Mechanisms of Ageing and Development 198 (2021) 111543

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Specific features of the oldest old from the Longevity Blue Zones in Ikaria and Sardinia

Michel Poulain[a,b,*], Anne Herm[b], Alessandra Errigo[c], Christina Chrysohoou[d], Romain Legrand[e], Giuseppe Passarino[f], Maria Antonietta Stazi[g], Konstantinos G. Voutekatis[h], Efstathios S. Gonos[h], Claudio Franceschi[i], Giovanni M. Pes[j]

[a] IACCHOS Université catholique de Louvain, Belgium
[b] Estonian Institute for Population Studies, Tallinn University, Estonia
[c] University of Sassari, Department of Biomedical Sciences, Sassari, Italy
[d] First Cardiology Clinic, Hippokratio Hospital, University of Athens, Greece
[e] Pôle Personnes âgées, Hospital of Champmaillot, University Hospital, Dijon, France
[f] Department of Cell Biology, University of Calabria, Rende, Italy
[g] Istituto Superiore di Sanità, Roma, Italy
[h] National Hellenic Research Foundation (N.H.R.F.), Institute of Biology, Medicinal Chemistry and Biotechnology, Athens, Greece
[i] Department of Applied Mathematics and Center of Bioinformatics, Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia
[j] University of Sassari, Department of Medical, Surgical and Experimental Sciences, Sassari, Italy

A R T I C L E I N F O

Keywords:
Population longevity
Longevity Blue Zones
Nonagenarians
Behavioral factors
Genetic markers

A B S T R A C T

Human longevity may be found in single individuals as well as in the population as a whole ("population longevity"). *Longevity Blue Zones* (LBZs), which are areas with an unusually high number of oldest old, have been identified in Sardinia and the Greek island of Ikaria. We compared the lifestyle, health status and some genetic markers of the LBZ populations with those of reference populations from Italy and Greece; the data were extracted from the GEHA database. In the LBZs, the proportion of individuals who never married or were married and still living with their spouse was significantly greater. Nonagenarians males and females with a high self–perception of optimism and/or a high score for self-rated health were also found in larger proportions in LBZs. Among the variables with lower frequency were the proportion of the widowed, the percentage of subjects who had suffered a stroke and the frequency of Apoε4 and Apoε2 and the TT genotype of FOXO3A gene. Compared to behavioral and health indicators, the impact of genetic factors might be relatively less important in the LBZs. Nevertheless, further research is needed to identify potential epigenetic traits that might play a predominant role due to the interaction between genetics and the human and physical environments.

1. Introduction

Some individuals may live considerably longer than most other people; this phenomenon is known as longevity. The age threshold at which a person is regarded as "long-lived" has changed over time, as the lifespan of human beings has increased. Decades ago, the age threshold for identifying the so-called "oldest old" was fixed at 85 years (Suzman et al., 1992), whereas more recent studies, such as the GEHA, an EU-funded research project, consider longevity as starting at the age of 90 years (Franceschi et al., 2007; Cevenini et al., 2014). Usually, longevity studies address *individual* longevity and are aimed at identifying specific traits or life trajectories of sporadic long-lived persons. Yet, populations in which longevity is shared by a large portion of their members have been identified, i.e., in these populations the percentage of oldest old and the average life expectancy are greater than usually expected. The term *population longevity* has been proposed as a more appropriate term in this context (Poulain et al., 2013; Poulain, 2019). The existence of such populations raises the question of whether the factors responsible for the high number of oldest old are the same as those responsible for individual longevity. In this article, we present the

Abbreviations: LBZs, Longevity Blue Zones; GEHA, Genetics of Healthy Aging in Europe; MMSE, Mini Mental State Examination; SRH, Self Rated Health; APOE, Apolipoprotein E; FOXO3A, Forkhead box O3; PON1, Serum Paraoxonase/Arylesterase 1 gene.

* Corresponding author at: IACCHOS Université catholique de Louvain, Belgium.
E-mail address: michel.poulain@uclouvain.be (M. Poulain).

https://doi.org/10.1016/j.mad.2021.111543
Received 25 April 2021; Received in revised form 17 June 2021; Accepted 9 July 2021
Available online 12 July 2021

results of a preliminary attempt to compare two populations displaying high levels of population longevity – one living in the mountainous area of the Sardinia, Italy, and the other living on the Greek island of Ikaria. The features of nonagenarians from these locations have been compared with corresponding data concerning nonagenarians' siblings in mainland Italy and Greece, obtained from the GEHA study (Skytthe et al., 2011), representative of individual longevity.

1.1. Individual versus population longevity

Considerable research has been devoted to the analysis of *centenarians* or *near–centenarians* taken as a model of extreme individual longevity (Franceschi et al., 2000, 2007; Poon et al., 1992; Motta et al., 2005; Sebastiani et al., 2010). In most cases the methodology of these studies entails a random selection of participants (both centenarians and younger controls). In such conditions, these very old persons are actually representative of individual longevity since they display an extreme phenotype, exceedingly rare among people living in the same physical and social environment. This approach seeks to find out why these persons live longer than their close relatives, co-residents and other members of the local community, and tries to outline the characteristics of individual longevity and any possible association between individual traits and extreme survival. Many of these studies were carried out from a single disciplinary perspective, such as demography, sociology, anthropology, psychology, epidemiology, nutrition or genetics, and the impact of other disciplines manifested only marginally through control covariates. Currently, investigations involving the collaborative efforts of researchers from several disciplines within an integrated approach are quite rare (Carey and Vaupel, 2005). This fact is unacceptable since successful aging is a complex achievement that requires the contribution of numerous and heterogeneous factors.

Population longevity is when the proportion of people surviving to the oldest ages is greater in a given population than in its neighboring areas according to a predetermined threshold deviation (Poulain, 2019). If the oldest members of such a population are also able to maintain a high standard of cognitive and physical functionality until the end of their lives, studying them may prove to be an innovative and promising model in longevity research. Hence, the study of specific long-lived populations is aimed at seeking determinants shared by a sizable percent of people within a given population. The determinants of population longevity could encompass either individual characteristics (factors or traits associated with individual longevity) or contextual factors (related to the global environment surrounding that population) conducive to longevity. A conventional study centered on individual longevity is hardly able to capture these "shared" components peculiar to population longevity. Analysis of population longevity should not be viewed as an alternative to the analysis of individual longevity, but it could potentially complement the more conventional approaches and facilitate the identification of longevity factors acting at the super–individual level. Although this new approach goes beyond searching for the factors associated with individual longevity, it is still strongly related to them.

Interest in the study of long-lived populations has remained limited due to the following reasons:

i Identification of a long-lived population and the age validation of its exceptional members are time-consuming and demanding tasks that are not easy to complete due to the lack of documentary evidence, as explained later.
ii Communities experiencing higher population longevity are often small in size, and the oldest old group may consist of a limited number of individuals; thus the risk of underpowered statistics exists.
iii More generally, searching for population longevity determinants requires the mastering of several disciplines, which is a relatively complicated task, as specific methods have yet to be developed. A consensus has still not been reached regarding a strategy of analysis that is consistent with the data collected in various disciplines.

1.2. Factors associated with population longevity

The present study is the first attempt to identify the longevity factors largely shared by the populations of two LBZs identified in Sardinia and in Greece. This objective was achieved by comparing the characteristics of nonagenarians living in these populations with the corresponding characteristics of nonagenarian siblings surveyed as part of the GEHA project in mainland Italy and Greece. Our working hypothesis was that long-lived individuals in the LBZs may be somewhat unique, as they may express differently those longevity traits already identified through studies targeted at individual longevity. The impact of these traits may be enhanced by the surrounding physical or social context and shared by several members of these long-lived populations. However, although these specific traits may play a significant role in favoring longevity in a long-lived population, they are likely overlooked in the context of individual longevity, as the subjects do not live in the same environment and social context.

Accordingly, we assumed that, by comparing nonagenarians in the LBZs with GEHA nonagenarians in the respective countries, we could identify the longevity traits in the LBZs as results of their specific favorable socio-cultural and physical environments.

1.3. The indispensable first step: identifying and validating long-lived populations

In March 2000, as a result of the validation of individual centenarians in Sardinia (which was done as part of the AKEA project), a specific area was identified in the island's mountains– in this area the proportion of centenarians was significantly higher than in the populations born in the same place (Poulain et al., 2004). This area was called a *Longevity Blue Zone* (LBZ), and the term has since come to be defined as an area where the population is characterized by a significantly higher level of longevity compared to neighboring regions, and the exceptional longevity of people in this population must be fully validated (Poulain et al., 2007). In practice, an LBZ is defined as a rather limited and homogenous geographical area in which the population shares the same lifestyle and environment and its longevity has been proved to be exceptionally high (Poulain et al., 2013). So far, validated LBZs have been identified in Okinawa (Japan), on the Nicoya peninsula (Costa Rica) and on the island of Ikaria (Greece) (Poulain, 2011; Poulain et al., 2013; Pes and Poulain, 2016).

To identify an LBZ and prove the exceptional longevity of its population, it is necessary to first validate the individual longevity of the people living in the candidate area, i.e., assess accurately the age at death or the extreme survival of the oldest old. Age misreporting and, more specifically, age exaggeration must be eschewed (Poulain et al., 2007). Several populations had claimed to live in longevity areas, but most of these alleged LBZs were later invalidated (Young et al., 2010). Perls (2006) concluded that "*such cases of extreme longevity required detailed scrutiny because they are so incredibly rare*". In addition to the validation methodology developed for individual centenarians (Poulain, 2010), the validation of an exceptional population longevity requires several further operational steps, which vary depending on the current availability of data sources specific to the population under scrutiny. Usually, the level of population longevity is estimated by deriving the average of individual survival of all subjects born in a given geographic area. To accomplish this task, exhaustive data on the births and deaths that occurred within that population during at least a century must be available.

The extreme longevity area identified in the mountainous part of Sardinia includes a group of 6 villages in the Barbagia and Ogliastra districts; they contain about 40,000 inhabitants who are mainly engaged in shepherdry and agricultural activities and follow a relatively traditional lifestyle. This population remained isolated for centuries, which contributed to make its gene pool more homogeneous and the preservation of its socio-cultural and anthropological characteristics throughout its history (Pes and Poulain, 2016). Among the non-genetic factors that might account for the exceptional longevity recorded in central Sardinia and the low gender ratio among the oldest people there, the role of physical activity (Pes et al., 2020; Fastame et al., 2020), life satisfaction, optimism, resilience and religiosity (Fastame et al., 2021) and nutrition have been investigated; in particular, the role of traditional foods, typical of a society centered on animal farming has been studied (Pes et al., 2013, 2015; Pes et al., 2021).

Ikaria, the other longevity area considered in this comparative study, is a mountainous Greek island (255 sq km) located in the Eastern Aegean Sea, located between Mykonos and Samos. Its nearly 8000 inhabitants live in three municipal units. Unlike other Greek islands, it has remained relatively poor and isolated. Its inhabitants have kept their traditions alive and preserved their manual occupations, and most of their food is produced locally (Panagiotakos et al., 2011; Georgiopoulos et al., 2017; Legrand et al., 2019; Chrysohoou et al., 2020; Foskolou et al., 2021).

1.4. The second step: measuring and comparing the level of longevity across populations

Conventionally, the apparent exceptional longevity of a population is inferred from the existence of an unusually large proportion of centenarians/nonagenarians. To compare longevity levels,centenarian prevalence (CP), i.e., the ratio of the number of living centenarians in a given population to the total resident population, is largely used in the literature as well by the media. However, the reliability of this indicator deserves critical evaluation as it is sensitive to a number of biases related to existing migration flows and changes in fertility behavior. For example, in the case of a population that experienced large-scale immigration flows in the younger generations, or a baby boom later, CP will fail to identify the remarkable survival of persons in old ages as the proportion of elders is artificially lowered. On the contrary, if the younger population drops in number due to emigration, the proportion of elderly may be artificially inflated. In such cases, the prevalence or proportion of the oldest old is no longer reliable for measuring longevity and should not be used for comparison across populations. Nevertheless, CP is still the indicator most frequently used by gerontologists as well as by national and regional authorities that are eager to claim a longevity status for the area of the population concerned.

A closely related indicator, the Centenarian Rate (CR), was proposed by demographers for comparing longevity levels. It was introduced by Robine and Caselli (2005) as the ratio of the number of persons aged 100 years and above, who were 60 and above 40 earlier, living within the same territory. This index can be easily calculated by using census data, and it minimizes the impact of the cohort size and the role of migration, naturalization, fertility and infant mortality as most of these confounding events occur before the age of 60.

Compared to CP and CR, life tables provide a better measurement of longevity and allow one to compare different populations more reliably. The *cohort life table* is computed from the mortality rates of a given birth cohort observed during one century or longer, while the *period life table* is calculated for a fictive cohort by considering the mortality rates for each age group at a given time. In most populations, longevity is rising so rapidly that only the same cohorts of different populations can be compared; thus cohort life tables are preferable in such cases. Nevertheless, building a cohort life table is not a straightforward task as different methodologies need to be used. Moreover, *ad-hoc* data is not always available. As a result, very few countries provide cohort life tables,[1] and no such tables currently exist for the two LBZs included in this study.

A last indicator named *Extreme Longevity Index* (ELI) was proposed by researchers when they were assessing the level of longevity in Sardinia (Poulain et al., 2004) as the ratio of the number of centenarians, dead or alive, born during a given period (e.g., in Sardinia, between 1880 and 1914) to the total number of births recorded during the same period. This index, expressed as the number of centenarians per 100,000 newborns, is equivalent to the probability for any person born in that municipality to reach 100 years of age, there or anywhere else. The advantage of such an indicator is that the bias can only result from an under-registration of the centenarians who emigrated, which leads to only a slight underestimation of ELI. A similar indicator has also been computed for assessing the probability of the studied population reaching 90 years of age.

In Sardinia, the municipality of *Villagrande Strisaili*, the epicenter of the LBZ, was the object of an in−depth demographic study (Poulain et al., 2011). All birth and death records for the period 1866–2016 were entered into an electronic database, which also includes information about all family links within the population. We also considered the administrative information available in the *anagrafe*, the Italian population registration system, to identify those who emigrated and died outside the village. By doing so, we succeeded in obtaining the date of and age at death or proof of survival for 98 % of the newborns identified.

In Ikaria, census data and age-at-death statistics were retrieved from the *Hellenic Statistical Authority*. These were compared with individual data extracted from the *δημοτολογίων*, a locally−based administrative registry that contains demographic information on all Greek citizens in a given municipality. Unfortunately, for the oldest old on the island, no birth records could be found. Therefore, the extinct-cohort method, which involves exhaustive identification of those surviving and aged above 90, as well as those who had been part of the same birth cohorts and died during the two last decades, was used to estimate the level of population longevity. Individual age validation was successfully achieved during interviews with all those aged 90 years and above in the North-Western part of the island by using a battery of questions on the occurrence of demographic events and the age of close relatives.

2. Material and methods

The study population included: (i) nonagenarians living in the village of *Villagrande Strisaili* which is characterized by the highest longevity level (ELI) in the region, especially among men, and (ii) nonagenarians living in the municipal units of *Raches* and *Evdilos* in Ikaria, which are characterized by the highest longevity level in Greece according to statistical data provided by the *Hellenic Statistical Authority*. The study protocol was approved by the local ethics committees. The participants gave written informed consent, permitting us to use their clinical and laboratory data in this study. The data collected in the Sardinian LBZ area were compared with those extracted from an Italian reference population, retrieved from the GEHA study, which was aimed at identifying the genes involved in healthy aging and longevity (Franceschi et al., 2007). The GEHA study included nonagenarian siblings from various groups within the Italian peninsula; these represented different geographical areas, particularly Northern Italy (80 % from the Emilia Romagna region), Central Italy (City of Rome) and subjects from Southern Italy (Calabria region). All 90+ sibling pairs who had agreed to participate in the study were recruited as deemed fit by the Ethics Steering Committee. For the sake of representativeness and to obtain a sufficient sample size, the three Italian recruiting sites were pooled together in a single category titled “Italy”. Internal differences between the three Italian locations were taken into account only in the descriptive statistics; these were not considered for comparison and were left for further study. The GEHA study also provided phenotypic and genetic data concerning nonagenarian siblings in Greece, mostly identified in rural areas of Peloponnese.

2.1. Choice of indicators

The overall goal of this study was to perform a comparison between the nonagenarians of the two long-lived populations in the Sardinian

[1] See the Human Mortality Database visited at www.mortality.org.

and Ikarian LBZs and the respective reference populations of Italy and Greece. For this purpose, we included a number of indicators associated with longevity: (i) demographic and lifestyle indicators, (ii) health indicators; (iii) some specific genetic markers investigated in previous studies. The latter are ApoE markers ε2 and ε4 (Schächter et al., 1994), the variant *rs2802292* of the FOXO3A gene (Willcox et al., 2008) and the variant *rs662* of the PON1 gene (Lescai et al., 2009). We are aware that this particular choice might have been reductive and somewhat arbitrary, and we ignored *a priori* the indicators that capture the information concerning the phenotype under investigation. Moreover, our choice was largely constrained by the data collected in the two LBZs, as well as the data already available in the GEHA database for the respective reference populations. The ApoE markers ε2 and ε4 were determined in DNA samples by a PCR-based method (Hixson and Vernier, 1990). The variant *rs662* of the PON1 gene was determined with the method of Bonafe et al. (2002). The TT genotype of FOXO3A was determined with the method of Anselmi et al. (2009).

2.2. Statistical analysis

Based on the original data collected for each of the indicators included in the analysis, basic statistics were calculated for each location and gender. We performed a descriptive analysis by calculating the means and standard deviations for continuous variables and the absolute and relative frequencies for dichotomic variables in the two high longevity areas, the Sardinian LBZ and the Ikarian LBZ, and in both reference populations. Differences between each indicator of the participants in the LBZs and those in the reference population were tested through Student's t test for independent samples, or by the two-sided Pearson χ^2 test for categorical variables. Odds ratios (ORs) and their 95 % confidence intervals (CI) were also calculated. All statistical analyses were performed using SPSS statistical software (version 22.0, Chicago, IL), and p−values <0.05 were considered statistically significant.

3. Results

3.1. Comparative levels of longevity

As stated earlier, the longevity of a given population can be assessed from different indicators. Table 1 illustrates relevant information about our study population. Unfortunately, it is unknown how many newborns Greece and Ikaria had at the turn of the 20th century, and no data is available for Greece in the *Human Mortality Database*[1]. Nevertheless, the longevity advantage of the two LBZs over the respective Italian and Greek populations is clearly evident. This advantage is larger for the Sardinian LBZ compared to the Ikarian one, and a unique characteristic of Sardinia emerges; here men live as long as women do (Poulain et al., 2011).

3.2. Descriptive statistics

A total of 1633 cases from 4 settings were available for comparative analysis (Sardinia and Italy, and Ikaria and Greece). For Italy and Greece, the data were extracted from the electronic database available from the GEHA study, while the data for Sardinia and Ikaria came from ongoing local surveys. The number of subjects involved in both LBZs and the mean age at the time of recruitment are displayed in Table 2; these did not show any significant difference across the locations.

3.3. Comparison of indicators

Overall, the variables analyzed in this study can be grouped into four groups: (i) variables showing a higher level in both genders and in LBZs compared to the reference populations – this category includes the proportion of never married or currently married individuals, the proportion of individuals living with their spouses, self-perception of optimism, self-rated health, the frequency of type 2 diabetes and the frequency of the PON1 RR genotype; (ii) variables showing positive association in both men and women in one of the two LBZs – in Sardinia, they include only mean body height, while in Ikaria, they include the average number of schooling years; (iii) variables showing negative association in both long-lived areas – they include the proportion of the widowed, the proportion of individuals living alone, the average number of disabilities, mean self-rated health score, the percentage of cerebrovascular accidents, the frequency of Apoε4 and Apoε2 and the TT genotype of the FOXO3A gene; (iv) variables showing differences in opposite direction in men and women – among them are included the average number of schooling years and drinking alcohol almost every day. These results are summarized in Table 3.

4. Discussion

Exceptional human longevity has become a field of growing interest as research in this field can potentially identify the relevant factors that can help people live a longer and healthier life (Franceschi et al., 2000, 2007; Fortney et al., 2015; Schoenhofen et al., 2006). However, identification of the major determinants that affect the duration of human life has proven intrinsically difficult as longevity is a complex trait resulting from the interaction between genetics, environment and stochasticity, whose relative contribution is still unclear (Avery et al., 2014). The study of single long−lived individuals may be complemented by the study of populations in which the proportion of very old individuals is demonstrably higher compared to neighboring populations. A clear advantage of this approach is that it exploits the usual genetic and ethnic homogeneity of these communities, which may help in overcoming some of the confounders affecting conventional studies. Furthermore, the contextual factors at play in the LBZs have the potential to enhance individual longevity factors, which may not be captured in the case of more heterogeneous physical and social environments.

To outline the characteristics of population longevity, in the present study, we compared a number of indicators in nonagenarians living in the two LBZs, with those in a reference population of nonagenarians from Italy and Greece, who had already been investigated as part of the multicenter study GEHA (Skytthe et al., 2011). As shown in Table 1, the

Table 1
Comparative levels of longevity across the populations investigated.

	Sardinian LBZ		Italy		Ikarian LBZ		Greece	
	Men	Women	Men	Women	Men	Women	Men	Women
Survival probability[a]	13.4	13.6	3.1	9.6	3.3	8.8	2.3	6.3
Life expectancy at birth (years)[b]	79.7	84.6	78.7	83.9	80.3	84.1	77.5	83.0
Extreme Longevity Index	1297	1402	124	558	n.a.	n.a.	n.a.	n.a.
Number of new-borns reaching 90 years per 100,000[b]	12,575	12,729	3506	8745	n.a.	n.a.	n.a.	n.a.

[a] Probability of surviving between 1970 and 2000 for people who were aged 60–69 in 1970 and 90–99 in 2000 (based on census data). Sources: the ISTAT database for Italy, and the *Hellenic Statistical Authority* for Greece.
[b] Source: the LBZ database for Sardinia and the Human Mortality Database for Italy (accessed on 22 April 2021 at www.mortality.org).

Table 2
Descriptive statistics of the population surveyed in the four population groups.

	Sardinian LBZ		Bologna GEHA		Rome GEHA		Calabria GEHA		Ikarian LBZ		Greece GEHA	
	M	F	M	F	M	F	M	F	M	F	M	F
Population (N)	51	44	163	386	58	158	154	258	42	46	186	87
Mean age at survey (yrs)	92.4	96.2	94.5	95.0	92.6	93.5	92.9	93.0	93.6	94.1	94.0	94.3
Never married (%)	13.7	18.2	10.4	13.5	1.7	15.8	3.9	8.2	4.8	6.5	2.2	5.7
Currently married (%)	54.9	13.6	36.2	1.8	43.1	1.9	40.3	5.0	54.7	13.1	44.0	3.5
Widowed (%)	31.4	68.2	53.4	84.7	55.2	82.3	55.8	86.8	40.5	80.4	53.8	90.8
Average number of years of education	4.4	3.0	5.6	4.5	11.2	7.1	2.9	2.4	7.3	6.2	5.4	4.0
Living alone (%)	5.9	11.4	18.4	25.9	12.1	19.6	20.1	18.2	19.0	30.4	17.7	23.0
Living with spouse (%)	52.9	11.4	34.4	1.8	43.1	1.9	40.3	5.0	54.8	13.0	44.1	2.3
ADL score	1.02	1.98	1.69	2.16	1.53	1.96	2.32	2.89	0.85	1.78	1.34	1.97
Standardized MMSE score	20.3	15.6	22.7	20.7	25.2	23.6	17.5	15.0	24.1	20.4	22.6	18.8
Ever smoker (%)	76.5	2.5	55.8	6.8	64.3	16.1	61.4	2.4	85.7	10.9	44.3	3.5
Drinking alcohol almost every day (%)	64.0	20.0	74.1	51.0	64.9	46.2	69.5	45.1	90.5	58.7	65.6	24.1
Mean SRH score (from 1, very good, to 5, very bad)	1.82	2.30	2.17	2.52	2.24	2.36	2.64	3.15	1.35	1.44	2.28	2.46
Self−perceived optimism (%)	72.6	52.3	63.8	51.5	52.8	49.3	20.1	18.2	85.7	71.7	49.5	44.1
Myocardial infarction (%)	8.0	3.0	9.8	4.4	8.6	3.2	5.2	2.3	7.1	4.4	7.5	5.7
Stroke, cerebral thrombosis, hemorrhage (%)	10.0	0.0	11.7	16.8	10.5	10.1	10.4	12.8	4.9	4.4	10.8	14.9
Cancer (%)	4.0	0.0	12.9	13.5	6.9	5.1	1.3	3.5	7.1	4.3	4.8	5.7
Hypertension (%)	18.0	27.3	47.9	55.4	56.9	62.0	42.2	52.7	69.0	78.3	64.5	58.6
Type 2 diabetes (%)	12.0	12.1	4.3	8.3	17.5	7.0	9.2	10.1	16.7	21.7	5.9	6.9
Mean body height (cm)	163.0	155.9	163.6	148.9	166.2	155.0	160.4	147.6	164.9	149.5	168.8	158.1
ApoE (ε4 genotype)	12.2	2.4	15.9	5.3	12.8	14.6	6.1	7.9	2.4	2.4	10.3	12.9
ApoE (ε2 genotype)	4.4	4.7	15.2	16.7	12.8	10.0	15.7	17.6	0.0	0.0	16.1	11.6
PON1 *rs662* (RR genotype)	9.8	9.1	5.5	10.6	5.2	8.2	10.4	8.1	9.5	8.7	5.4	4.6
FOXO3A *rs2802292* (TT genotype)	15.7	11.4	25.2	26.2	32.7	30.5	28.6	27.3	14.6	17.1	19.7	19.5

force of mortality is stronger in Italy and Greece than in their respective LBZs, and in the latter, the probability of people's reaching the age of 90 is greater, especially among men. Thus, the GEHA nonagenarians are probably more selected than those identified in the two LBZs; this means that *a priori*, some longevity factors may be more common in the GEHA nonagenarians, and this may affect the global interpretation of the phenomenon of population longevity.

4.1. Behavioral and lifestyle variables

4.1.1. Marital status and living arrangements

Among the variables collected in our study and pertaining to behavior and life, marital status and living arrangements, i.e., with whom a person lives, are two key variables. The descriptive findings (Table 2) show a larger proportion of currently married nonagenarians in both Sardinia and Ikaria than in Italy and Greece. The proportion of nonagenarians who never married was also slightly higher but that of the widowed was remarkably lower. As for the living arrangements, the proportion of nonagenarians living with their spouses was also larger in the LBZs, a condition related to the higher proportion of individuals currently married. Of the never−married and widowed nonagenarians, a smaller proportion was living alone, and a higher proportion was living with others in both LBZs. There could be multiple reasons why a larger proportions of nonagenarians live with their spouse: (i) in the LBZs, since both men and women live longer, there are more couples of advanced age; (ii) the age difference between spouses could be larger in the LBZs compared to in the general population, and this increases the proportion of male nonagenarians still married and having relatively younger wife; (iii) remarriage is more frequent among older men, especially in Sardinia, due to traditional cultural norms (Mazzoni et al., 2013).

Our investigation found a larger difference in the proportion of nonagenarians living with spouses among Sardinian women, and this could be explained by the fact that in the Sardinian LBZ, the men live as long as women. A number of studies have shown that marriage is associated with better health (Verbrugge, 1979; Johnson et al., 2000) and protection against premature mortality (Martikainen et al., 2005; Manzoli et al., 2007; Grundy and Tomassini, 2010; Rendall et al., 2011). These findings have been further supported by research on mortality variation by living arrangements among older adults; it has confirmed that being married and living with a spouse in old age is positively associated with longevity; this was observed in Belgium (Herm et al., 2016). However, the advantage of being married or living with a spouse is more remarkable in men than in women among whom being never-married is related to an equal chance to become a centenarian (Poulain and Herm, 2016). Living with one's spouse may lead to the employment of a healthier lifestyle, which, in turn, protects the couple against the risk of premature death and shorter survival for both spouses (Ben-Shlomo et al., 1993; Murphy et al., 1997; Seeman, 2000).

4.1.2. Education

Nonagenarians in Sardinia were less educated than their Italian GEHA counterparts, whereas in the case of Ikaria, the opposite was true. The education level, measured by the number of schooling years was significantly higher in the Ikarian LBZ for both genders, whereas in Sardinia, it was low among women (Nieddu et al., 2020). These divergent findings could be explained by the negative education gradient (Hardarson et al., 2001), which results in larger proportions of higher educated people among the oldest old. Accordingly, the lower proportion of higher educated people in Sardinia could be the result of a lower selection. In Ikaria, the larger proportion of the higher educated could be related to the lower level of schooling of Greek nonagenarians from GEHA.

4.1.3. Attitudes and behavior

Self-perception of being optimistic is the only variable that is consistent in both genders and in both LBZs; therefore, it is the first one that should be considered as a major factor associated with population longevity. There is a positive relationship between optimism and well-being, and longevity (Ben-Zur, 2012; Diener and Chan, 2011; Kim et al., 2019; Lee et al., 2019; Fastame et al., 2021). Moreover, the way subjective well-being modulates longevity could interact with marital status, living arrangements and the health promotion that comes with these conditions. Some studies have found a positive association between optimism and better health, and this may be related to a healthy lifestyle and food (Giltay et al., 2007; Steptoe et al., 2006). Since lifestyles are promoted by family and social relationships, being a spouse or parent protects one against premature death (Kobrin and Hendershot,

Table 3
Odds ratio (95 % CI) for each of the indicators selected in the study, for 1633 nonagenarians from the LBZs and reference populations.

Indicators	Sardinian LBZ vs Italy GEHA				Ikarian LBZ vs Greece GEHA			
Gender	Men		Women		Men		Women	
Odd ratio	OR (95 %CI)	p-value	OR (95 %CI)	p-value	OR (95 %CI)	p-value	OR (95 %CI)	p-value
Never married (%)	2.33 (0.95–5.71)	0.079	1.60 (0.72–3.53)	0.242	2.28 (0.40–12.85)	0.305	1.14 (0.26–5.02)	1.000
Currently married (%)	1.91 (1.06–3.44)	**0.034**	5.35 (2.06–13.91)	**0.003**	1.54 (0.78–3.01)	0.233	4.20 (1.00–17.66)	0.064
Widowed (%)	0.38 (0.20–0.71)	**0.003**	0.39 (0.20–0.75)	**0.009**	0.58 (0.30–1.15)	0.128	0.42 (0.15–1.17)	0.105
Average number of schooling years		0.152		**0.016**		**0.001**		**<0.0001**
Living alone (%)	0.26 (0.08–0.85)	**0.018**	0.43 (0.17–1.11)	0.093	1.09 (0.46–2.57)	0.826	1.47 (0.66–3.27)	0.405
Living with spouse (%)	1.80 (1.00–3.25)	0.049	4.34 (1.57–12.03)	**0.012**	1.54 (0.78–3.01)	0.233	6.38 (1.23–32.99)	**0.020**
ADL		**<0.0001**		1.168		**0.010**		0.591
Standardized MMSE		0.525		**<0.0001**		0.077		0.179
Ever smoker (%)	2.22 (1.13–4.39)	**0.021**	0.33 (0.05–2.48)	0.515	7.54 (3.02–18.86)	**<0.0001**	3.37 (0.77–14.81)	0.126
Drinking alcohol almost every day (% yes)	0.80 (0.43–1.50)	0.328	0.27 (0.11–0.66)	**0.002**	4.98 (1.70–14.58)	**0.001**	4.47 (2.08–9.60)	**<0.0001**
Mean SRH (score from 1 very good to 5 very bad)		**<0.0001**		**0.003**		1.000		1.000
Self–perceived optimism (%)	3.47 (1.81–6.64)	**<0.0001**	1.67 (0.91–3.07)	0.114	6.13 (2.46–15.26)	**<0.0001**	3.22 (1.49–6.98)	**0.003**
Myocardial infarction (%)	1.04 (0.35–3.08)	1.000	0.86 (0.11–6.55)	1.000	0.95 (0.26–3.45)	1.000	0.76 (0.14–4.10)	1.000
Stroke, cerebral thrombosis, hemorrhage (%)	0.12 (0.05–0.34)	1.000		**0.016**	0.94 (0.34–2.65)	0.383	0.26 (0.06–1.23)	0.087
Cancer (%)	0.54 (0.12–2.33)	0.557		0.102	1.51 (0.39–5.85)	0.467	0.75 (0.14–4.00)	1.000
Hypertension (%)	0.25 (0.12–0.53)	**<0.0001**	0.30 (0.14–0.65)	**0.002**	1.23 (0.60–2.52)	0.720	2.54 (1.12–5.77)	**0.034**
Type 2 diabetes (%)	1.50 (0.59–3.81)	0.421	1.47 (0.50–4.29)	0.522	3.18 (1.15–8.78)	**0.028**	3.75 (1.27–11.10)	**0.022**
Mean body height (cm)		0.707		**<0.0001**		**0.003**		**<0.0001**
ApoE (ε4 genotype)	1.07 (0.42–2.72)	0.812	0.27 (0.04–2.03)	0.240	0.22 (0.03–1.69)	0.202	0.17 (0.02–1.35)	0.087
ApoE (ε2 genotype)	0.26 (0.06–1.12)	0.061	0.26 (0.06–1.10)	**0.048**		**0.003**		**0.024**
FOXO3A (rs2802292) (TT genotype)	0.48 (0.22–1.07)	0.087	0.34 (0.13–0.87)	**0.021**	0.70 (0.27–1.80)	0.656	0.85 (0.32–2.29)	0.809
PON1 rs662 (RR genotype)	1.35 (0.50–3.66)	0.575	0.97 (0.34–2.78)	1.000	1.64 (0.49–5.52)	0.297	1.98 (0.47–8.30)	0.447

*The empty cells reflect those instances in which the OR could not be calculated as the numerators were zero or the variable was continuous.

1977). Marriage supports wellbeing by acting as a buffer against the emotional effects of life problems and economic constraints (Kessler and Essex, 1982). Behavioral factors influencing health, (e.g. smoking, alcohol consumption and physical activity) have been shown to have a direct association with self-rated optimism. On the other hand, optimism is also positively related to self-rated health regardless of health behaviors (Steptoe et al., 2006) or other factors, such as demographic, chronic conditions, medication etc. Among the optimistic oldest old, the risk of cardio-vascular death is reduced by 50 % (Giltay et al., 2007; Tindle et al., 2009).

Oddly enough, the condition of being a current or former smoker is more frequent among men in both LBZs, and also in women in Ikaria. There is no explanation for this finding at the moment, but it may be reflecting traditional norms. Gaining a better understanding will require further study, which must take into consideration the intensity, the time of cessation of smoking and the period after the termination.

Drinking alcohol every day, in general, emerged as a negative factor in the Sardinian LBZ, but it was a positive factor in the Ikarian LBZ. The putative association of alcohol with exceptional longevity has given rise to persistent controversies. Red wine consumption has been hypothesized as one of the possible factors contributing to Sardinian male longevity because of its high antioxidant content (Corder et al., 2006). However, in an ecological study, we have reported that the average intake of red wine is equal in the Sardinian LBZ and the rest of the island, where the longevity level is lower (Pes et al., 2013). Although we acknowledge that among Ikarian nonagenarians the situation may be different, at the moment, we can conclude that the association of alcohol consumption with population longevity is not supported by empirical evidence.

4.2. Health status indicators

The cognitive status as measured by the MMSE score was not significantly different; the only exception is that in the case of Sardinian women, this variable was heavily dependent on education. Regarding the frequency of major comorbidities, no differences were observed between the two LBZs and their respective motherlands for ischemic heart disease and cancers in both sexes. The frequency of cerebrovascular accidents was significantly lower among women from the Sardinian LBZ compared to their Italian peers. As regards type 2 diabetes, unexpectedly, a higher frequency was observed in both LBZs and it even reached statistical significance in the case of Ikaria (Table 3). As underlined earlier (Pes et al., 2019), overall, the health indicators examined in this study describe a population mainly consisting of delayers and, to a lesser extent, escapers or survivors, i.e., most of the long-lived in the Blue Zones do not escape major age-related diseases, yet they have resources that enable them to significantly delay their appearance. In our study, self-rated health showed higher scores for the Sardinian and Ikarian people, irrespective of gender, compared to the average score among the nonagenarians recruited for the GEHA study. Body height showed opposite effects in Sardinia and Ikaria. A lot of literature deals with the association between height and longevity (Samaras, 2012). In a previous study we attempted to explore this relationship in Sardinia (Salaris et al., 2012); we had advanced the hypothesis that the shorter stature of Sardinians, mostly genetically determined, is related to their longevity. However, this association seems peculiar to the LBZ only, not to Sardinia as a whole (Pes et al., 2018).

4.3. Genetic markers

Genetic factors are considered essential for attaining an advanced age. Several studies have been conducted by using the model of centenarians with the aim of identifying the key gene variants that confer survival advantages (Franceschi et al., 2007; Sebastiani and Perls, 2012; Broer et al., 2015). Twin studies and family studies have shown that 20–25 % of a person's lifespan is a result of genetic factors (Herskind et al., 1996; Iachine et al., 1998). Recently, two studies conducted on large genealogical trees claimed that the heritability of human longevity has been overestimated, i.e., it is lower than previously thought, as the inflationary effect of assortative mating was not duly considered before (Kaplanis et al., 2018; Ruby et al., 2018). In our study, we found that the frequency of Apoε4 was the same in the LBZ and motherland cohorts, while the frequency of Apoε2 was significantly lower in both sexes in Ikaria and in women in Sardinia. In the latter subgroup, the frequency of the TT genotype of FOXO3A was also lower. These modest discrepancies were probably a result of the small sample size, which is especially noticeable when we consider the two sexes separately. However, a reduction in the frequency of the minor allele due to genetic drift in these isolated populations cannot be excluded as a potential reason. The role of genetic factors in Sardinian longevity raises some controversies. It is theoretically possible that some genetic traits that favor longevity and are highly prevalent in this population may have been selected because of high endogamy in the past. Sardinian centenarians have been the subject of several genetic association studies focusing on Y chromosome SNP (Passarino et al., 2001) and markers associated with cardiovascular mortality, cancer, and inflammation (Pes et al., 2004; Lio et al., 2003), and none of these markers have been shown to diverge significantly from those of the general Sardinian population. Similarly, we found that the frequency of Apoε4 was very low in the general population of Sardinia as a whole compared to Italy, probably due to genetic drift, and it was even lower among the LBZ nonagenarians, which is in line with previous studies.

Our study has some limitations. They mainly concern the choice of genetic variants for comparison between the studied populations. This was limited by two constraints: the need to include major variants previously found to be associated with longevity in independent studies and the need to include the variants available in the GEHA study. This led to our including only ApoE, FOXO3A and PON1. Thus, there is a need for more specific, genetically-oriented future investigations. We also recognize that self-rated optimism can be influenced by the current health and wellbeing of the person and may not necessarily reflect their usual psychological status. Nevertheless, we hope that our findings initiate further discussion on the factors favoring longevity.

5. Conclusions

Populations with exceptionally high longevity could be a promising model in mortality research. LBZs are unique because the populations living there have a significantly higher proportion of long-lived individuals than the population living anywhere else. Our study revealed that nonagenarians from such populations display demographic, lifestyle and genetic traits that are different from the traits of the nonagenarians selected at random from other populations. For example, a greater proportion of the oldest old in the Sardinian and Ikarian LBZs are still married or share their lives with relatives or partners; this helps them live healthier. In our study, they also received higher scores in self-rated health and optimism. These features may be seen as possible positive factors related to staying healthy and, maybe, living longer, despite the existence of other unfavorable conditions such as a greater prevalence of chronic diseases and smoking.

From the theoretical standpoint, a concentration of the oldest old in the population can result from two main processes: (i) the effect of specific genetic and/or non-genetic factors that give their carriers a particular resistance to early mortality during the aging process – these individuals may outweigh selection with greater ease and lead to an increase in the number of oldest old people; (ii) the lifetime mortality selection may be less stringent, and this enables frail individuals to outweigh selection. However, these two hypothetical mechanisms would produce distinct phenotypes: In the first case, the growth of an *élite* of highly functioning elderly people, and in the second case, a group of older people who are relatively frailer and have age-related diseases. Based on the results of our study, we believe that LBZ nonagenarians underwent less stringent selection during the ageing process compared to the nonagenarians from the GEHA study. In other words, the former group of people seem to be more like survivors than escapers, as per the definition of Evert et al. (2003). This raises a question: Which contextual or behavioral factors (not yet investigated) could have lowered the selection threshold in the LBZ? Several health-promoting lifestyle factors (social support, diet, physical activity) that are more prevalent in the LBZ populations may be potential candidates. Traditional socio-demographic norms for matrimonial and fertility behavior as well as efficient community support have been maintained in the LBZ populations. Indeed, a large part of the population has long-term living arrangements entailing better care of elderly people, more optimism in the advanced age, perhaps, longer survival. These favorable factors are more common in the populations of the LBZ although they can be detected but less frequently in other populations. Moreover, the existence of the LBZ itself is likely the result of an optimal combination of multiple transitions, namely economical, demographical, nutritional and sanitarian, which might act in synergy to create a *milieu* favorable to life extension.

The several caveats in our study should be taken into consideration: (i) the reference population compared with Sardinia might pose some problems, which could be a result of the pooling of the three populations of Northern, Central and Southern Italy. Historically, these three populations showed major cultural and genetic differences, which have also been noted in our dataset. Nonetheless, we decided to keep this population pool to ensure a larger sample size for statistical analysis; (ii) the selection criterion for the nonagenarians recruited for the GEHA study was the presence of at least one sibling older than 90 years in age – this could have theoretically increased the genetic component of longevity in the reference populations; (iii) the difference between the mortality rates of the two LBZs' populations and the reference populations could have led to a differential selection of the people who became nonagenarians – this may have led to a different distribution of the characteristics favoring longevity in the populations under scrutiny; (iv) finally, it can be assumed that the overall longevity in an LBZ is also affected by contextual variables linked to the shared environment. Unfortunately, it was not possible to include these variables in this analysis because the nonagenarians from the GEHA study were from heterogeneous locations, and no contextual data have been collected yet. Moreover, in the two LBZs, the variability of contextual data is almost absent; this precludes the possibility of testing its alleged beneficial effect on longevity. Definitely, further research is needed to investigate a larger number of long-lived populations and compare the potential impact of lifestyle and crucial contextual factors to explain their exceptional longevity. Moreover, epigenetic traits should be investigated very thoroughly as these are a result of the interaction between genetics and both physical and human environments; thus, the LBZs are the best places for further in-depth investigations.

Funding

This research was partially supported by an Estonian Research Council grant (PRG71).

Acknowledgments

The authors are very grateful to the Municipality of Villagrande Strisaili especially Mrs. Rita Usai and Simona Rubiu, for their invaluable help to this research. We also thank Dany Chambre, who helped with the data collection and the revision of the text. This research was partially supported by an Estonian Research Council grant (PRG71). Particular thanks go to the GEHA project consortium, whose work was funded by the EU GEHA (GEnetics of Healthy Ageing) Project contract no. LSHM-CT-2004-503-270.

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