genet Epidemiol 2000; 19:202–10

Gilbert-Diamond D, Moore JH. Analysis of gene-gene interactions. Curr Protoc Hum Genet 2011; Chapter 1: Unit 114.

Glass D, Vinuela A, Davies MN, Ramasamy A, Parts L, Knowles D, Brown AA, Hedman AK, Small KS, Buil A, Grundberg E, Nica AC, Di Meglio P, Nestle FO, Ryten M; UK Brain Expression consortium.; MuTHER consortium., Durbin R, McCarthy MI, Deloukas P, Dermitzakis ET, Weale ME, Bataille V, Spector TD. Gene expression changes with age in skin, adipose tissue, blood and brain. Genome Biol. 2013;14:R75.

Govindaraju D, Atzmon G, Barzilai N. Genetics, lifestyle and longevity: Lessons from centenarians. Appl Transl Genom. 2015; 4:23-32.

Grammatikakis I, Panda AC, Abdelmohsen K, Gorospe M. Long noncoding RNAs (lncRNAs) and the molecular hallmarks of aging. *Aging (Albany NY)* 2014; 6:992-1009.

Hahn LW, Ritchie MD, Moore JH. Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions. Bioinformatics 2003; 19, 376–82.

Hancks, D. C. & Kazazian, H. H. Jr. Active human retrotransposons: variation and disease. Curr. Opin. Genet. Dev. 2012; 22, 191–203.

Herskind AM, McGue M, Holm NV, Sorensen TI, Harvald B, Vaupel JW. The heritability of human longevity: A population-based study of 2872 Danish twin pairs born 1870-1900. American Journal of Human Genetics. 1996;97(3):319–23.

Hjelmborg JB, Iachine I, Skytthe A, Vaupel JW, McGue M, Koskenvuo M, Kaprio J, Pedersen NL, Christensen K. Genetic influence on human lifespan and longevity. American Journal of Human Genetics. 2006;119: 312–21

Huffman DM, Deelen J, Ye K, Bergman A, Slagboom EP, Barzilai N, Atzmon G. Distinguishing between longevity and buffered-deleterious genotypes for exceptional human longevity: the case of the MTP gene.J Gerontol A Biol Sci Med Sci. 2012;67:1153-60.

Ibáñez-Ventoso C, Yang M, Guo S, Robins H, Padgett RW, Driscoll M. Modulated microRNA expression during adult lifespan in Caenorhabditis elegans. Aging Cell. 2006; 5:235–46.

Johnson SC, Rabinovitch PS, Kaeberlein M. mTOR is a key modulator of AGING and age-related disease. Nature. 2013; 493:338–45.

Kim J, Kim KM, Noh JH, Yoon JH, Abdelmohsen K, Gorospe M. Long noncoding RNAs in diseases of aging. Biochim Biophys Acta. 2016; 1859:209-21.

Kour S, Rath PC. Long noncoding RNAs in aging and age-related diseases. Ageing Res Rev. 2016; 26:1-21.

Kulminski AM, Arbeev KG, Culminskaya I, Ukraintseva SV, Stallard E, Province MA, Yashin AI. Trade-offs in the effects of the apolipoprotein E polymorphism on risks of diseases of the heart, cancer, and neurodegenerative disorders: insights on mechanisms from the Long Life Family Study. Rejuvenation Res. 2015; 18:128-35.

Kulminski AM, Raghavachari N, Arbeev KG, Culminskaya I, Arbeeva L, Wu D, Ukraintseva SV, Christensen K, Yashin AI. Protective role of the apolipoprotein E2 allele in age-related disease traits and survival: evidence from the Long Life Family Study. Biogerontology. 2016; 1:893-905.

Kwee LC, Liu Y, Haynes C, Gibson JR, Stone A, Schichman SA, Kamel F, Nelson LM, Topol B, Van den Eeden SK, Tanner CM, Cudkowicz ME, Grasso DL, Lawson R, Muralidhar S, Oddone EZ, Schmidt S, Hauser MA. A high-density genome-wide association screen of sporadic ALS in US veterans. PLoS One 2012;7(3): e32768.

Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R, Gordon JI. Evolution of mammals and their gut microbes. Science. 2008; 320:1647-51.

Li N, Bates DJ, An J, Terry DA, Wang E. Up-regulation of key microRNAs, and inverse down-regulation of their predicted oxidative phosphorylation target genes, during aging in mouse brain. Neurobiol Aging. 2011;32:944-55.

Liu N, Landreh M, Cao K, Abe M, Hendriks GJ, Kennerdell JR, Zhu Y, Wang LS, Bonini NM. The microRNA miR-34 modulates ageing and neurodegeneration in Drosophila. Nature. 2012;482(7386):519-23.

Longo VD, Antebi A, Bartke A, Barzilai N, Brown-Borg HM, Caruso C, Curiel TJ, de Cabo R, Franceschi C, Gems D, Ingram DK, Johnson TE, Kennedy BK, Kenyon C, Klein S, Kopchick JJ, Lepperdinger G, Madeo F, Mirisola MG, Mitchell JR, Passarino G, Rudolph KL, Sedivy JM, Shadel GS, Sinclair DA, Spindler SR, Suh Y, Vijg J, Vinciguerra M, Fontan, L. Interventions to slow aging in humans: are we ready? Aging Cell 2015; 14:497-510.

Olivieri F, Rippo MR, Monsurrò V, Salvioli S, Capri M, Procopio AD, Franceschi C. MicroRNAs linking inflamm-aging, cellular senescence and cancer. Ageing Res Rev. 2013;12(4):1056-68.

Maes OC, An J, Sarojini H, Wang E. Murine microRNAs implicated in liver functions and aging process. Mech Ageing Dev. 2008;129:534–41.

Minster RL, Sanders JL, Singh J, Kammerer CM, Barmada MM, Matteini AM, Zhang Q, Wojczynski MK, Daw EW, Brody JA, Arnold AM, Lunetta KL, Murabito JM, Christensen K, Perls TT, Province MA, Newman AB. Genome-Wide Association Study and Linkage Analysis of the Healthy Aging Index. J Gerontol A Biol Sci Med Sci 2015; 70:1003-8

Montesanto A, Latorre V, Giordano M, Martino C, Domma F, Passarino G. The genetic component of human longevity: analysis of the survival advantage of parents and siblings of Italian nonagenarians. Eur J Hum Genet 2011; 19:882-6

Murphy CT. The search for DAF-16/FOXO transcriptional targets: approaches and discoveries. Exp Gerontol 2006; 41: 910–21.

Mustafina OE. The possible roles of human Alu elements in aging. Front Genet. 2013; 4:96.

Nebel A, Kleindorp R, Caliebe A, Nothnagel M, Blanché H, Junge O, Wittig M, Ellinghaus D, Flachsbart F, Wichmann HE, Meitinger T, Nikolaus S, Franke A,Krawczak M, Lathrop M, Schreiber S. A genome-wide association study confirms APOE as the major gene influencing survival in long-lived individuals. Mech Aging Dev 2011; 132: 324–30.

Neish AS. Microbes in gastrointestinal health and disease. Gastroenterology 2009;136: 65-80

Newman AB, Walter S, Lunetta KL, Garcia ME, Slagboom PE, Christensen K, Arnold AM, Aspelund T, Aulchenko YS, Benjamin EJ, Christiansen L, D'Agostino RB Sr, Fitzpatrick AL, Franceschini N, Glazer NL, Gudnason V, Hofman A, Kaplan R, Karasik D, Kelly-Hayes M, Kiel DP, Launer LJ, Marciante KD, Massaro JM, Miljkovic I, Nalls MA, Hernandez D, Psaty BM, Rivadeneira F, Rotter J, Seshadri S, Smith AV, Taylor KD, Tiemeier H, Uh HW, Uitterlinden AG, Vaupel JW, Walston J, Westendorp RG, Harris TB, Lumley T, van Duijn CM, Murabito JM. 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. J Gerontol A Biol Sci Med Sci. 2010 May;65(5):478-87.

Newton JP, Yemm R, Abel RW, Menhinick S. Changes in human jaw muscles with age and dental state. Gerontology 1993; 10:16–22.

O'Hara AM, Shanahan F. The gut flora as a forgotten organ. EMBO Rep 2006; 7:688-93

Perls TT, Wilmoth J, Levenson R, Drinkwater M, Cohen M, Bogan H, Joyce E, Brewster S, Kunkel L, Puca A. Life-long sustained mortality advantage of siblings of centenarians. Proc. Natl. Acad. Sci. USA 2002a; 99: 8442 – 47.

Perls T, Kunkel LM, Puca AA. The genetics of exceptional human longevity. J Mol Neurosci 2002b; 19: 233.

Poulain M, Pes GM, Grasland C, Carru C, Ferrucci L, Baggio G, Franceschi C, Deiana L. Identification of a geographic area characterized by extreme longevity in the Sardinia island: the AKEA study. Exp Gerontol. 2004; 39:1423-9.

Poulain M, Herm A, Pes G. The Blue Zones: areas of exceptional longevity around the world. Vienna Yearbook of Population Research 2013; 11: 87-108.

Ponsuksili S, Murani E, Trakooljul N, Schwerin M, Wimmers K. Discovery of candidate genes for muscle traits based on GWAS supported by eQTL analysis. Int J Biol Sci 2014; 10: 327–37

Postmus I, Deelen J, Sedaghat S, Trompet S, de Craen AJ, Heijmans BT, Franco OH, Hofman A, Dehghan A, Slagboom PE, Westendorp RG, Jukema JW. LDL cholesterol still a problem in old age? A Mendelian randomization studyInt J Epidemiol 2015;44: 604-12.

Rampelli S, Candela M, Turroni S, Biagi E, Collino S, Franceschi C, O'Toole PW, Brigidi P. Functional metagenomic profiling of intestinal microbiome in extreme ageing. Aging (Albany NY). 2013; 5:902-12.

Raule N, Sevini F, Li S, Barbieri A, Tallaro F, Lomartire L, Vianello D, Montesanto A, Moilanen JS, Bezrukov V, Blanché H, Hervonen A, Christensen K, Deiana L, Gonos ES, Kirkwood TB, Kristensen P, Leon A, Pelicci PG, Poulain M, Rea IM, Remacle J, Robine JM, Schreiber S, Sikora E, Eline Slagboom P, Spazzafumo L, Stazi A, Toussaint O, Vaupel JW, Rose G, Majamaa K, Perola M, Johnson TE, Bolund L, Yang H, Passarino G, Franceschi C. The co -occurrence of mtDNA mutations on different oxidative phosphorylation subunits, not detected by haplogroup analysis, affects human longevity and is population specific. Aging Cell 2014; 13:401-7.

Ridley, M., 2003. Nature via Nurture: Genes, Experience and What Makes us Human. Fourth Estate, Ltd., London.

Rose G, Crocco P, De Rango F, Montesanto A, Passarino G. Further support to the uncoupling-to-survive theory: the genetic variation of human UCP genes is associated with longevity. PLoS One 2011;6: e29650.

Ross OA, Curran MD, Crum KA, Rea IM, Barnett YA, Middleton D. Increased frequency of the 2437T allele of the heat shock protein 70-Hom gene in an aged Irish population. Experimental gerontology 2003; 38:561-5.

Sanders JL, Minster RL, Barmada MM, Matteini AM, Boudreau RM, Christensen K, Mayeux R, Borecki IB, Zhang Q, Perls T, Newman AB. Heritability of and mortality prediction with a longevity phenotype: the healthy aging index. J Gerontol A Biol Sci Med Sci. 2014; 69:479-85.

Schächter F, Faure-Delanef L, Guénot F, Rouger H, Froguel P, Lesueur-Ginot L, Cohen D. Genetic associations with human longevity at the APOE and ACE loci. Nat Genet 1994; 6: 29–32

Schoenmaker M, de Craen AJ, de Meijer PH, Beekman M, Blauw GJ, Slagboom PE, Westendorp RG. Evidence of genetic enrichment for exceptional survival using a family approach: the Leiden Longevity Study. Eur J Hum Genet 2006; 14:79-84.

Sebastiani P, Riva A, Montano M, Pham P, Torkamani A, Scherba E, et al. Whole genome sequences of a male and female supercentenarian, ages greater than 114 years. Front Genet. 2011;2: 90.

Sebastiani P, Solovieff N, Dewan AT, Walsh KM, Puca A, Hartley SW, Melista E, Andersen S, Dworkis DA, Wilk JB, Myers RH, Steinberg MH, Montano M, Baldwin CT, Hoh J, Perls TT. Genetic signatures of exceptional longevity in humans. PLoS One 2012; 7: e29848.

Skytthe A, Pedersen NL, Kaprio J, Stazi MA, Hjelmborg J, Iachine I, Vaupel JW, Christensen K. Longevity studies in GenomEU twin. Twin Research and Human Genetics. 2003; 6:448–54.

Singh A, Babyak MA, Nolan DK, Brummett BH, Jiang R, Siegler IC, Kraus WE, Shah SH, Williams RB, Hauser ER. Gene by stress genome-wide interaction analysis and path analysis identify EBF1 as a cardiovascular and metabolic risk gene. Eur J Hum Genet. 2015;23: 854-62.

Smith-Vikos T, Slack FJ. MicroRNAs and their roles in aging. J Cell Sci 2012; 125: 7–17.

Soerensen M, Dato S, Christensen K, McGue M, Stevnsner T, Bohr VA, Christiansen L. Replication of an association of variation in the FOXO3A gene with human longevity using both case-control and longitudinal data. Aging Cell 2010; 9:1010– 17

Soerensen M, Thinggaard M, Nygaard M, Dato S, Tan Q, Hjelmborg J, Andersen-Ranberg K, Stevnsner T, Bohr VA, Kimura M, Aviv A, Christensen K, Christiansen L. Genetic variation in TERT and TERC and human leukocyte telomere length and longevity: a cross-sectional and longitudinal analysis. Aging Cell 2012a;11: 223-7

Soerensen M, Dato S, Tan Q, Thinggaard M, Kleindorp R, Beekman M, Jacobsen R, Suchiman HE, de Craen AJ, Westendorp RG, Schreiber S, Stevnsner T, Bohr VA, Slagboom PE, Nebel A, Vaupel JW, Christensen K, McGue M, Christiansen L. Human longevity and variation in GH/IGF-1/insulin signaling, DNA damage signaling and repair and pro/antioxidant pathway genes: cross sectional and longitudinal studies. Exp Gerontol 2012b; 47:379-87

Terry DF, Wilcox M, McCormick MA, Lawler E, Perls TT. Cardiovascular advantages among the offspring of centenarians. J Gerontol A Biol Sci Med Sci 2003;58:M425-31.

Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. Nature. 2009; 22:480-4.

Van Meter M, Kashyap M, Rezazadeh S, Geneva AJ, Morello TD, Seluanov A, Gorbunova V. SIRT6 represses LINE1 retrotransposons by ribosylating KAP1 but this repression fails with stress and age. Nat Commun. 2014;5:5011.

Vance KW, Ponting CP. Transcriptional regulatory functions of nuclear long noncoding RNAs. Trends Genet. 2014;30: 348-55.

Vaupel JW. Biodemography of human AGING. Nature. 2010; 25;464(7288):536-42.

Vijg J, Suh Y. Genome instability and aging. Annu Rev Physiol. 2013;75:645-68.

Wald A. Constipation and fecal incontinence in the elderly. Gastroenterol Clin North Am 1990; 19: 405-18.

Wang K, Li M, Bucan M. Pathway-based approaches for analysis of genomewide association studies. Am J Hum Genet 2007; 81: 1278–83

Wang L, Jia P, Wolfinger RD, Chen X, Zhao Z. Gene set analysis of genome-wide association studies: methodological issues and perspectives. Genomics 2011; 98: 1-8

Weiffenbach JM, Bartoshuk LM. Taste and smell. Clin Geriatr Med. 1992; 8:543-55.

Wijburg OL, Uren TK, Simpfendorfer K, Johansen FE, Brandtzaeg P, Strugnell RA. Innate secretory antibodies protect against natural Salmonella typhimurium infection. J Exp Med. 2006; 203: 21-6.

Willcox DC, Willcox BJ, Hsueh W-C, Suzuki M. Genetic determinants of exceptional human longevity: insights from the Okinawa Centenarian Study. Age. 2006;28(4):313-32.

Willcox BJ, Donlon TA, He Q, Chen R, Grove JS, Yano K, Masaki KH, Willcox DC, Rodriguez B, Curb JD. FOXO3A genotype is strongly associated with human longevity. Proc Natl Acad Sci 2008; 105: 13987–992.

Wood JJ, Helfand SL. Chromatin structure and transposable elements in organismal aging. Front. Genet. 2013; 4, 274.

Yerges-Armstrong LM, Chai S, O'Connell JR, Curran JE, Blangero J, Mitchell BD, Shuldiner AR, Damcott CM. Gene Expression Differences Between Offspring of Long-Lived Individuals and Controls in Candidate Longevity Regions: Evidence for PAPSS2 as a Longevity Gene. J Gerontol A Biol Sci Med Sci. 2016; 71:1295-9.

Zeng Y, Nie C, Min J, Liu X, Li M, Chen H, Xu H, Wang M, Ni T, Li Y, Yan H, Zhang JP, Song C, Chi LQ, Wang HM, Dong J, Zheng GY, Lin L, Qian F, Qi Y, Liu X, Cao H, Wang Y, Zhang L, Li Z, Zhou Y, Wang Y, Lu J, Li J, Qi M, Bolund L, Yashin A, Land KC, Gregory S, Yang Z, Gottschalk W, Tao W, Wang J, Wang J, Xu X, Bae H, Nygaard M, Christiansen L, Christensen K, Franceschi C, Lutz MW, Gu J, Tan Q, Perls T, Sebastiani P, Deelen J, Slagboom E, Hauser E, Xu H, Tian XL, Yang H, Vaupel JW. Novel loci and pathways significantly associated with longevity. Sci Rep 2016; 6:21243.

Zhang C, Zhang M, Wang S, Han R, Cao Y, Hua W, Mao Y, Zhang X, Pang X, Wei C, Zhao G, Chen Y, Zhao L. Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. ISME Journal 2010; 4: 232–41.

Table 1: Summary of the main models of data analysis in studies on the genetic component of longevity. Study design and principal evidences are shown for each different approach, as well as some references are indicated. * Healthy Aging Index" (HAI) is built summing five measures, systolic blood pressure, forced vital capacity, Mini-Mental State Exam, serum creatinine, and serum fasting glucose.

	Survival studies	**Study design**	**Principal evidences**	**References**
1	Analysis of survival advantage	Analysis of survival and health characteristics in families of long-lived subjects	Healthy aging and longevity have a hereditary component	Perls et al, 2002; Schoenmaker et al, 2006; Montesanto et al, 2011
2	Estimation of variation in the age of death	Analysis of lifespan in monozygotic and dizygotic twins	A) about 25 % of the variation in human longevity is due to genetic factors B) genetic component is higher at older ages C) genetic component is more important in males than in females	Herskind et al, 1996; Skytthe et al, 2003; Hjelmborg et al, 2006;
	Genetic studies			
3	Single-gene association studies by candidate approach	Comparison of allele frequency between long-lived subjects and younger controls; estimation of genotype-specific relative mortality risks	*Apoe* and *FOXO3A* are the only genes replicated in different populations	Gerdes et al, 2000; Bathum et al, 2006; Dato et al, 2013 and references therein
5	Genome-wide association studies(GWAS)	Whole-genome analysis of genetic variants in long-lived samples	Association with longevity for *APOE/TOMM40/APOC1* loci; new locus on chromosome 5q33.3	Newman et al, 2010; Deelen et al, 2011; Nebel et al, 2011; Sebastiani et al, 2012; Deelen

				et al., 2014; Broer et al, 2015; Zeng et al, 2016
6	Large population-based linkage studies	Analysis of linkage with longevity in candidate genomic regions	Association with longevity for *APOE/TOMM40/APOC1* locus; additional loci at 14q11.2, 17q12-q22, and 19p13.3-p13.1	Beekman et al., 2013
7	iGWAS (informed GWAS)	Analysis of disease-related genetic variants, selected among high-ranked results of large studies of disease and disease-related traits	Association with longevity for: *APOE/TOMM40* (associated with Alzheimer's disease), *CDKN2B/ANRIL* (regulating the cellular senescence), *ABO* (tagging the O blood group), and *SH2B3/ATXN2* (regulatind Drosophila's lifespan and involved in neurological disease).	Fortney et al, 2015
8	GWAS in exceptionally healthy aging families	Genetic analysis in families stratified for "Healthy Aging Index" (HAI)*	*ZNF704* is a candidate gene for aging in women	Minster et al, 2015
9	Functional-association approach	Measure of differential gene expression between offspring of long-lived Amish and their spouses	*PAPSS2* expression is higher in offspring of nonagenarians	Yerges-Armstrong et al, 2016
10	Study of mitocondrial genome	Re-sequencing of mtDNA in long-lived sample from different population	Co-occurrence of mtDNA mutations affects longevity in population-specific way	Raule et al, 2014
11	Pathway-based approach	Combined analysis of SNP sets at multiple genes by different methods:	Conserved mechanisms are associated with survival along the	Wang et al, 2011;

		PLINK set-based test, Global test, GRASS, SNP ratio test, competitive gene-set enrichment analysis (GSEA). Analysis of sub-processes to discriminate epistatic effects.	evolutionary scale: stress response, telomere maintenance, DNA repair, INS/IGF1 pathways	Deelen et al, 2013; Debrabant et al, 2014