A Trait of Longevity: The Microbiota of Centenarians

A trait of longevity: the microbiota of centenarians

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Abstract

Centenarians, as striking examples of individuals who reach the extreme limits of human lifespan, are a valuable model for studying how the microbiota-component can successfully maintain or re-establish a mutualistic relationship with the human host, along with the occurrence of age-related variations. Indeed, the gut microbiota of centenarians emerges as a peculiar ecosystem, different from that of younger elderly and adults, specifically adapted to an extremely aged host. The study of the centenarians' gut microbiota provided in the last decade a large amount of remarkable data, from different populations across the world, summarized in the present chapter. Comparison between data from different study populations pointed out that, while lifestyle, ethnicity and geography surely have an impact on such extreme microbiota adaptive variations, common signatures of longevity emerge among the studied populations. The possibilities to exploit such data for human health maintenance during aging are still being explored and interesting scenarios are being envisioned, from the

bioprospecting of age-specific probiotic bacteria to the possibility of using microbiota transplantation to promote those features in the gut ecosystem that are known to be linked to longevity.

Glossary

Aging: the intricate and complex biological phenomenon represented by an age-dependent decline in intrinsic physiological function and adaptive capacity leading to an increase in age-specific diseases and mortality rate (i.e., a decrease in survival rate) (Franceschi et al., 2018b).

Longevity: represents the successful side of aging and is a complex, dynamic and multifactorial phenomenon resulting from the peculiar reciprocal interaction between environment, genetics, epigenetics and stochasticity (Franceschi et al., 2018b).

Centenarians: people who have reached the extreme limits of human life by successfully adapting to a variety of stressors who were exposed lifelong and remodelling to increase robustness and thus escape, postpone or survive to the major age-related diseases (Franceschi et al., 2000; Franceschi et al., 2017).

Lifespan: The length of time for which an organism lives (from birth to death).

Healthspan: The length of time for which an organism lives without diseases.

Immunosenescence: the progressive decline in the functionality of the immune system that accompany the advancement of age (Fulop et al., 2018).

Inflammaging: the peculiar, low-grade, chronic, and sterile inflammatory state that characterizes old age and substantially contribute to the progression of the aging process and age-associated diseases (Franceschi et al., 2018a).

1. The centenarians as model: why do we study the microbiota of the long lived?

A relatively small portion of the human population shows the capability to reach and cross the extreme limits of the species lifespan, a phenotypical trait that is called longevity (van den Berg et al., 2017). Few other mammals have been studied in this perspective and decades of observations allowed to define longevity as a complex phenomenon resulting from the intricate network of gene-environment (G×E) interactions, as well as other stochastic factors (Giuliani et al., 2018). Such complexity is even intensified by the fact that the human nuclear genome is not the only one residing within the human organism. Indeed, the mitochondrial DNA (mtDNA) and the cumulative genome of our microbial counterpart (microbiome) actively impact on our health along our whole life and during the aging process, consequently affecting our chances to attain longevity. The different genetic components interact among themselves, as well as with the external environment (Goodrich et al., 2017; Giuliani et al., 2018; Han et al., 2019). The microbiota has been described as an entity being "at the interface between the environment and our internal world", and having a marked ability to adapt itself (i.e. its functionality, as well as its temporary composition in terms of bacterial species) to a changing environment. This huge adaptive potential contributes to the maintenance of an optimal relationship between our body and the external world (Flandroy et al., 2018).

In this scenario, variations in the microbiota have been proposed to be part of a dynamic, adaptive process of the human "superorganism" (i.e. the sum of the human and all microbes that inhabits its body niches, also called "metaorganism") to both physiological and pathological unavoidable changes that occur lifelong. In other words, the gut microbiota is an element of higher plasticity, with respect to the human genetic asset, that helps in guaranteeing the superorganism adaptation to age-specific demands and thus to health and fitness (Santoro et al., 2020). According to this ecological and evolutionary view, the centenarians, as striking example of individuals who "successfully" get to the extreme limits of human lifespan, surely represent an incredibly valuable study model. Indeed, in this perspective, the centenarians microbiota somehow managed to efficiently and progressively re-

establish a mutualistic relationship with the host, along with the occurrence of progressive, age-related internal (physiological and pathological) and environmental (lifestyle and diet among others) variations (Biagi et al., 2017).

Long-living people are considered the paradigm of "healthy aging", being able to escape or survive most of the age-accompanying co-morbidities (Cevenini et al., 2008). Centenarians are indeed characterized by an uncommonly healthy phenotype with low blood pressure, optimal metabolic and endocrine markers, low number of diseases, and very wholesome nutritional and lifestyle habits (Franceschi et al., 2018b). The exceptional phenotype of centenarians has been described as extremely complex, context-dependent and very dynamic, uniquely merging the aspects of adaptive robustness and accumulating frailty, with the centenarian's metaorganism manifesting the capability to respond/adapt to both internal and external damaging stimuli (Santoro et al., 2018). For these reasons, it has been a natural course for the study of human aging and longevity, to direct the attention towards the microbiota of exceptionally old people, with the aim to provide insights on how the gut microbiota successfully adapts and contribute to the maintenance of health and promotion of survival beyond the human average life expectancy. Given the increasing importance that the scientific community is devoting to the lifelong relationship between human and microbes for our health (Knight et al., 2017), the questions that studies on centenarians' microbiomes are attempting to answer are the following:

a) what happens when this lifelong relationship is prolonged beyond the average human life duration?
b) does it happen in all human population or it depends on lifestyle, geography, ethnicity and so on?
c) what are the consequences for human physiology?
d) which is the contribution to longevity?
e) and, finally, can such knowledge be exploited in promoting healthy aging?

2. The state of the art for microbiota and longevity: what has been done (how, where and when).

In order to answer to the first question (what happens when the microbiome-host relationship is prolonged beyond the average life) researchers had to face the fact that longitudinal studies on human aging and longevity are, up to date, unfeasible: such studies would need a timeframe that is longer than the life of the researchers themselves. Moreover, studies should focus on a cohort impressively large to be sure to "obtain", at the end, an appreciable number of centenarians. However, centenarians are a model of extreme phenotype, a sort of highly informative "supercontrol" that is supposed to maximise the results when the aim is to study age-related and longevity-related traits, increasing the research power. For this reason, many clinical studies have demonstrated that the study of centenarians themselves, even without the possibility to obtain measurements or samples from their past, provides priceless information about human aging, including the last 20–30 years of human life that are usually neglected (Santoro et al., 2018).

Thus, first and foremost, you need to get centenarian's samples. In particular, since the gut microbiota is the most extensively studied and the one offering the wider expertise and comparative datasets worldwide, you often need to obtain fecal samples.

Human life expectancy has increased with an impressive rate during the last couple of centuries, making it ever less exceptional to encounter people counting more than 100 years (Partridge et al., 2018), even if they continue to represent a very small percentage of the total population. Demographic projections estimated to reach 3.7 million centenarians worldwide in 2050, with the higher concentration located in China, followed by Japan, the United States, Italy and India (Department of Economic and Social Affairs, 2015). In fact, the distribution of the longevity population is geographically clustered, defining what is known as longevity regions (also called "blue zones", i.e. where the percentage of people reaching the age of 100 is several times greater than in other areas).

The populations living in the blue zones have been thoroughly studied for what concern their lifestyle, genetics, environment and cultural aspects (Buettner & Skemp, 2016).

Thanks to the higher prevalence of centenarians and the availability of metadata, some longevity regions have been chosen to study the relationship between gut microbiota and longevity during the last decade. Indeed, the available studies on the gut microbiota of centenarians have been carried on by enrolling subjects in Italy (Biagi et al., 2010; Biagi et al., 2016; Wu et al., 2019; Rampelli et al., 2020; Wu et al., 2020), China (Wang F et al., 2015a, 2015b; Kong et al., 2016; Wang et al., 2019; Luan et al., 2020), Japan (Odamaki et al., 2016), India (Tuikhar et al., 2019) and, only lately, Russia (Kashtanova et al., 2020) (Fig. 1).

The first study published on the microbiota of centenarians was the one from Biagi et al. (2010). The authors chose to compare a group of Italian centenarians, with elderly people (65 years old approximately) and young adults from the same, small geographic area, the Bologna surrounding area in Italy. This area, even if it is not traditionally considered a "blue zone" like Sardinia island (Buettner & Skemp, 2016) showed one of the highest centenarian's prevalence (i.e. number of centenarians per 100,000 inhabitants) in Europe (Istat, 2015, http://demo.istat.it/; Biagi et al., 2016). The choice of using elderly and young adults from the same geographic area and belonging to the same ethnicity as control groups was aimed at reducing the lifestyle and environment confounding effects in exploring the association between microbiota features and longevity. Such design has been replicated in many of the following studies on longevity and microbiota (Biagi et al., 2016; Kong et al., 2016; Tuikhar et al., 2019; Wu et al., 2019; Wu et al., 2020), even in full awareness that it is not free of possible biases: for instance, this design does not allow to understand if the peculiarities of the gut microbiota found in long-living individuals were already present at a younger age or, on the contrary, they were (re)acquired later on (Biagi et al., 2017), with considerable impact on the interpretability of the results. The problem is that, when human longevity is concerned, longitudinal studies are far from being feasible, leading the scientific community to currently accept this type of comparative studies as the best possible approximation of gut microbiota trajectory along the human life.

The first study of Biagi et al. (2010) was performed using a microarray-based technique, which is hardly comparable with the following studies based on 16S rRNA gene sequencing technologies. However, the study allowed to highlight that centenarians were to be considered a separate group from adults and younger elderly in terms of gut microbiota composition, with their own compositional peculiarities. The same cohort was reanalysed few years later using Illumina sequencing technique based on 16S rRNA gene, which was considered the golden standard for human gut microbiome analysis (Biagi et al., 2016). An even more exceptional group of subjects was added to the cohort, *i.e.* a group of semi-supercentenarians, namely people >105 years old, enrolled from the same restricted geographic area. The elongation of the covered lifespan, and the updated microbiota characterization technique, allowed the Authors to describe a trajectory of the microbiota compositional modifications along with aging and extreme aging. In this work, the Authors reported that exceptional survivors, like centenarians and supercentenarians, showed all the modifications that are known to be associated with aging itself (Biagi et al., 2012, 2013), namely the reduction in the abundance of known health-promoting bacteria belonging to the genera *Faecalibacterium, Roseburia, Coprococcus*, associated to an increase in the proportion of subdominant species, including putative pro-inflammatory bacteria, such as those belonging to the *Enterobacteriaceae* and *Desulfovibrionaceae* families. At the same time, the gut microbiota of centenarians and, especially, semi-supercentenarians showed some peculiarities that, based on the available literature, might be able to contribute somehow to the maintenance of health during the extreme phases of aging. In fact, the gut microbiota of the extremely old presented higher prevalence of *Bifidobacterium*, a long time known probiotic group of bacteria, as well as higher abundances of subdominant members of the human gut ecosystem that have been explored only recently, namely *Akkermansia* and uncultured members of the family *Christensenellaceae*. *Akkermansia muciniphila* is a mucin-degrading bacterium of the phylum Verrucomicrobia, whose abundance in the human gut has been inversely correlated to several disease states, especially related to the host's metabolic homeostasis (Everard et al., 2013; Greer et al., 2016; Derrien et al., 2017; Geerlings et al., 2018). *Christensenellaceae* are a less known family of the human

gut that, like *Akkermansia*, has been associated to a state of metabolic health for the host, for instance being correlated to a lean phenotype and a reduced visceral adipose tissue even if the biological mechanisms of such correlation are yet to be discovered (Oki et al., 2016; Tavella et al., 2021). Curiously, *Christensenella* also resulted one of the mostly heritable taxa in the human gut microbiota (Goodrich et al., 2014, 2017) offering an interesting parallel with the fact that human longevity has a recognized genetic component (Schoenmaker et al., 2006; Sebastiani & Perls, 2012). The mechanism by which such bacteria could have been involved in health preservation during aging is currently under exploration, even if the studies need to rely mostly on animal models. For instance, in a recent work the microbiota of old mice was transferred in young germ-free mice: the microbiota of old mice induced inflammation in the young one, but inflammation levels were negatively correlated with the abundance of *Akkermansia* in the original microbiota (Fransen et al., 2017). The counteraction of systemic inflammation is indeed one of the most hypothesized methods by which the microbiota is supposed to contribute to healthy aging, *i.e.* by dampening the progression of the low-grade chronic inflammatory states that characterizes the elderly, called "inflammaging" (Biagi et al., 2013; Franceschi et al., 2018a). *Akkermansia* and *Christensenella* are indeed listed among the so-called "next generation probiotics", *i.e.* bacteria other than the traditional *Lactobacillus* and *Bifidobacterium*, which could become part of more modern and targeted probiotic strategies (O'Toole et al., 2017; Chang et al., 2019).

Such interesting findings were paralleled by the publication of analogous studies focused on the microbiota of centenarians living in other longevity spots, in particular in rural or isolated areas of China and Japan (Kong et al., 2016; Odamaki et al., 2016). These long living populations have a very different nutritional habits, lifestyle pattern, as well as genetic background, from the Italian population analyzed by Biagi et al. (2010, 2016). In such scenario, an important question arose: does a universal longevity signature exist in the human gut microbiota? Or is it masked by the impact of confounding variables, *i.e.* lifestyle, geography, and genetics, on microbiota composition? The comparison between all the long-living subjects analyzed by the international scientific community

is offering interesting answers, which will become even more reliable with the increasing amount of data produced by microbiome studies worldwide. Indeed, next-generation sequencing and bioinformatics, thanks to approaches able to correct for possible study biases, allow for analyzing data coming from different studies and, possibly, for providing new, interesting conclusions.

Kong et al. (2016) provided the comparison between the study performed on Italian centenarians and the data obtained from Sichuan province, in China. The microbiota of both Italian and Chinese centenarians showed higher biodiversity with respect to the young controls from the same country and, most interestingly, in both cohorts centenarians showed higher abundance of sequences assigned to *Akkermansia*, and to uncultured bacteria belonging to the families *Christensenellaceae* and *Ruminococcaceae*.

The comparison between Italians and Japanese was concomitantly provided by Biagi et al. (2017), using the data published by Biagi et al. (2016) and Odamaki et al. (2016). A common trajectory was indeed found by this analysis, that confirmed that the reduction of *Faecalibacterium* could be a shared aging signature and that the oldest old people seems to be enriched in unclassified members of the *Ruminococcaceae* family, finding in this family a common ground with the signature provided by Kong et al. (2016) in Chinese and Italian centenarians.

Later on, a study involving centenarians from a rural area of India was published (Tuikhar et al., 2019) and the results were compared to all those available at the moment: Italian (Biagi et al., 2016), Chinese (Kong et al., 2016) and Japanese centenarians (Odamaki et al., 2016). The Indian study population showed its own peculiarity, with an unprecedented enrichment in *Erysipelotrichaceae*, *Enterobacteriaceae* and *Lactobacillaceae*, traits possibly due to the different lifestyle and nutritional habits. In spite of this distinctiveness, the Authors confirmed that centenarians showed higher gut biodiversity than younger people (as previously reported by Kong et al., 2016, 2019) and highlighted that the internal biodiversity within the family *Ruminococcaceae* was most strikingly higher in centenarians from all considered countries. Moreover, offering a parallel with the observation of Kong et al. (2016) and Biagi et al. (2017), a high abundance of an unclassified *Ruminococcaceae*

species, previously reported as putative major butyrate producer, was found being a common longevity signature across four populations that are very different in terms of ethnicity, genetics, lifestyle, diet and culture. As stated by the Authors, this increased biodiversity within such a metabolically relevant family of the human gut ecosystem, might point to a high metabolic plasticity and versatility of the microbiome of long-living individuals.

Even more recently, Russian centenarians were taken into account (Kashtanova et al., 2020). They showed more similarity to the Italian centenarians' cohort (Biagi et al., 2016) than to the Japanese centenarians (Odamaki et al., 2016) and were reported to be enriched in *Ruminococcaceae* and *Christensenellaceae* with respect to younger elderly from the same geographic area.

In conclusion, looking at all the data and comparisons available to date for the centenarians gut microbiota composition, we could summarize that, even if the microbiota signature of longevity seems to be as context-dependent as the genetics of human longevity (Giuliani et al., 2018), common aspects can be found among populations that are very far from each other in terms of geography, culture and genetics. However, the population living in the longevity spots that have been taken into account so far represent only a minor portion of the human population (Fig. 1). Since ethnicity and geography were reported to strongly impact the gut microbiota composition (Deschasaux et al., 2018; He et al., 2018), long living groups of people from other parts of the world (especially African and American continents) will hopefully be analyzed in future, progressively allowing for the definition of an ever more global microbiota longevity signature.

During the last few years, the study of the human microbiome progressively entered the so-called "metagenomics era", in which, beside the composition of the gut ecosystem, the functions performed by the microorganisms are explored, bringing the researchers potentially closer at answering others of the above listed questions about microbiota and longevity: what are the consequences for the human physiology? how can it contribute to longevity? On the long term, when additional metagenomics studies will be available on long-living individuals from different places across the

globe, functional common signature of longevity might reveal how bacteria, even if belonging to different species, can contribute with their metabolism to the longevity phenotype.

Up to date, only Italian centenarians have been explored in this perspective, from two different cohorts: the previously discussed Bologna cohort (Rampelli et al., 2020) and a cohort from Sardinia island, a well-known blue-zone in the Mediterranean Sea (Wu et al., 2019). Both the Italian cohorts were explored also by 16S sequencing and proved to have similarities in the age-dependent trajectory of gut microbiota composition and in the presence of age-associated, as well as longevity associated, signatures (Wu et al., 2020). The work performed by Wu et al (2019) on the Sardinian elderly and centenarians was mostly focused on variations on metabolic pathways in the gut metagenome. They highlighted that the gut metagenome of centenarians showed potential health-promoting signatures, most importantly an augmented capability for glycolysis and short-chain fatty acids (SCFA) production, the latter being health-promoting fermentation products of gut microbes, able to positively influence immune system homeostasis and inflammatory status of the host (Koh et al., 2016). In the opinion of the Authors, such features of microbial metabolisms might be involved in boosting the chances of the host to maintain and prolong its metabolic and immunological health, ultimately favoring longevity. Indeed, such variation would be balancing other pro-inflammatory traits, such as a shrinkage in the abundance of genes involved in the degradation of carbohydrates, that were still found in centenarians and could be considered “maladaptive” for the extremely aged human host (Wu et al., 2019). The work of Rampelli et al. (2020) on the Bologna cohort, partly confirming the decrease in carbohydrate metabolism in centenarians highlighted by Wu et al (2019), also pointed out that long living individuals showed a gut metagenome enriched in functions related to the degradation of xenobiotics, such as ethylbenzene, chlorobenzene, toluene and other pervasive environmental contaminants. Such molecules are generated during the processing of petroleum products, i.e. plastics, and are known to be more concentrated in indoor environments. The Authors postulates that the reduced mobility of centenarians, which bring them to a mostly indoor lifestyle, together with the long history of exposure to xenobiotic stressors, derived from their long life, might

have concurred in progressively selecting for a gut microbiome enriched in bacteria able to degrade such chemicals. This observation offer an interesting parallel with the results reported by Tuikhar et al (2019) from their analysis of gut metabolome of Indian centenarians: the intestinal environment of long living individuals showed indeed a lower load of some environmental chemical contaminants, and the Authors hypothesized that the gut microbiota of centenarians could provide enzymes to degrade such compounds.

Metagenomics was very recently used on the gut microbiome of centenarians to explore a different aspect, by changing, for the first time, the study design. Luan and colleagues (2020) proposed the first longitudinal sampling of gut microbiota of centenarians along more than one year, without comparing them with people of different age group, but stratifying them retrospectively based on the date of death. The Authors showed that significant changes in the gut microbiota composition become detectable from 7 months prior to death, with significant decrease of the abundance of several species, including the already associated to healthy aging *Akkermansia muciniphila*. The Authors speculated that such changes might occur before the clinical symptoms of deterioration in the health status of the enrolled long living individuals, pointing at the gut metagenome as a possible alarm bell for the health decline in aged people. It is still hard to place the findings coming from this unique work in a larger picture, but it is undeniable that this change in the approach can pave the way to future studies that tackle the mystery of longevity from a new perspective.

Concluding, it is quite clear that, from the functional point of view, many pieces are missing from the puzzle and it is hard to understand what the complete picture will be, i.e. how microbiome is connected to all the other aspects (i.e. genetics and environment) that define and influence the longevity phenotype. In particular, the progresses in computational science will have a strong impact on this aspect of aging sciences, since the more studies from across the world are published, the more variables are thrown on the plate together with the actual data. In this scenario, it is worth reminding that the gut microbiota is only part, even if the most thoroughly explored, of the total human microbiome, and that some other human body ecosystems (such as the skin, and the genito-urinary

tract) are now starting to be included in microbiome studies on long-living individuals (Wu et al., 2020). However, our ability to deal with big and complex data is increasing every day with an unprecedented speed, meaning that the day in which such knowledge could be sorted with and finally put into use, i.e. be exploited for actual health maintenance strategies, might not be so far.

3. Towards exploitation: how this knowledge might become of use?

In order to answer to this last, but not least, question (how the knowledge gained in the last decade about the gut microbiota of centenarians can become useful) we need to go back again to why exploring the gut microbiome of long living, successfully-aged people is of particular interest. Microbiome of aged people is featured by traits that strongly correlate with age-associated phenomena, such as immunosenescence, the progressive decline of the immune system functionality, and inflammaging which are both necessary to extend longevity (Fulop et al., 2018). This aspect is thoroughly explored in several review articles published in the last few years (such as Biagi et al., 2012, 2013; O'Toole and Jeffery, 2015; Partridge et al., 2018; Bana and Cabreiro, 2019; Badal et al., 2020; Santoro et al., 2020). Such traits can be briefly, even if not exhaustively, summarized in the reduction of biodiversity of the gut microbiota, a decrease in known health-promoting bacteria able to produce SCFA and positively influence the immunological homeostasis of the host, accompanied by an increase in species able to thrive in an inflamed environment and contribute to the overall inflammation, such as enterobacteria and other LPS-producing groups. Such variations occur in association to the physiological changes of the aging gut, supporting inflammation in turn, in a sort of self-sustaining loop that makes the resulting "age-associated" microbiota structure hardly modifiable (Biagi et al., 2013; DeJong et al., 2020). The hope that drove the research in the field of microbiota and longevity was to find how the gut microbiota can successfully adapt to such

compromised situation, and acquire or preserve – hard to tell which one, due to the unfeasibility of longitudinal studies – other, different traits that could sustain healthy aging and promote longevity.

The state of the art summarized in the previous paragraph show that this might indeed be the case: the centenarians gut microbiome has peculiarities that could prove to be health promoting and that are not found in younger elderly, the most recurring of which is the presence of a higher biodiversity, regarding the whole gut microbiota (Kong et al., 2016, 2018) or only a bacterial subpopulation, as highlighted by (Tuikhar et al., 2019). The biodiversity of a bacterial ecosystem is strictly related to its resilience, i.e. its resistance against disruptive, disbiotic changes (Sommer et al., 2017) and tendency to return to a previous, balanced structure after a stressing event. Resilience is a relevant concept when we are trying to define health not as merely the absence of disease, but as the capacity of a living being to respond and recover after relevant stresses. Indeed, it has been proposed that successfully aged individuals do not escape physiological decline and age-related diseases, but they are characterized by a higher enough resilience to effectively slow down these processes (Borras et al., 2020). Indeed, the phenotype of old people is very dynamic because it is characterized by a high capacity to respond and adapt to internal and external detrimental stimuli that an individual is exposed lifelong. This phenomenon has been known as “remodelling” (Franceschi et al., 1995).

The resilience of the gut bacterial ecosystem and the resilience of the aging human hosts might be interconnected. In other words, the biodiversity of the gut microbiota, by guaranteeing a good enough level of resilience of the gut ecosystem, could be part of the complex process of maintaining the resilience, and the health, of the human being. As a consequence, strategies to maintain health as long as possible for the elderly should surely include means for maintaining elevated levels of biodiversity in the gut microbiota.

In this perspective, it has been repeatedly proposed that interventions based on diet and supplements of pro/prebiotics could be useful to maintain microbiota diversity, it is a very general concept and there are still few evidences regarding the pervasiveness of such effects on the long term (Leeming et al., 2019). Indeed, the hope in studying centenarians was to obtain more specific hints regarding

aging, such as identifying commensal microbes strongly related to the maintenance of health during aging that could be isolated, studied and reintroduced (DeJong et al., 2020).

Akkermansia is among those bacteria that were found enriched in centenarians (Biagi et al., 2016; Kong et al., 2016) and its decrease was recently pointed out as a marker for health decline (Luan et al., 2020) in extremely aged people. In addition, several studies on animal models seems to confirm the ability of *Akkermansia* to provide benefits to the host, marking this bacterium as a promising candidate for the so-called next generation probiotics (Gomez-Gallago et al., 2016; Cani & de Vos, 2017; Sanders et al., 2019). Taken together, the data available point at *Akkermansia* as an excellent candidate for "aging-specific probiotics" and, surely, studies performed on other species, such as *Christensenella*, will, in due time, add candidates to such list.

However, this is not the only possible path towards the development of probiotic strategies. Indeed, instead of focusing on known species and strains that studies reported as associated to the longest living individuals, a possibility is to directly isolate strains from samples taken from healthy centenarians. This approach is not novel: strains of *Bifidobacterium* and *Lactobacillus* have been isolated from centenarians fecal samples by different research group, and they were brought to the public attention as having increased health promoting functionalities of being able to perform specific metabolic functions (Hao et al., 2011; Shen et al., 2011; An et al., 2014; Liu et al., 2014, 2015; Sun et al., 2015; Nicola et al., 2016; Jiang et al., 2019; Zhang et al., 2019; Dong et al., 2020; Huang et al., 2020; Jin et al., 2020). For instance, the strains of *Bifidobacterium* isolated from centenarians by Shen and colleagues (2011) and by Huang et al (2020) have been observed to possess marked antioxidant activity both in vitro and in vivo. Other *Bifidobacterium* strains isolated from centenarians have been studied for their peculiar ability to stimulate immune system (Nicola et al., 2016), for their high acid resistance ability (Sun et al., 2015) or for their bile stress response (An et al., 2014). Such strains could prove to be specifically adapted to the aging host and could be able to provide specific benefits, in framework of their specific inflammatory status, metabolic balance and peculiar lifestyle. The

translation of such strains can end up in proposing innovate solutions in the context of targeted prevention and personalization of medical care.

On the other hand, currently, the most promising strategies for geroprotection tends to include the possibility of transferring the whole microbiome, instead of trying to modulating it by means of diet or probiotics. Recently, data have been provided showing effectiveness and safety of fecal microbiota transplantation (FMT) in the prevention and treatment of age-related pathological conditions (i.e. atherosclerosis, type 2 diabetes and Parkinson's disease) (Vaiserman et al., 2017). It has been shown that by transplanting the gut microbiota of long-living individual into mouse models improved their general health, reduces aging-related indices and transfers beneficial bacteria (Chen et al., 2020). Studies on mice and other animals have also been performed in order to understand if fecal transplantation might also impact on promoting longevity, by increasing lifespan (Callaway, 2017). For instance, by transplanting wild-type, normal microbiota into mouse model of accelerated aging (progeroid mice) Barcena and colleagues (2019) demonstrated that healthspan and lifespan of the progeroid individuals increased, and that similar results could be obtained also by transferring *Akkermansia* only, a species reported to be associated to longevity and one of the most interesting putative next-generation probiotics. Such interesting results seems to point at a possible future in which fecal transplantation or other innovative microbiome-modulating strategies (e.g. isolation and transplantation of reduced communities from healthy donors, artificial evolution of microbial communities, separation and administration of beneficial and modulatory metabolites from feces, etc) might become used for improving and maintaining health during aging, as well as enlarging human health span and lifespan. However, despite the great therapeutic potential of the FMT procedure, its implementation in clinical practice, especially in aging people, is still limited by several concerns, including donor screening, limited viability of fresh stool samples, fears about potential pathogen transmission, lack of a standardized treatment regimen, and patients not consenting to be treated (Choi & Cho, 2016).

4. Conclusions and perspectives

The first three paragraphs of this chapter were aimed at summarizing the actual knowledge about the gut microbiota of the oldest people, as well as give a comprehensive view of the reasons for the interests that the scientific community showed for this particular research field and the possible applications of the knowledge that this relatively young research field is gaining.

Before concluding, we would like to focus also on possible misinterpretation of the results obtained by the studies presented above, using hypothetical questions.

1) The fact that the microbiota of centenarians shows potentially health-promoting peculiarities makes it somehow "younger" that the microbiota of "normal elderly"? The answer is no: the microbiota of centenarians seems to retain all the modifications commonly induced by the age advancement, it is equally compromised by the age-related phenomena as the microbiota of people 70 or 80 years old with comparable health status. Also, the health promoting peculiarities detected by the studies performed up to date could be partially population-specific and be linked to the specific context of the enrolled long-living individuals.
2) Is the microbiome of centenarians somehow "better" than the microbiota of people of different age or with poor health status? Again, the answer is no. The gut microbiota of centenarians could be considered "better adapted" to the physiological changes that accompany the aging process. In other words, centenarians' microbiome might "find alternative solution" (i.e. provide alternative metabolic functions) to obtain an improved performance than that of not-long-lived elderly.
3) Would the microbiome of centenarians be "good for everyone"? The answer is another "no". By transplanting the microbiota of centenarians into young people not only would not provide any benefit, but we would probably damage the recipient. The microbiota found in long lived individuals is specifically adapted to the extreme aging process

Regarding this last point, however, the gut microbiota of centenarians might become a good place to look at for operating a sort of "bioprospecting", i.e. the search for unknown microbial functions in the metagenome. Indeed, centenarians, expanding the average duration of the symbiosis between human and microbiome and providing a sort of "extreme environment" (i.e. extremely old, extremely modified physiology) could allow for the emergence of microbial functions that are not detected in the microbiome of younger people. Such functions could not just be useful to the centenarians but, if thoroughly explored and correctly exploited, they could become relevant for promoting health in different situations.

In conclusion, the questions that studies on centenarians' microbiomes are attempting to tackle (see first paragraph) have not been answered completely, yet. However, many steps forward have been taken and this particular research field can now count on a small body of solid literature (see second paragraph), the peculiarity of which is the common effort put by the different research group into comparing each other results (Kong et al., 2016; Biagi et al., 2017; Tuikhar et al., 2019; Luan et al., 2020). Such attempt is surely going to ensure that more studies in the field are correctly designed to provide pieces of information that are still missing from the puzzle. The exploitation of the data obtained by studies on centenarians microbiome is still in its infancy (see paragraph number three), even if the progressively increasing age of the human population, as well as the public interest into maintaining health as long as possible while people grow old, is prompting the research field to move from simple observation to more focused studies, exploring for instance, the relationship between microbiome variations in healthy centenarians and the type of medications they underwent, the features of the environment in which they lived in the past, as well as in the present, and, most importantly, their dietary habits.

References

An, H., Douillard, F. P., Wang, G., et al. (2014). Integrated transcriptomic and proteomic analysis of the bile stress response in a centenarian-originated probiotic *Bifidobacterium longum* BBMN68. *Molecular & Cellular Proteomics* **13(10),** 2558–2572.

Badal, V.D., Vaccariello, E.D., Murray, E.R., et al. (2020). The gut microbiome, aging, and longevity: a systematic review. *Nutrients* **12(12)**, 3759.

Bana, B., & Cabreiro, F. (2019). The microbiome and aging. *Annual Review of Genetics* **53,** 239–261. Ht

Bárcena, C., Valdés-Mas, R., Mayoral, P., et al. (2019). Healthspan and lifespan extension by fecal microbiota transplantation into progeroid mice. *Nature Medicine* **25(8),** 1234–1242.

Biagi, E., Nylund, L., Candela, M., et al. (2010). Through aging, and beyond: gut microbiota and inflammatory status in seniors and centenarians. *PloS One* **5(5),** e10667.

Biagi, E., Candela, M., Fairweather-Tait, S., Franceschi, C., & Brigidi, P. (2012). Aging of the human metaorganism: the microbial counterpart. *Age (Dordrecht, Netherlands)* **34(1),** 247–267.

Biagi, E., Candela, M., Turroni, S., et al. (2013). Aging and gut microbes: perspectives for health maintenance and longevity. *Pharmacological Research* **69(1),** 11–20.

Biagi, E., Franceschi, C., Rampelli, S., et al. (2016). Gut microbiota and extreme longevity. *Current Biology* **26(11),** 1480–1485.

Biagi, E., Rampelli, S., Turroni, S., et al. (2017). The gut microbiota of centenarians: signatures of longevity in the gut microbiota profile. *Mechanisms of Aging and Development* **165(Pt B),** 180–184.

Borras, C., Ingles, M., Mas-Bargues, C., et al. (2020). Centenarians: An excellent example of resilience for successful aging. *Mechanisms of Aging and Development* **186,** 111199.

Buettner, D., & Skemp, S. (2016). Blue Zones: Lessons From the World's Longest Lived. *American Journal of Lifestyle Medicine* **10(5),** 318–321.

Callaway E. (2017). 'Young poo' makes aged fish live longer. *Nature* **544(7649),** 147.

Cani, P. D., & de Vos, W. M. (2017). Next-Generation beneficial microbes: the case of *Akkermansia muciniphila*. *Frontiers in Microbiology* **8,** 1765.

Cevenini, E., Invidia, L., Lescai, F., et al. (2008). Human models of aging and longevity. *Expert Opinion on Biological Therapy* **8(9),** 1393–1405.

Chang, C. J., Lin, T. L., Tsai, Y. L., et al. (2019). Next generation probiotics in disease amelioration. *Journal of Food and Drug Analysis* **27(3),** 615–622.

Chen, Y., Zhang, S., Zeng, B., et al. (2020). Transplant of microbiota from long-living people to mice reduces aging-related indices and transfers beneficial bacteria. *Aging* **12(6),** 4778–4793.

Choi, H. H., & Cho, Y. S. (2016). Fecal microbiota transplantation: current applications, effectiveness, and future perspectives. *Clinical Endoscopy* **49(3),** 257–265.

DeJong, E. N., Surette, M. G., & Bowdish, D. (2020). The gut microbiota and unhealthy aging: disentangling cause from consequence. *Cell Host & Microbe* **28(2),** 180–189.

Department of Economic and Social Affairs, Population Division (2015) World population prospects: The 2015 revision. Key findings and advance tables. Working paper no. ESA/P/WP.241. United Nation, New York

Derrien, M., Belzer, C., & de Vos, W. M. (2017). *Akkermansia muciniphila* and its role in regulating host functions. *Microbial Pathogenesis* **106,** 171–181.

Deschasaux M, Bouter KE, Prodan A, et al. (2018). Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. *Nature Medicine* **24(10),** 1526-1531.

Dong, Y., Zhu, J., Zhang, M., Ge, S., & Zhao, L. (2020). Probiotic *Lactobacillus salivarius* Ren prevent dimethylhydrazine-induced colorectal cancer through protein kinase B inhibition. *Applied Microbiology and Biotechnology* **104(17),** 7377–7389.

Everard, A., Belzer, C., Geurts, L., et al. (2013). Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proceedings of the National Academy of Sciences of the United States of America* **110(22),** 9066–9071.

Flandroy, L., Poutahidis, T., Berg, G., et al. (2018). The impact of human activities and lifestyles on the interlinked microbiota and health of humans and of ecosystems. *The Science of the Total Environment* **627,** 1018–1038.

Franceschi, C., Monti, D., Barbieri, D., et al. (1995). Immunosenescence in humans: deterioration or remodelling? *International Reviews of Immunology* **12(1),** 57–74.

Franceschi, C., Valensin, S., Bonafè, M., et al. (2000). The network and the remodeling theories of aging: historical background and new perspectives. *Experimental Gerontology* **35(6-7),** 879–896.

Franceschi, C., Passarino, G., Mari, D., & Monti, D. (2017). Centenarians as a 21st century healthy aging model: A legacy of humanity and the need for a world-wide consortium (WWC100+). *Mechanisms of Ageing and Development* **165(Pt B),** 55–58.

Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., & Santoro, A. (2018a). Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nature Reviews Endocrinology* **14(10),** 576–590.

Franceschi, C., Ostan, R., Santoro, A. (2018b) Nutrition and inflammation: are centenarians calorie-restricted like individuals? *Annual Review of Nutrition* **38,** 329-356.

Fransen, F., van Beek, A. A., Borghuis, T., et al. (2017). Aged gut microbiota contributes to systemical inflammaging after transfer to germ-free mice. *Frontiers in Immunology* **8,** 1385.

Fulop, T., Larbi, A., Dupuis, G., et al. (2018). Immunosenescence and inflamm-aging as two sides of the same coin: friends or foes? *Frontiers in Immunology* **8,** 1960.

Geerlings, S. Y., Kostopoulos, I., de Vos, W. M., & Belzer, C. (2018). *Akkermansia muciniphila* in the human gastrointestinal tract: when, where, and how? *Microorganisms* **6(3),** 75.

Giuliani, C., Garagnani, P., & Franceschi, C. (2018). Genetics of human longevity within an eco-evolutionary nature-nurture framework. *Circulation Research* **123(7),** 745–772.

Gómez-Gallego, C., Pohl, S., Salminen, S., De Vos, W. M., & Kneifel, W. (2016). *Akkermansia muciniphila*: a novel functional microbe with probiotic properties. *Beneficial Microbes* **7(4),** 571–584.

Goodrich, J. K., Waters, J. L., Poole, A. C., et al. (2014). Human genetics shape the gut microbiome. *Cell* **159(4),** 789–799.

Goodrich, J. K., Davenport, E. R., Clark, A. G., & Ley, R. E. (2017). The relationship between the human genome and microbiome comes into view. *Annual Review of Genetics* **51,** 413–433.

Greer, R. L., Dong, X., Moraes, A. C., et al. (2016). *Akkermansia muciniphila* mediates negative effects of IFNγ on glucose metabolism. *Nature Communications* **7,** 13329.

Han, B., Lin, C. J., Hu, G., & Wang, M. C. (2019). 'Inside Out'- a dialogue between mitochondria and bacteria. *The FEBS Journal* **286(4),** 630–641.

Hao, Y., Huang, D., Guo, H., et al. (2011). Complete genome sequence of *Bifidobacterium longum* subsp. *longum* BBMN68, a new strain from a healthy chinese centenarian. *Journal of Bacteriology* **193(3),** 787–788.

He Y, Wu W, Zheng HM, et al. (2018). Regional variation limits applications of healthy gut microbiome reference ranges and disease models. *Nature Medicine* **24(10)**, 1532-1535.

Huang, G., Pan, H., Zhu, Z., & Li, Q. (2020). The complete genome sequence of *Bifidobacterium longum* LTBL16, a potential probiotic strain from healthy centenarians with strong antioxidant activity. *Genomics* **112(1),** 769–773.

Jiang, J., Feng, N., Zhang, C., et al. (2019). *Lactobacillus reuteri* A9 and *Lactobacillus mucosae* A13 isolated from Chinese superlongevity people modulate lipid metabolism in a hypercholesterolemia rat model. *FEMS Microbiology Letters* **366(24),** fnz254.

Jin, Z., Li, W., Wang, W., & Sun, B. (2020). Complete genome sequence of *Bifidobacterium adolescentis* ZJ2, isolated from a centenarian in Anhui, China. *Microbiology Resource Announcements* **9(29),** e00710-20.

Kashtanova, D. A., Klimenko, N. S., Strazhesko, I. D., et al. (2020). A cross-sectional study of the gut microbiota composition in Moscow long-livers. *Microorganisms* **8(8),** E1162.

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell* **165(6),** 1332–1345.

Knight, R., Callewaert, C., Marotz, C., et al. (2017). The Microbiome and human biology. *Annual Review of Genomics and Human Genetics* **18,** 65–86.

Kong, F., Hua, Y., Zeng, B., et al. (2016). Gut microbiota signatures of longevity. *Current Biology* **26(18),** R832–R833.

Kong, F., Deng, F., Li, Y., & Zhao, J. (2019). Identification of gut microbiome signatures associated with longevity provides a promising modulation target for healthy aging. *Gut microbes* **10(2),** 210–215.

Leeming, E. R., Johnson, A. J., Spector, T. D., & Le Roy, C. I. (2019). Effect of diet on the gut microbiota: rethinking intervention duration. *Nutrients* **11(12),** 2862.

Liu, L., Qin, Y., Wang, Y., et al. (2014). Complete genome sequence of *Bifidobacterium animalis* RH, a probiotic bacterium producing exopolysaccharides. *Journal of Biotechnology* **189,** 86–87.

Liu, S., Zhao, L., Ren, F., et al. (2015). Complete genome sequence of *Bifidobacterium adolesentis* BBMN23, a probiotic strain from healthy centenarian. *Journal of Biotechnology* **198,** 44–45.

Luan, Z., Sun, G., Huang, Y., et al. (2020). Metagenomics study reveals changes in gut microbiota in centenarians: a cohort study of hainan centenarians. *Frontiers in Microbiology* **11,** 1474.

Nicola, S., Amoruso, A., Deidda, F., et al. (2016). Searching for the perfect homeostasis: five strains of *Bifidobacterium longum* from centenarians have a similar behavior in the production of cytokines. *Journal of Clinical Gastroenterology* **50 Suppl 2,** S126–S130.

Odamaki, T., Kato, K., Sugahara, H., et al. (2016). Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. *BMC Microbiology* **16,** 90.

Oki, K., Toyama, M., Banno, T., et al. (2016). Comprehensive analysis of the fecal microbiota of healthy Japanese adults reveals a new bacterial lineage associated with a phenotype characterized by a high frequency of bowel movements and a lean body type. *BMC Microbiology* **16(1),** 284.

O'Toole, P. W., & Jeffery, I. B. (2015). Gut microbiota and aging. *Science* **350(6265),** 1214–1215.

O'Toole, P. W., Marchesi, J. R., & Hill, C. (2017). Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. *Nature Microbiology* **2,** 17057.

Partridge, L., Deelen, J., & Slagboom, P. E. (2018). Facing up to the global challenges of aging. *Nature* **561(7721),** 45–56.

Rampelli, S., Soverini, M., D'Amico, F., et al. (2020). Shotgun metagenomics of gut microbiota in humans with up to extreme longevity and the increasing role of xenobiotic degradation. *mSystems* **5(2),** e00124-20.

Sanders, M. E., Merenstein, D. J., Reid, G., Gibson, G. R., & Rastall, R. A. (2019). Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. *Nature Reviews. Gastroenterology & Hepatology* **16(10),** 605–616.

Santoro, A., Ostan, R., Candela, M., et al. (2018). Gut microbiota changes in the extreme decades of human life: a focus on centenarians. *Cellular and Molecular Life Sciences* **75(1),** 129–148.

Santoro, A., Zhao, J., Wu, L., et al. Microbiomes other than the gut: inflammaging and age-related diseases. *Seminars in Immunopathology*. doi: 10.1007/s00281-020-00814-z, In press.

Schoenmaker, M., de Craen, A. J., de Meijer, P. H., et al. (2006). Evidence of genetic enrichment for exceptional survival using a family approach: the Leiden Longevity Study. *European Journal of Human Genetics* **14(1),** 79–84.

Sebastiani, P., & Perls, T. T. (2012). The genetics of extreme longevity: lessons from the new England centenarian study. *Frontiers in Genetics* **3,** 277.

Shen, Q., Shang, N., & Li, P. (2011). In vitro and in vivo antioxidant activity of *Bifidobacterium animalis* 01 isolated from centenarians. *Current Microbiology* **62(4),** 1097–1103.

Sommer, F., Anderson, J. M., Bharti, R., Raes, J., & Rosenstiel, P. (2017). The resilience of the intestinal microbiota influences health and disease. *Nature Reviews Microbiology* **15(10),** 630–638.

Sun, E., Zhao, L., Ren, F., et al. (2015). Complete genome sequence of *Bifidobacterium animalis* subsp. *lactis* A6, a probiotic strain with high acid resistance ability. *Journal of Biotechnology* **200,** 8–9.

Tavella, T., Rampelli, S., Guidarelli, G., et al. (2020). Elevated gut microbiome abundance of *Christensenellaceae*, *Porphyromonadaceae* and *Rikenellaceae* is associated with reduced visceral adipose tissue and healthier metabolic profile in Italian elderly. *Gut Microbes* **13(1),** 1-19.

Tuikhar, N., Keisam, S., Labala, R. K., et al. (2019). Comparative analysis of the gut microbiota in centenarians and young adults shows a common signature across genotypically non-related populations. *Mechanisms of Aging and Development* **179,** 23–35.

Vaiserman, A. M., Koliada, A. K., & Marotta, F. (2017). Gut microbiota: a player in aging and a target for anti-aging intervention. *Aging Research Reviews* **35,** 36–45.

van den Berg, N., Beekman, M., Smith, K. R., Janssens, A., & Slagboom, P. E. (2017). Historical demography and longevity genetics: Back to the future. *Aging Research Reviews* **38,** 28–39.

Wang, F., Yu, T., Huang, G., et al. (2015a). Gut microbiota community and its assembly associated with age and diet in chinese centenarians. *Journal of Microbiology and Biotechnology* **25(8),** 1195–1204.

Wang, F., Huang, G., Cai, D., et al. (2015b). Qualitative and semiquantitative analysis of fecal *Bifidobacterium* species in centenarians living in Bama, Guangxi, China. *Current Microbiology* **71(1),** 143–149.

Wang, N., Li, R., Lin, H., et al. (2019). Enriched taxa were found among the gut microbiota of centenarians in East China. *PloS One* **14(10),** e0222763.

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4. https://ggplot2.tidyverse.org.

Wu, L., Zeng, T., Zinellu, A., et al. (2019). A cross-sectional study of compositional and functional profiles of gut microbiota in sardinian centenarians. *mSystems* **4(4),** e00325-19.

Wu, L., Zeng, T., Deligios, M., et al. (2020). Age-related variation of bacterial and fungal communities in different body habitats across the young, elderly, and centenarians in Sardinia. *mSphere* **5(1),** e00558-19.

Zhang, J., Wang, S., Zeng, Z., Qin, Y., & Li, P. (2019). The complete genome sequence of *Bifidobacterium animalis* subsp. *lactis* 01 and its integral components of antioxidant defense system. *3 Biotech* **9(10),** 352.

Figures

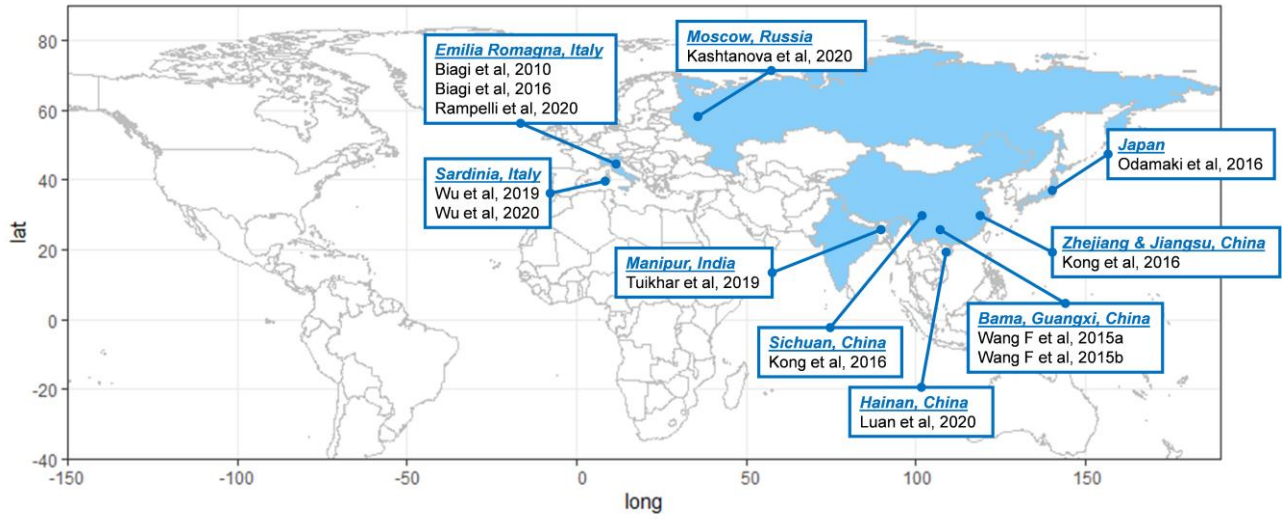

Figure 1. Geographical distribution of studies focused on the microbiota features of centenarians around the world. Represented countries are depicted in light blue. References and indication of the geographical region in which centenarians were enrolled are provided. World map has been obtained using the R statistical software (www.r-project.org), and the R packages ggplot2 (Wickham, 2016) and maps (https://cran.r-project.org/web/packages/maps/index.html).