Alma Mater Studiorum – Università di Bologna

Scuola di Dottorato in Scienze Mediche e Chirurgiche Cliniche

Dottorato di Ricerca in Scienze Biomediche:

Progetto n. 1 "Biotecnologie mediche"

Ciclo XXI

MED/04

C.I.G. – Centro Interdipartimentale "L. Galvani"

Dipartimento di Patologia Sperimentale

The Integrated European Project *"GEHA - GEnetics of Healthy Aging"*: recruitment, health status assessment and survival of the Italian 90+ sibpairs

Dott.ssa Elisa Cevenini

Coordinatore:

Prof.ssa Marialuisa Zerbini

Relatore:

Prof. Claudio Franceschi

Correlatori:

Prof.ssa Maria Antonietta Stazi

Prof. Bernard Jeune

Esame finale anno 2009

To all GEHA nonagenarian siblings

[…] Fructum ferent etiam in senectute, sucosi et vegeti erunt […]
(…nella vecchiaia daranno ancora frutti, saranno vegeti e rigogliosi…)

- Salmo 92 (91), 15 -

INDEX

The present study is part of the Integrated European Project ***"GEHA – GEnetics of Healthy Aging"*** (Franceschi *et al.*, 2007a), whose aim is to identify **genes involved in healthy aging and longevity**, which allow individuals to survive to advanced age in good cognitive and physical function and in absence of the major age-related diseases. To achieve this aim the working plan is to: (a) collect information on health status and DNA from 2650 long-lived (90+) sibpairs and 2650 younger ethnically-matched controls from eleven European countries; (b) perform a genome-wide linkage scanning in all the sibpairs (a total of 5300 individuals) and a linkage disequilibrium mapping (LD mapping) of the candidate chromosomal regions; (c) compare the three genomic regions (chromosome 4, D4S1564, chromosome 11, 11.p15.5, and chromosome 19, around APOE), which were identified in previous studies as possible candidates to harbour longevity genes in cases (i.e. the 2650 probands of the sibpairs) and controls (2650 young people); (d) genotype all recruited subjects for apoE polymorphisms; and (e) genotype all recruited subjects for inherited as well as epigenetic variability of the mitochondrial DNA (mtDNA).

In order to reach this goal a common recruiting procedure was adopted in all the eleven countries: the recruited subjects were interviewed according to a standardized questionnaire, comprising extensively utilized questions that have been validated in previous European studies on elderly subjects and covering **demographic information, life style, living conditions, cognitive status (SMMSE), mood, health status and anthropometric measurements**. Moreover, subjects were asked to perform some **physical tests (Hand Grip Strength test and Chair Standing test)** and a sample of about 24 mL of blood was collected and then processed according to a common protocol for the preparation and storage of DNA aliquots.

Finally, the **vital status** of the GEHA participants was also checked at the end of the recruitment period to allow a survival analysis on this selected population and possibly to assess the impact of the identified genetic loci on 90+ people mortality.

Within the framework of the whole GEHA project, in this thesis we will describe the **recruitment activity performed by UNIBO (University of Bologna) and ISS (Istituto Superiore di Sanità, Rome)** recruiting units and the phenotypic characteristics of the recruited

90+ Italian siblings, by paying particular attention to the evaluation of their health status, their functional status and mortality. Since the peculiarity of GEHA population which is composed of nonagenarian siblings (i.e. subjects belonging to the same families) we will also present the concordance among siblings for health and functional status in order to find the phenotypic variables that are concordant in families.

It is worth pointing out that all the data included in this thesis were obtained as a part of the EU FP6 Integrated Project on Genetics of Healthy Aging (GEHA). Permission to use these data in this thesis has been granted by the GEHA Consortium. It should be noted that future publications by the GEHA Consortium may include these results possibly with additional data and/or analyses. Should this occur, the results presented in the publications by the GEHA Consortium and not this thesis shall be regarded as definitive.

1. INTRODUCTION

1.1 AGING AND LONGEVITY

1.1.1 The Demographic Revolution

Human aging and longevity are complex and multi-determined traits whose study has became a very hot topic in the last years. Some of the reasons can be traced on the actual demographic scenario: after the demographic phenomena of the 19th century, characterized by an increase of the world population, we are now in the middle of a second demographic revolution, represented by the **increase in the number of elderly people**, especially in Western countries (including Europe), but also in countries such as the demographic giants India and China.

Moreover, the improvement in public health has reduced the principal causes of mortality in the elderly, allowing an extraordinary **lengthening of the average human lifespan**. The life expectancy of *Homo Sapiens* has been approximately 20–40 years for the most part of its evolutionary history, and very few subjects survived enough to be appreciably affected by aging. Only in the last 200 years, and most dramatically during the last century, life expectancy doubled, especially in economically developed Western countries. In fact, at the beginning of the 19th century, the mean life expectancy was about 40 years (Abbott, 2004). Currently, life expectancy in Italy is 76.8 years for men and 82.9 for women. In the most developed regions, the life expectancy at birth in 2000–2005 is 71.9 years for men and 79.3 years for women. The highest values are in Japan, i.e. 79.3 and 86.3 years for men and women, respectively (Candore *et al.*, 2006) and it does not seem to decrease (forecast at 2050 are very high). Until now all the attempts to fix the maximum lifespan were denied, leading to think that probably lifespan is not limited at all.

In the last 50 years the mortality of people over 80 years decreased dramatically (each year we have gained 2.7 months in life-expectancy). Moreover, the Gompertz's law of mortality, which was one of the central tenets of the aging research, showed some weakness: he reported that the death rate of humans increased in an exponential manner with age, and he suggested that this was a feature of all organisms. Together with this observation, it came also the convincement for a species-specific limit to the lifespan. However, in the last years demographic studies showed that the mortality curve is not exponential, but it shows a late-life plateau in mortality in many species. Humans, fruit-flies, nematodes as well as yeasts revealed a levelling off, if not a decline, in the mortality rate instead of a constant increase. In particular, in humans the deceleration rate does not begin before than 80s and the plateau is not seen before 110, as shown in **Figure 1.1** (Kirkwood and Franceschi, 1992). There is still not a clear explanation of this phenomenon.

Practically, the consequence of these phenomena was the remarkable increase in the number of people over the age of 65 or 80 years living in all European countries. In 2000, 69 million people

world wide were aged 80 or over. By 2050 the 80+ year-olds are projected to increase 5 fold to 377 million and represent 4.4% of the population. Similarly the number of nonagenarians will reach 63 million by 2050 which is an 8 fold increase. Centenarians currently estimated at 167,000 will reach a projected 5.3 million worldwide. Europe is the area of the world where population aging is most advanced. The proportion of people aged more than 60 years in the European Union (EU) is currently close to a quarter and it is likely to rise to a third within three decades.

Thus, this scenario indicates that at the dawn of the third millennium one of the most important demographic phenomena is the increasing aging of the population, mainly due to a reduction in both birth rate and mortality rate, this latter being especially evident for the cohort of the over 80-years people.

The progressive increase of oldest old people brought to a new condition, i.e. the **increase of different age groups such as octogenarians, nonagenarians and centenarians**. This situation leads to extremely complicated demographic phenomena together with new problems regarding the allocation of resources for old age pensions and care for the elderly and it makes critically important the identification of factors (biological and non-biological) involved in aging devoid of major diseases and disabilities, thus contributing to increase the number of old European citizens in good health.

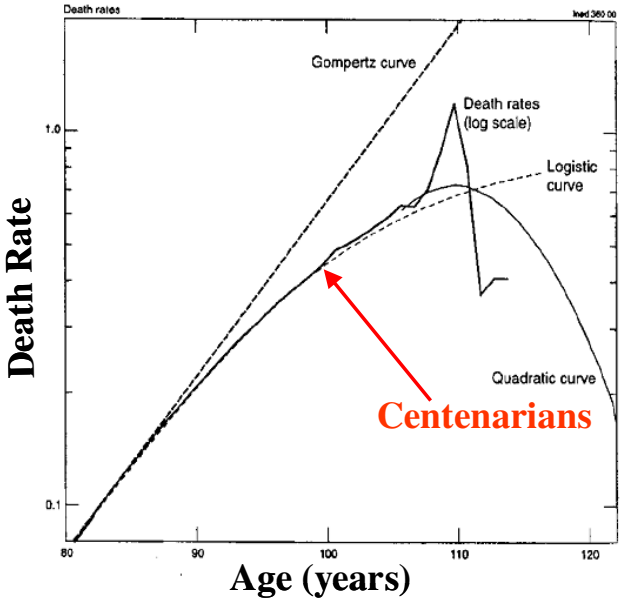


Figure 1.1 – Gompertz's curve of mortality

1.1.2 The Aging Process

In recent decades the research on aging has expanded quickly, probably as a consequence of the lengthening of the average human lifespan and the increasing percentage of elderly population. Biological, epidemiological and demographic data generated a huge number of theories trying to explain in part or completely the complex phenomenon of the aging process.

Many of them have been divided according to the basic idea of aging being a programmed process or not, an evolutionary determined process or not (Weinert and Timiras 2003). A summary is presented in the **Table 1.1**.

Table 1. *Classification and brief description of main theories of aging*

Biological Level/Theory	Description
Evolutionary	
Mutation accumulation*	Mutations that affect health at older ages are not selected against.
Disposable soma*	Somatic cells are maintained only to ensure continued reproductive success; after reproduction, soma becomes disposable.
Antagonistic pleiotropy*	Genes beneficial at younger age become deleterious at older ages.
Molecular	
Gene regulation*	Aging is caused by changes in the expression of genes regulating both development and aging.
Codon restriction	Fidelity/accuracy of mRNA translation is impaired due to inability to decode codons in mRNA.
Error catastrophe	Decline in fidelity of gene expression with aging results in increased fraction of abnormal proteins.
Somatic mutation	Molecular damage accumulates, primarily to DNA/genetic material.
Dysdifferentiation	Gradual accumulation of random molecular damage impairs regulation of gene expression.
Cellular	
Cellular senescence-Telomere theory*	Phenotypes of aging are caused by an increase in frequency of senescent cells. Senescence may result from telomere loss (replicative senescence) or cell stress (cellular senescence).
Free radical*	Oxidative metabolism produces highly reactive free radicals that subsequently damage lipids, protein and DNA.
Wear-and-tear	Accumulation of normal injury.
Apoptosis	Programmed cell death from genetic events or genome crisis.
System	
Neuroendocrine*	Alterations in neuroendocrine control of homeostasis results in aging-related physiological changes.
Immunologic*	Decline of immune function with aging results in decreased incidence of infectious diseases but increased incidence of autoimmunity.
Rate-of-living	Assumes a fixed amount of metabolic potential for every living organism (live fast, die young).

*Discussed in the text.

J Appl Physiol • VOL 95 • OCTOBER 2003 • www.jap.org

Table 1.1 – Main Theories of aging

Many of the proposed theories can actually explain only part of the complex phenomenon. Most of the mechanisms underlying aging, on the other hand, seem to be closely tangled each other.

It is quite difficult to find the optimal definition of "aging", since it is continuously challenged by new discoveries and insights in the paradoxes characterizing the aging process. However, **aging** could be defined as the process of **intrinsic deterioration of an organism that is reflected at the population level as an increase in the death probability and a decline in the production of the offspring** (Partridge and Gems, 2002).

Until some decades ago, it was believed that all the physiological functions of the organism underwent a simultaneous age-related decline (Maynard Smith, 1966). Other authors tried to quantify such a decline on the basis of cross-sectional comparison of data obtained from groups

of subjects of different age belonging to different cohorts, who showed a decrease of about 1% per year for most of the physiological functions, and these data were considered valid for the great majority of the organs of the body. Such a decrease would be detectable from 30 years of age onwards according to some authors (Andres and Tobin, 1977), whilst for some others, it would become evident even earlier, since the age of sexual maturation (Bafitis and Sargent, 1977). Longitudinal studies suggested that the most striking age-related changes occur after the age of seventy (Svanborg *et al.*, 1982). An updated vision of the phenomenon proposes that **human aging** should be considered as a **dynamic process leading to a continuous adaptation of the body to the life-long exposure to harmful stresses**. This vision has been conceptualised in the so-called "remodelling theory of aging" (Franceschi and Cossarizza, 1995; Franceschi *et al.*, 1995), which is mostly based on evidences obtained from studies on immunosenescence. In particular, these results show that immune functions are differently affected by aging, being some parameter strongly affected whereas some other remain unchanged or even increased (Wack *et al.*, 1998; Fagnoni *et al*., 2000; Franceschi *et al*., 2000c).

1.1.3 The Extreme Longevity

Owing to the increasing aging of the population and the increasing number of centenarians, we can state that human longevity, that is the attainment of the extreme limits of potential lifespan, is a reality. The highest life span ever scored and properly validated is that reached by the French lady Jeanne Calment, who died in 1997 at the remarkable age of 122 years and 164 days (Abbott, 2004). Longevity is considered to be the result of the interaction between environmental factors, genetics, epigenetics and stochasticity, each making variable contributions to the overall presentation of the phenotype (Candore *et al.*, 2006).

$$L = En + G + Ep + S$$

(Longevity = Environment + Genetics + Epigenetics + Stochasticity)

By **environment** we mean the early life events, societal and social factors and physical environment (personality and intelligence, health behaviour and everyday activities, mental and physical health), each contributing to attain longevity. Curiously, it was found a deviation in the remaining life span of people born in specific months from the average remaining life span at the age of 50 (in the Northern hemisphere countries the people born in the fourth quarter of the year live longer than those born in the second quarter; for Australia the pattern is shifted by half a year).

By **stochasticity** we mean the wide variation of life span of genetically identical organisms even if reared in a constant environment. For example isogenic population of the nematode *C. Elegans*

shows a striking intrinsic variability of life span (from 8 to 32 days, depending also on the strain) (Kirkwood *et al.*, 2005). In particualer, we intend that the whole process contains an element of chance, but not that the outcome is entirely random. Although the individual stochastic event is random, the distribution of the events in space and time is modulated by other factors: genetics and environment (Kirkwood *et al.*, 2005).

Understanding the interplay between genetics, epigenetics, environment and stochasticity is one of the most interesting challenge in gerontological research. In this perspective, it is conceivable that longevity can be achieved by different combinations of these three components, that vary, quantitatively and qualitatively, in different geographic areas according to the population-specific gene pool and to the socio-economic level of the population. (De Benedictis and Franceschi, 2006), thus indicating that no one of these factors is probably either necessary or sufficient to determine the aging phenotype at the individual level.

It seems that the importance of each component changes with the passing of time: the age of 60 years appears as a discriminatory point after which the role of environmental factors, stochasticity and also genetics increases, contributing to reaching very old ages. The rate of the age-related modifications occurring in each component is missing and it is difficult to be quantified because it also depends on the population differences in terms of genetics, life style, cultural habits, economic status and social networks.

Extreme longevity could be considered as a new phase of life, different from the previous one, which is characterised by **two types of remodelling**: A) immunological remodelling (immunosenescence); B) genetic remodelling (post-reproductive genetics).

A) During aging the immune system progressively changes in a dynamic process (**immunosenescence**) which mainly depends on the evolutionary unpredicted, chronic antigenic load persisting lifelong. This leads to the development of a chronic, low grade inflammatory process (called inflammaging), which however is compatible with 100 years of age or more because centenarians have also high levels of anti-inflammatory markers and protective genotypes of important molecules.

B) A complex genetic remodelling also occurs with age (**post-reproductive genetics**), whose main characteristics indicate that: the same alleles likely have different (beneficial or detrimental) effect at different ages -"Antagonist Pleiotropy"- (genes involved in IGF-1/Insulin pathway), protective genes become progressively more and more important with age (the case of IL-10), increased homozygosity at several polymorphic sites occurs with age, contrary to the accepted advantage of heterozygosity for survival at younger age (for example inter*Alu* sequence).

Therefore, the age-associated remodelling is associated with **increased robustness and frailty which occur concomitantly** (for example, the increase of memory and effector T cells that occurs with age becames deleterious if in excess).

The robustness of a complex system cannot be infinite and fully pervasive: somewhere in the system there is always a hidden frailty dictated by evolution. On the whole, the **aging process** (both physical and cognitive) is to be considered as an **adaptative process**: during life we are continuously exposed to antigens, stressors, emotions and we have to adapt to them (it is a darwinian fitness problem). Centenarians are individuals who adapted more and better than the rest of the population, therefore they are more robust (from a biological point of view), but at the same time they are frailer (from a geriatric point of view).

Inflammaging and the consequent change of body microenvironment is a major example of the concomitant accumulation of robustness and frailty. Inflammatory responses are physiological crucial for survival and constitute an essential part of our robustness, but at the same time inflammation is a basic components of frailty and most age-related major pathologies.Within this scenario, we can argue that robustness and frailty occur concomitantly. Moreover, together with an increased robustness and an increased frailty, the age-associated remodelling is associated also with a **loss of complexity**. To this regard, we should remember the loss of complexity of trabecular bone that occurs with aging, or the age related decrease of the absolute number of virgin T cells (non antigen-experienced) (CD95- CD28+) and the exhaustion of such cells in centenarians which is correlated to an increased risk of mortality (Fagnoni *et al.*, 2000). In summary, the global remodelling is composed of an accumulation of robustness, an accumulation of frailty and a loss of complexity which occur concomitantly. The three factors act independently in three dimensions until when they meet each other and the subject dies. The environment can shift further the moment of the meeting of the three factors and the role of stochasticity increases with age.

With increasing age, also individuality increases. Each organ of the body but also every tissue and cell type composing the organ are affected differently by the aging process: we have a great organ and individual variability which let us speak of the **"Aging Mosaic"** (Cevenini *et al.*, 2008).

1.1.4 Healthy Aging

As discussed, extreme longevity is a new phase of life characterised by a strong heterogeneity, due to sex, geographical, demographic, clinical and genetics differences (Franceschi *et al.*, 2008), which influence the rate of the of the physical, cognitive and psychological modification that

occur with age in each individual. Therefore, it is difficult to give a universal definition of "healthy aging". The concept of "healthy aging" was proposed for the first time by Cicero in 44 B.C, when he wrote: "Aging is not a phase of decline and loss, but, if properly faced, it becomes a fundamental source of positive changes" (Logan J, 1744). From this definition, many studies were performed in last years in order to distinguish "successful" from "unsuccessful aging".

Now, in a realistic way, "successful aging" can be defined as absence overt or severe diseases and disabilities, maintenance of high levels of physical and cognitive abilities and preservation of the social and productive activities. In this perspective, recent studies on Italian centenarians indicate that it is possible to identify a consistent subgroup of centenarians devoid of clinically overt major diseases, maintaining good physical and cognitive abilities and rather autonomous in their daily life. However, none of them fitted the criteria of "maintaining the social and productive ability" and in this sense they cannot strictly be considered as "successfully aged". Nonetheless, assuming less strict criteria, and avoiding any reference to any working activity, about 20% of the Italian centenarians could be considered as in "good health status for their age". This is now the best definition for the top subgroup of centenarians. It combines the awareness that centenarians are de facto extremely old and show the sign of aging, but at the same time it clearly indicates that they are in good shape notwithstanding their very advanced age, on the basis of standardized criteria regarding the cognitive and physical abilities. With all these methodological limitations in mind, we can argue that "healthy aging" is a real possibility for human beings and cast some doubt on the pessimistic view that extreme age must always be accompanied with severe diseases and/or disabilities. To conclude, at present aging must be considered an unavoidable end point of the life history of each one, nevertheless our increasing knowledge about the mechanisms it is regulated by, allows us to envisage many different strategies to cope with, and delay it, in order to endow everybody with a long and good final part of the life.

1.2 THE GENETICS OF HUMAN LONGEVITY

1.2.1 Recent advances in the genetics of human longevity

The two main concepts arisen from recent studies on the genetics of human longevity are the following:

(1) human longevity clusters in families;

(2) long-living siblings are likely enriched in longevity genes.

Actually, an impressive and coherent series of epidemiological data from different populations (White Americans from New England, Mormons from Utah, Ashkenazi Jewish living in the United States, Icelanders, Japanese from Okinawa, Netherlanders from Leiden, Danish collected in the entire nation, Italians from Southern Italy) suggests the presence of a strong FAMILIAR component of human longevity. All these studies demonstrate that **first-degree relatives (parents, siblings, and offspring) of long-lived subjects** (but not the spouses of the long-lived subjects who shared with them most part of their adult life) **have a significant survival advantage,** a higher probability to have been or to become long-living people and to have a lower risk regarding the most important age-related diseases, such as cardio- and cerebral-vascular diseases (CVD), diabetes, and cancer, when compared to appropriate controls (Terry *et al.*, 2004a; Terry *et al.*, 2004b; Atzmon *et al.*, 2004; Karasik *et al.*, 2004; Ikeda *et al.*, 2006). Thus, literature indicates that longevity is present in many generations of a single family in spite of the great variations in lifestyle and life expectancy as it occurred in the last century. In particular, it is remarkable that in the most recent studies on this topic, spouses of long-lived subjects were added as additional control group. The results indicate that this control group does not have any advantage/benefit in terms of survival and protection from the above-mentioned diseases, even if they shared with the long-lived partner most of their adult life.

In particular, as far as centenarians, parents, siblings, and offspring of centenarians are concerned, the available data indicate that:

(1) ***CENTENARIANS*** have the following characteristics:

• A lower prevalence of cancer, CVD, insulin-resistance and diabetes, and a delay of about 1–2 decades of the onset of others pathologies, such as dementia and hip fractures (Passeri *et al.*, 2003);

• Most of them do not show insulin-resistance and have anthropometric (BMI), metabolic (cholesterol, LDL-C, HDL-C, triglycerides, etc.), and cardiovascular (systolic and diastolic pressure) features that are optimal for their age (Barbieri at al., 2004);

• Their successful aging seems to be largely influenced by their optimal balance between inflamm-aging and anti-inflammaging (Franceschi *et al.*, 2007b). Centenarians appear to have

the capability to set up responses capable of neutralizing or at least diminishing the deleterious effect of the low-grade, chronic inflammatory status, characteristics of the aging process (inflammaging), which in turn is largely a consequence of the level of subclinical antigenic stimulation sustained by bacteria, viruses, and other pathogens;

• The above-mentioned characteristics can explain the finding in centenarians of a different frequency of a variety of polymorphisms of genes involved in immune response, inflammation, coagulation, and lipid and glucose metabolism, in comparison with younger controls (association studies). (Tan *et al.*, 2001; Barbieri *et al.*, 2003; Bonafè *et al.*, 2003; Bonafè *et al.*, 2001; Lio *et al.*, 2004; Carrieri *et al.*, 2004; Marchegiani *et al.*, 2006; Christiansen L *et al.*, 2004; Franceschi *et al.*, 2005; De Martinis *et al.*, 2005). However, most of these studies need to be replicated in different populations and contrasting data have been obtained in different studies;

• A different frequency of germ line variants of mtDNA (Tanaka *et al.*, 1998).

To this regard it is important to remind that **it is still unclear** whether and how much the different populations of long-lived individuals (centenarians and nonagenarians) studied so far (Ashkenazi Jewish, Danish, French, Finnish, German, Irish, Icelanders, Italians, Japanese, Mormons, among others) share the same genetic markers of longevity and **whether "public" and/or "private" (population specific) longevity genes and polymorphisms do exist in different populations and/or individuals**.

(2) ***PARENTS OF CENTENARIANS*** have a higher "risk" (about 7 times) to have reached extreme longevity (90–99 years old) (Atzom *et al.*, 2004). Parents' longevity is probably important and interesting from a biomedical point of view, as demonstrated by two recent studies:

• According to an investigation performed on 1402 members of 288 pedigrees within the framework of the Framingham Heart Study, genetic factors explained an additional 57% of biological age variability (Karasik *et al.*, 2004);

• According to a study performed in 51,485 men and women aged 40–79 years, the risk of mortality from all death causes including stroke and CVD was 20–30% lower in men and women with parents who died at age equal or higher than 80 years (fathers) and equal or higher than 85 years (mothers), compared with subjects having parents whose age at death was lower than 60 years (fathers) and lower than 65 years (mothers). These findings indicate that parental longevity could be a predictor for reduced risk of mortality from stroke, CVD, and all causes of death (Ikeda *et al.*, 2006).

(3) ***SIBLINGS OF CENTENARIANS*** also have an advantage for survival and for attaining extreme longevity:

• In a study on 2092 centenarian siblings, it has been demonstrated that both males and females have a mortality 50% lower than that of 1900 subjects of the same birth cohort, and their relative survival probabilities increase markedly at older ages, reflecting the cumulative effect of their mortality advantage throughout life. Male siblings of centenarians were at least 17 times as likely to attain the age of 100 years, while female siblings were at least 8 times as likely (Perls *et al.*, 2002);

• From the analysis of the pedigrees of 348 Okinawan centenarian families with 1142 siblings it resulted that both male and female centenarian siblings experienced approximately half mortality of their birth cohort-matched counterparts of the general Okinawan population (Willcox *et al.*, 2006). Remarkably, this mortality advantage of centenarians siblings was sustained at all ages and decades, and did not diminish or disappear with age in contrast to many environmentally based mortality gradients (gender, ethnicity, nutritional factors, such as cholesterol, physical activity, economical status, education level), suggesting that the familiar component is mostly genetically related;

• In families with at least two long-living siblings (men aged 89 years or more and women aged 91 years or more), the rest of their siblings, their parents, and their offspring, but not their spouses (husbands and wives), showed a major survival and a mortality rate for all causes of death that was 35% less than in the general population (Schoenmaker *et al.*, 2006) (see later).

(4) ***OFFSPRING OF CENTENARIANS*** presents a lower prevalence of CVD (56%), hypertension (66%), and diabetes (59%) (Terry *et al.*, 2003) and their median ages of onset for CVD, hypertension, diabetes, and stroke were significantly shifted forward by 5.0, 2.0, 8.5, and 8.5 years, respectively, indicating an increased age of onset of the major age-related diseases (Terry *et al.*, 2004a);

• They had a 62% lower risk of all causes mortality, a 71% lower risk of cancer-specific mortality, and an 85% lower risk of coronary heart disease-specific mortality (Terry *et al.*, 2004b);

• They had a favourable lipoprotein profile characterized by significantly larger HDL and LDL particle size and significantly increased homozygosity for the 405 valine allele (V allele) in the CETP gene (Cholesteryl Ester Transfer Protein) (Barzilai *et al.*, 2003), and the-641Callele in APOC3 gene (Atzmon *et al.*, 2004), similar to what has been observed in parents of centenarians.

At present, it is still unknown how much this familiar component of longevity and successful aging is due to genetics. This is a crucial issue from a theoretical (biology) and practical (biomedicine and public health) point of view, and the GEHA project is aimed to contribute to its clarification.

On the whole, the above-mentioned data would suggest that the familiar component of longevity is fundamentally a GENETIC component. At the same time, they indicate that families enriched in long-living members and, in particular, in very old siblings, and offspring of long-lived parents represent study groups particularly suitable to investigate the determinants of the human longevity.

In the relatively large literature on the genetics of longevity, three recent papers are of particular interest.

Schoenmaker *et al.* (Schoenmaker *et al.*, 2006) *s*tudied families with at least two long-living siblings (men: 89 years and over; women: 91 years and over) and showed that the standardized mortality ratio for all siblings of the long-living participants was 0.66 and that a similar survival benefit was also observed in the parents (0.76) and in the offspring (0.65) of the long-living participants. The standardized mortality ratios of the spouses of the long-living subjects was 0.95. The authors conclude that: (*a*) it is unlikely that the familiar clustering of extended survival is caused by environmental factors, because the spouses of the long-living participants had a mortality risk comparable with the general Dutch population, whereas they share the same environment; and (*b*) families with two long-living siblings are genetically enriched for extreme survival.

Hjelmborg *et al.* (Hjelmborg *et al.*, 2006) start from the consideration that although human family studies have indicated that a modest amount of the overall variation in adult life span (approximately 20–30%) is accounted for by genetic factors, it is not known if they become increasingly important for survival at the oldest ages. The genetic influence on human life span and how it varies with age was studied in cohorts of Danish and Finnish twins born between 1870 and 1910 (20,502 individuals) followed until 2003–2004. Mean life span for male monozygotic (MZ) twins increases 0.39 years for every year his cotwin survives over age 60 years, and this rate is higher than the rate of 0.21 for dizygotic (DZ) males. Females and males have similar rates and these are negligible before age 60 for both MZ and DZ pairs. Having a cotwin surviving to old ages substantially and significantly increases the chance of reaching the same old age and this chance is higher for MZ than for DZ twins. The authors conclude that: (*a*)

such a large population-based study shows genetic influence on human life span; (*b*) this influence is minimal prior the age of 60 years but increases thereafter; and (*c*) these findings provide a support for the search for genes affecting longevity in humans, especially at advanced ages; linkage studies in large samples of extremely long-lived siblings may be among the best approaches to identify such genes.

Christensen *et al.* (Christensen *et al.*, 2006) published a rich and comprehensive review which deliver several take home messages, including the followings:
(1) The determinants of life span are extraordinarily complex and human studies of longevity face theoretical and logistic challenges;
(2) Longevity clusters in some families but it is difficult to disentangle the effect of the shared environment and that of genetics;
(3) Owing to the complexity of the long-living phenotype, there is the possibility that different variants are involved in life-span variation in different populations;
(4) As the effect of the genetic component on longevity increases after the age of 60 years, nonagenarians and centenarians are particularly informative about longevity genes;
(5) Large sample size are needed to uncover alleles which occur only in a few percent of the population and that have a modest effect on survival;
(6) Large-scale and carefully designed study assessing long-lived siblings and controls, as well as studies on large cohorts of elderly people followed longitudinally, will be essential to progress in genetic studies of human longevity, especially if combined with high-throughput genotyping techniques;
(7) Genome-wide association studies are becoming feasible and are promising but logistically and financially demanding.

1.2.2 Putative Longevity Genes in Chromosome 4

An American group lead by Puca performed a **genomewide scan on 308 individuals belonging to 137 sibships** demonstrating exceptional longevity and observed a **borderline significant evidence** (P = 0.044) **for linkage for chromosome 4 near microsatellite D4S1564** (4q25) that was underrepresented among long-living individuals when compared with younger controls (Puca *et al.*, 2001). This candidate region in chromosome 4 (D4S1564) spans 12 million bp and contains approximately 50 putative genes. To identify the specific gene and gene variants impacting life span, the same group performed a haplotype-based fine-mapping study of the interval. The resulting genetic association study identified a haplotype marker within

microsomal transfer protein (MTP) as a modifier of human life span. This same variant was tested in a second cohort of French centenarians from CEPH, and **the association was not replicated** (Geesaman *et al.*, 2003). MTP has been identified as the rate-limiting step in lipoprotein synthesis. The **low number of sibships used in this study**, together with the impossibility to replicate the results in the French samples, prompted several labs to replicate the study in different populations and in a larger sample of long-living individuals. However, these studies **failed to replicate the original observation of the American group** in different European populations.

Nebel *et al.* (Nebel *et al.*, 2005) performed a study on 1039 unrelated subjects of German ancestry between 95 and 109 years of age (mean age, 98.2 years), 373 (36%) being centenarians. In comparison with all other U.S. and European subjects analysed in the literature, the MTP "risk" haplotype was found to be over-represented only in U.S. controls, implying that the putative association reported by Geesaman *et al.* (Geesaman *et al.*, 2003) was more likely to reflect recent changes in the genetic structure of the U.S. Caucasian population as a whole, rather than genetic effects upon survival to old age.

Bathum *et al.* (Bathum *et al.*, 2005) tested the hypothesis that MTP gene polymorphisms were associated with extreme longevity in a longitudinal study of nonagenarians and in an association study. Participants in the Danish 1905 cohort study (1651 participants aged 92–93 years) were genotyped for the two SNPs (rs2866164 and Q95H) in the MTP gene recently reported to be associated with longevity. The 1905 Cohort has been followed for 6.5 years, during which 83% of the cohort has died. Furthermore, a group of 575 middle-aged Danish twins (mean age 53.7 years) were tested as a younger control group. The risk haplotype had no significant survival disadvantage (*P* values: 0.56, 0.31, and 0.97 in the total population of nonagenarians, males, and females, respectively) after 6.5 years of follow-up. The distributions of the suggested risk alleles (rs2866164-G and Q95) and the resulting haplotypes were very similar and not statistically different between the two age cohorts. In conclusion, this longitudinal study of survival in the tenth decade of life and this association study in a genetically homogeneous population provided no support for an association between the MTP gene polymorphisms and extreme longevity.

Beekman *et al.* (Beekman *et al.*, 2006) investigated the linkage to 4q25 in 164 nonagenarian sibships of the Leiden Longevity Study (LLS). Moreover, the MTP -493G/T and Q95H allele and haplotype frequencies were compared in 379 nonagenarians, 525 of their offspring and 251 partners of their offspring of the LLS, and in 655 octogenarians and 244 young controls of the Leiden 85+ Study followed for at least 7 years and providing an opportunity to perform a prospective analysis. Both the linkage analysis and the association study were negative and the

authors, after performing a meta-analysis arrived to the same conclusions of Nebel *et al.* (Nebel *et al.*, 2005), i.e. that the problem of the original report was the admixture of the U.S. control population.

These data, on the whole, are important for research studies aimed at finding genes associated with longevity and suggest that:

(1) Linkage analysis to detect longevity genes must be performed in a large number of sibpairs;

(2) Association studies are useful and more sensitive than linkage analysis, but must be performed and replicated in different ethnically homogeneous populations, and particular attention must be paid to population stratification in the control groups.

1.2.3 Longevity Genes in Chromosome 11

It is becoming more and more evident that the **candidate region in chromosome 11 (11.15.5) could play a role in human longevity** because several studies point out that **polymorphic variants of an unusually large number of genes present in such a region** of about 2Mbases, such as Sirtuin 3 *(SIRT3),* v-Ha-ras Harvey rat sarcoma viral oncogene homologue 1 (*HRAS1*), Insulin-like Growth Factor 2 (*IGF2*), Insulin (*INS*), and Tyrosine Hydroxylase (*TH*) **are associated with human longevity** (De Benedictis *et al.*, 1998; De benedictis *et al.*, 2001; De Luca *et al.*, 2002; Bonafè *et al.*, 2002; Tan *et al.*, 2002; Rose *et al.*, 2003). It is important to remember that these genes are the human homologues of genes that, in a variety of animal models, appear to play an important role in life-span extension and in protection from a variety of stressors.

Moreover, new data published on humans (Bellizzi *et al.*, 2005; Bellizzi *et al.*, 2007) reinforce the interest for such a region of chromosome 11. Therefore it could be interested to test if the capability of some genes to be involved in life-span extension might have been conserved throughout evolution from yeast and worms to humans.

1.2.4 The genetics of healthy aging and longevity and the mtDNA variants

The mtDNA germline variants (haplogroups, subhaplogroups), and mutations (C150T) seem to play a role in human longevity, (Santoro A *et al.*, 2006) as well as their interaction with the newly emerging longevity nuclear genes. Indeed, a remarkable result from studies of long-lived individuals is the association found between mtDNA-inherited variants (**haplogroup J**) and healthy aging and longevity in **Italian centenarians** (De Benedictis *et al.*, 1999). Further data showed that **this association is likely population specific**, being present in long-lived subjects

from **Ireland** (Ross *et al.*, 2001; Niemi *et al.*, 2003), but *not in those from southern Italy* (Dato *et al.*, 2004). Moreover, a **C150T mutation** was found at a **much higher frequency in centenarians** than in young people (Zhang *et al.*, 2003). The data also showed that *C150T* variant causes a **remodelling of the replication origin at position 151** and can be either inherited (polymorphism) or somatically acquired (mutation). A commentary to this article was published by Wallace and co-workers (Coskun *et al.*, 2003) suggesting that **mtDNA-inherited variants (haplogroups) are likely not neutral** and subjected to climatic adaptation, and that C150T variant and/or J haplogroup **might have changed (reduced) oxidative phosphorylation (OXPHOS) efficiency** and thus reactive oxygen species (ROS) production, reducing oxidation stress, and increasing longevity. The higher frequency of 150T in aged subjects has been *confirmed* in a total of 321 very old individuals and 489 middle-aged controls from Finland and Japan (Niemi *et al.*, 2005). In addition, **150T was shown to be associated with longevity in subhaplogroup J2**, in accordance with a specific study on mtDNA haplogroup J in centenarians (Rose G *et al.*, 2001). Thus the available data concordantly point out that mtDNA variants (C150T polymorphism and haplogroup J or subhaplogroup J2) are associated with longevity in a population-specific way. The reason(s) and geographic extension are still unclear. Another open question regards the degree of heteroplasmy of the C150T variant and its tissue specificity.

It is therefore envisaged **to confirm and further extend those data**, which indicate a strong role of mtDNA variants in human longevity, starting from samples of Caucasian origin and from different geographic areas. Such a role of mtDNA (maternally inherited) is in line with data on the genealogy of supercentenarians (people older than 110 years of age), who show **a great survival advantage in the maternal lineage** (Caselli *et al.*, 2006). Furthermore, the classification of mtDNA variants is undergoing continuous modifications and updating which eventually redefine the mtDNA phylogenetic tree. The most recent paper redefining haplogroups classification and names also suggests that the complete sequencing of mtDNA would be preferable instead of the mere haplogroup identification (Torroni *et al.*, 2006). Unfortunately this kind of approach is not feasible at large scale due to the still high cost of mtDNA resequencing and it should be performed among homogeneous populations in order to confirm possible interactions between genetics and environment.(Dato *et al.*, 2004). Moreover, it is emerging that **mtDNA haplogroups interact with polymorphisms of nuclear genes** (Carrieri *et al.*, 2001; Bellizzi *et al.*, 2006).

1.2.5 The post-reproductive genetics of human longevity

The genetics of longevity appears to be quite peculiar, owing to the fact that it regards the post-reproductive period of life, a period largely non predicted by evolution and characterized by a progressive decrease of the force of selection (De Benedictis and Franceschi, 2006). This can explain some paradox of the genetics of longevity, such as the **increase of homozygosity** in several polymorphisms regarding a variety of candidate genes in centenarians with respect to younger subjects and the possibility that today centenarians may have originated from an initial frail part of the cohort which was able to survive at younger (reproductive) age and it was later allowed to exploit genes useful in the post-reproductive period of life. Again, it emerges that genetic traits which are useful in coping with stressors and are important for survival at younger age may became detrimental later in life. Vice versa it can be hypothesized that genes neutral or dangerous at younger age can became useful at old or extremely old age, according to a phenomenon defined as **"Antagonistic Pleiotropy"** (Williams and Nesse, 1991, Franceschi *et al.*, 2005; Salvioli *et al.*, 2006). It is thus evident that, if a genetic variant confers a selective advantage during young age, it will be selected even if it is unfavourable for longevity (for example by conferring a higher risk for age-related diseases). This seems to be the case for the inflammatory gene polymorphisms responsible for a higher responder status that were selected to fight infections in young age (Caruso *et al.*, 2005; Licastro *et al.*, 2005). In this perspective, the apparent paradoxes emerged from the studies on centenarians can be generated not only by the lack of validated scales for centenarians, but also by the fact that a genetic variant can play different roles in young age and in old age.

1.3 THE GEHA PROJECT

1.3.1 The origins of the GEHA Project

As previously discussed, the proportion of people aged more than 60 years in the European Union (EU) is currently close to a quarter and it is likely to rise to a third within three decades. This demographic explosion makes critically important the identification of factors (biological and nonbiological) involved in aging devoid of major diseases and disabilities, thus contributing to increase the number of old European citizens in good health. Clues concerning such healthy aging can be found by studying the selected group that survives over the age of 90 years and by searching for the genetic determinants of healthy aging in humans with a critical mass of human and technological resources.

Thus, it was in this scenario that the **5-year European Union (EU)-Integrated Project GEnetics of Healthy Aging (GEHA)** could rise, since its main aim is *to* **identify genes involved in healthy aging and longevity**, which allow individuals to reach advanced old age in good cognitive and physical function and in the absence of the major age-related diseases. The large size and vision of the GEHA project fits within the ambition and concept of integrating and strengthening the European Research Area. Indeed, GEHA coordinates a well-integrated network of demographers, physicians and gerontologists, geneticists, molecular biologists, statisticians, genetic epidemiologists, and bioinformaticians who are at the cutting edge of their various specialities. To our knowledge, GEHA represents **the strongest and most competitive consortium ever assembled in Europe** (and not only in Europe) **to investigate the genetic basis of the aging process and longevity in humans***,* capable of reaching a critical mass from a technological and interdisciplinary point of view which is impossible to attain in single European countries.

In July 2003 the 5-year GEHA-Integrated Project, supported through Priority 1 (Life Sciences, Genomics and Biotechnology for Health) of EU's FP6**,** Project Number LSHM–CT–2004–503270, was preliminaryly approved by the European Commission. The project officially started on May 1, 2004 after a negotiation of several months, during which a Consortium Agreement among the participating Partners was agreed. It will end on April 30, 2009.

The GEHA structure is conceived as a pipeline, where the first phase is the **recruitment of subjects (90+ sibpairs and younger unrelated controls)** over all Europe, that is the collection of information on their phenotype (health status) as well as of biological samples (blood and/or cheek swab); the second phase is the **DNA extraction***,* from the collected biological samples, **its quality control** and shipment to the GEHA partners in charge of the genetic analysis; the third

phase is the **genetic analysis**, and, finally, the forth phase is the **analysis of data** by mean of new analytical methods and *ad hoc* developed mathematical models.

As far as we know, the GEHA consortium is the largest international collaborative study on the genetics of human longevity, and eventually will provide the largest database on this topic.

1.3.2 The GEHA Consortium and its Bodies

The GEHA project is a large consortium of **25 partners** (24 partners from Europe and 1 partner from China). All these countries have traditions and laws quite different regarding privacy protection, ethical recommendations for genetic studies, access to demographic sources, Intellectual Property Rights (IPR) rules, among others. The GEHA project regarding the **genetics of human longevity** requires the recruitment of very old sibpairs and the donation of their blood or other biological material on which to carry out the genetic analysis. Thus, GEHA deals with sensitive issues (ethics, privacy, etc.), which requires as much attention and care as possible. For all these reasons, the first phases of the project were devoted to the **standardization of all the necessary tools**, and the **fulfilment or ethical requirements** both essential to start the recruitment of 90+ sibpairs and younger controls. A great effort was done to overcome the heterogeneity of the legislations established in the various countries involved in the project to guarantee the respect of privacy and confidentiality laws of the European citizens involved in the project.

In order to fulfil all the scientific, ethical, financial, and IPR requirements, and following the guidelines of the EU, the GEHA project was endowed with a complex organization structure composed by the following bodies:

Coordinator: Professor Claudio Franceschi; **Project Manager**: Dr. Alessandra Malavolta; **Scientific Manager**: Dr. Silvana Valensin;

General Assembly (GA) composed by 25 members (i.e., all the Principal Investigators, one person per Partner);

Steering Committee (SC) composed by 9 members (i.e., the leaders of the 12 Work Packages);

Ethics Steering Group (ESG) composed by 3 internal members plus 2 external members;

External Advisory and Gender Board (EAGB) composed by eminent scientists from the United States and Europe;

Legal and IPR Board (LIPR) composed by 3 members;

Financial Management Board (FMB) composed by 5 members.

The Institutions (Principal Investigator in parentheses) constituting the GEHA Consortium are:
(1) UNIBO-CIG, Interdepartmental Centre "L.Galvani," University of Bologna, Italy (Claudio Franceschi);
(2) CRLC, Department of Biostatistics, University of Montpellier, Val d'Aurelle Cancer Research Center, Montpellier, France (Jean Marie Robine);
(3) CAU, Kiel Center for Functional Genomics, University Hospital Schleswig Holstein, Kiel, Germany (Stefan Schreiber);
(4) CEPH, Centre Polymorphisme Humaine, Fondation Jean Dausset, Paris, France (Hélène Blanché);
(5) ISS, Istituto Superiore di Sanità, Rome, Italy (Maria Antonietta Stazi);
(6) LUMC, Molecular Epidemiology, Leiden University Medical Centre, Leiden, the Netherlands (Pieternella Eline Slagboom);
(7) MPIDR, Max Planck Institute for Demographic Research, Rostock, Germany (James W. Vaupel);
(8) NHRF, National Hellenic Research Foundation, Athens, Greece (Efsthatios Gonos);
(9) KTL, Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland (Leena Peltonen);
(10) NENCKI, Laboratory of Molecular Bases of Aging, Department of Cellular Biochemistry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland (Ewa Sikora);
(11) QUB, Department of Geriatric Medicine, The Queen's University Belfast, Belfast, United Kingdom (Irene Maeve Rea);
(12) UNICAL, Department of Cell Biology, University of Calabria, Rende, Italy (Giovanna De Benedictis);
(13) IFOM, Fondazione Istituto FIRC di Oncologia Molecolare, Milano, Italy (Pier Giuseppe Pelicci);
(14) UNISS, Department of Anesthesiologic Surgery, University of Sassari, Sassari, Italy (Luca Deiana);
(15) UCL, Research Centre of Demographic Management for Public Administrations, UCL—GéDAP, Louvain-la-Neuve, Belgium (Michel Poulain);
(16) FUNDP, Department of Biology, Facultes Universitaire Notre Dame de la Paix, Namur, Belgium (Olivier Toussaint);

(17) UNEW, School of Clinical Medical Sciences, Gerontology "Henry Wellcome" & PEALS Research Institute, Bioscience Centre, International Centre for life, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom (Tom B.L. Kirkwood, Erica Haimes);
(18) SDU, Institute of Public Health, University of Southern Denmark, Odense C, Denmark (Kaare Christensen, Bernard Jeune);
(19) TAMPERE, Laboratory of Gerontology, Tampere School of Public Health, University of Tampere, Tampere, Finland (Antti Hervonen);
(20) R&I, Research & Innovation Soc.Coop.a r.l., Padova, Italy (Alberta Leon);
(21) INRCA-Italian National Research Centre on Aging, Molecular Genetic Laboratory, Ancona, Italy (Liana Spazzafumo);
(22) UAAR, Department of Molecular Biology, University of Aarhus, Aarhus C, Denmark (Peter Kristensen);
(23) BGI, Department of Genome Dynamics and Bioinformatics, Beijing Genomics Institute, Chinese Academy of Sciences, Beijing, China (Huanning Yang, Lars Bolund);
(24) EAT, Eppendorf Array Technologies, SA - EAT Research and Development, Namur, Belgium (Jose Remacle);
(25) IG, Institute of Gerontology, Kiev, Ukraine (Vladyslav V. Bezrukov).

1.3.3 The Major Objectives of the GEHA Project

Europe is the oldest continent and is rapidly aging. Currently, the percentage of people in the EU who are 90 years old or older is about half a percent, with 90+ year-old-males comprising 0.29% of the male population and 90+ year-old-females 0.88% of the female population (data of 2003). Even if, collectively, age-related diseases (cardiovascular diseases, stroke, type II diabetes, cancer and dementia) affect most of the elderly, there is a minority which apparently undergoes an aging process that is free from such diseases (**"successful" or "healthy" aging**). The objective of the GEHA project is to identify genes that influence healthy aging and longevity in humans, and that protect individuals from major age-related diseases and disabilities, thus allowing them to survive to advanced old age in good cognitive and physical condition.
Accordingly, the major goals of the GEHA project are the following:
(1) To **overcome the fragmentation of the research on the genetics of aging in Europe**;
(2) To **set up a coherent, tightly integrated program of research** that unites demographers, geriatricians, geneticists, genetic epidemiologists, molecular biologists, bioinformaticians and statisticians;

(3) To **recruit an unprecedented number of long-living sibpairs** (***n* = 2650**) both aged 90 years of age or more (90+) **from 11 European countries** in 15 geographic areas;
(4) To **perform a genome-wide scan on the DNA of all recruited sibpairs** (Affected SibPair analysis, ASP analysis) in order to identify chromosomal regions involved in longevity and healthy aging;
(5) To **recruit a large number (*n* = 2650) of ethnically-matched control subjects** (50–75 years of age) from the same geographic areas, necessary to fine-map the chromosomal regions identified by ASP analysis and the three candidate chromosomal regions (see n.8), and to allow large scale association studies;
(6) To **perform bioinformatics, functional genomics, proteomics and molecular biology** studies on the identified/putative longevity regions/genes and gene variants resulting from ASP analysis and LD mapping;
(7) To test whether **ethnically different European populations** (including those from **Sardinia** and **Finland**) share the same genes involved in aging and longevity;
(8) To ascertain the **role played in human longevity by three candidate regions** (**D4S1564** in chromosome 4, **11p15.5** in chromosome 11 and **around the ApoE gene** in chromosome 19) once ascertained the LD block structure in CEPH families;
(9) To verify in a variety of European populations and at a large scale the **role of mitochondrial DNA (mtDNA) germline variants (haplogroups, subhaplogroups)**, and mutations (C150T) in human longevity, and to study their interaction with the newly emerging longevity nuclear genes;
(10) To **identify gender-specific genes** differently involved in the healthy aging and longevity of women and men;
(11) To stratify the samples according to **ApoE genotype**, i.e. the only genetic marker which so far has been found to be associated with reduced longevity in a variety of populations;
(12) To **develop innovative analytical strategies** (based on statistical method and mathematical models) **capable of combining all the data collected** (demographic, clinical, socio-economical, genetic and related to lifestyle), to highly increase the power of genetic analysis;
(13) To **perform a longitudinal study** to evaluate the importance of genetic factors on mortality of the recruited 90+ sibpairs.

1.3.4 Standardization of Recruitment Tools and Procedures

The overall success of the GEHA Project largely depends on the success of the recruitment of 90+ sibpairs and younger controls all across Europe; thus, at the beginning of the project, a

particular effort was made in order to standardize the recruitment strategy among GEHA Partners to allow the collection of homogeneous data that could be compared at the end of the study to answer the critical questions the project is aiming to answer.
In particular, the following activities were performed:
(1) Set up and standardization of **two Informed Consent Forms**, the first for 90+sibpairs, and the second for the younger controls (in all the collecting country National languages and in English).
(2) Set up and standardization of **three Questionnaires**, one for 90+sibpairs, one for younger controls, and the last for the family of the 90+ sibpair (in all the collecting countries National language and in English).
(3) Set up of the **GEHA phenotypic and genetic databases** plus a **database for mtDNA**. All databases strictly respect the privacy protection requirements established upon suggestions of the ESG and based on the European legislation.
(4) Set up and standardization of the procedures for the **collection, labelling and processing of the biological material** (blood samples and cheek swabs) in a way suitable to guarantee the privacy respect, and assure a suitable shipment and storage of the samples.
(5) Identification of the **centralised facilities for DNA extraction from peripheral blood and blood cells and DNA permanent banking** at KTL, Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland (Partner N.9).
(6) Identification of the **centralised facilities for DNA quality controls, quantification, preparation of DNA plates** and their shipment to genetotyping platforms at KTL, Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland (Partner N.9).
(7) Set up and standardization of the **protocol for DNA extraction** for nuclear DNA and for mtDNA from peripheral blood lymphocytes and granulocytes.

Moreover, before starting the real enrolment, all European recruiting units performed the following preliminary and preparatory activities:
(1) Identification of the **geographic areas suitable for the recruitment** of 90+ sibpairs and ethnically matched younger controls, and assessment of the procedures to **access the demographic data** when available and/or to contact the candidate sibpairs and controls directly or through the General Practitioner.
(2) Preparation of the documents (in both National and English language) for obtaining the **approval of the local ethical committees**.
(3) Participation to a specific **Recruitment Course** organized in Bologna in October 2004.

1.3.5 GEHA Databases

The GEHA project highly depends on a complex bioinformatics environment that ensures full availability of samples, phenotypes and molecular data to the Partners, but also ensures data privacy to the participating EU citizens. In order to fulfil the requirements related to privacy protection, security, easy access and implementation, GEHA envisages a peculiar centralization of the different types of data collected. Indeed, the three main types of GEHA data (phenotypic, genetics and related to the mtDNA) are stored on three physically separate servers:

- the **Phenotypic Database** (containing clinical and demographic data on the basis of GEHA questionnaires), localized in **Odense (Denmark)**;
- the **Genotypic Database** (containing genotyping data), localized in **Kiel (Germany)**;
- the **mtDNA Database** (containing data related to mtDNA), localised in **Tampere (Finland)**.

Thus, these geographically separated databases strictly separate phenotype data (phenotype database and phenotype server) and genotyping data (genotyping database and server). However, they are largely interconnected: this peculiar structure allows GEHA Partners to perform all types of analysis (cross-analysis) and at the same time it protects privacy (**Figure 1.2**).

The general criteria of GEHA databases can be summarised as follow: not access from outside, air conditioned system, localization in locked server room, daily backups and networks protected by a firewall.

As regards the ***Phenotypic Database***:

• Data are entered using the PC application **EPIDATA** on the server;
• **Each centre** enter **locally** all the data related to each recruited subject;
• EPIDATA provides **immediate validation** while entering data (Web solutions will NOT give immediate validation);
• The system **speed** is **satisfactory**;
• Access of **several users contemporary** (tested with 5 users);
• **Central backup** of the data;
• **Access control**: each partner can only access his own data;
• EPIDATA stores data in **text files** (ability to track changes in data and easier merging of the data from the different centers);• The Oracle application makes it possible to **download and view your own data**.

The ***Genotypic Databases*** was built up for high throughput SNP genotyping and for the storage of genotype data, such as chromosome, locus, oligo sequences and genotypes.As regards the ***mtDNA Database***, it should be remembered that the GEHA consortium will eventually match all

the data obtained on mtDNA genetics with those obtained as a result of the genotyping of the nuclear genome, in the 90+ sibpairs and in the controls. For this purpose, a new database was created in order to allow storage, retrieval, and analysis of all the collected mtDNA as well as the cross-matching of these data with those coming from the nuclear DNA genotyping. This database will represent one of the largest collection of mtDNA sequence data, and by adding to it other already published sequences will constitute one of the largest mtDNA database worldwide. The consortium will also work to implement in the database some new functions which are currently not available in any other mtDNA database such as the automatic haplogroups classification. This feature is the first step into the direction of making such a software a permanent service available, in due time, to any other user worldwide.

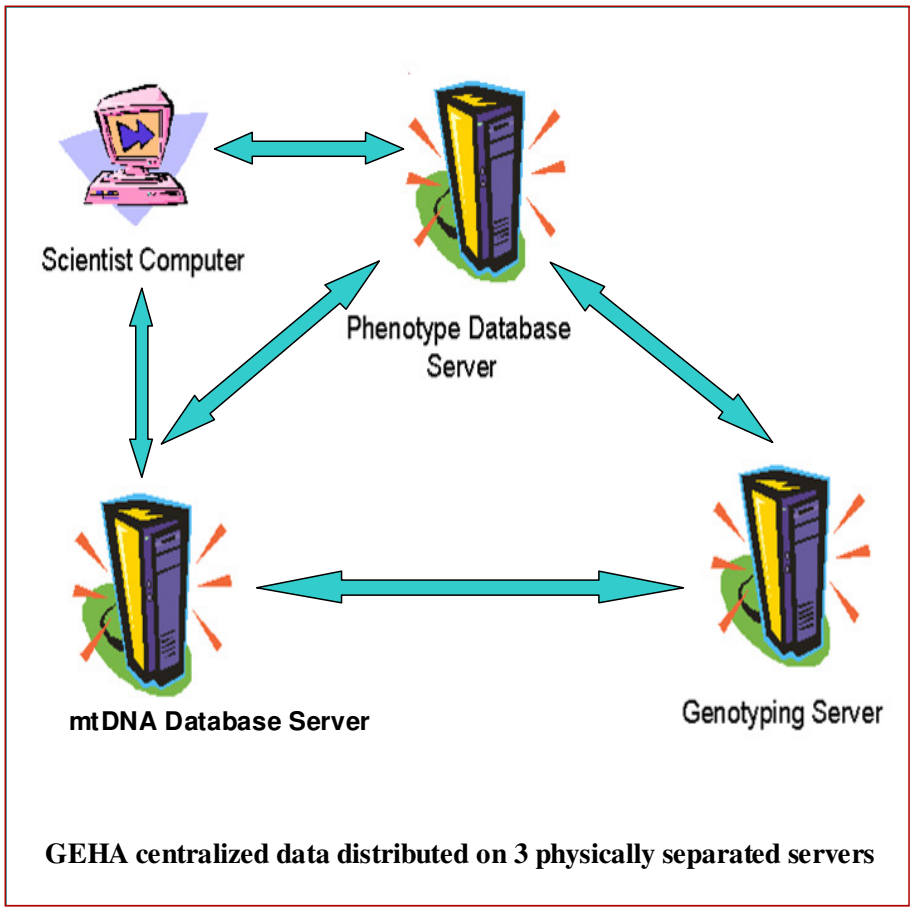


Figure 1.2 - GEHA databases: physically separated but largely interconnected

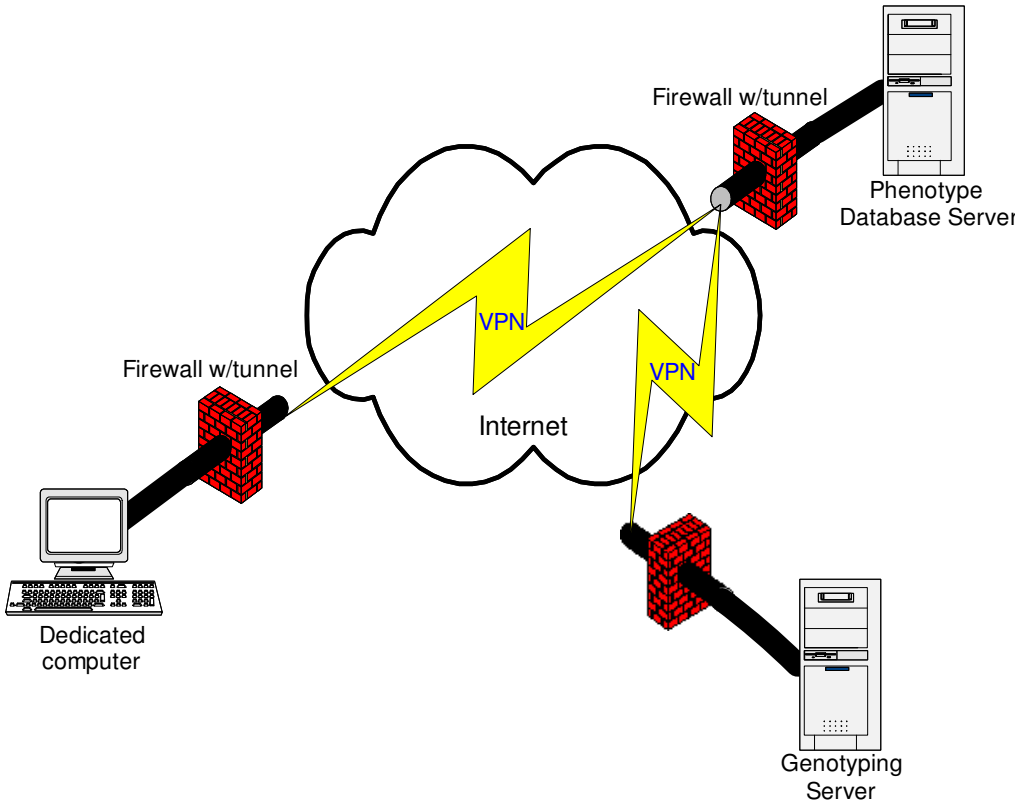


Figure 1.3 - Security of GEHA databases

1.3.6 The GEHA design and the genetic analysis (nuclear and mitochondrial genome)

GEHA genome-wide linkage scanning

In the last few years an enormous amount of data became available regarding the human genome, including data on millions of new single-nucleotide polymorphism (SNP) variants in different human populations (HAPMAP Project). Such an unprecedented extremely fast progress has been possible owing to the continuous refinement of the genetic methodologies as well as the methods of data analysis. Concomitantly, the conceptualisation about genome-wide studies, their possibilities and limitations, has also progressed in a very fast way so that the entire scenario of genetic studies on complex traits has completely changed.

The GEHA project took an enormous advantage from such a rapid advancement in the field and the GEHA geneticists, after a careful examination of the most recent available literature in the field, decided the genetic strategy and the platform to adopt according to reliability of the results, cost per SNPs and technician time, as well as the direct experience and the expertise of the GEHA partners.

Even if the main goal of the GEHA project is to perform a **Linkage analysis** with several thousand of highly informative SNPs using the 2650 90+sibpairs, it is important to stress that the GEHA design allows to perform both linkage and **association studies** (using one member of the sibship and the younger unrelated control), according to the most advanced genetic approaches to complex traits, as illustrated in **Figure 1.4**. The last possibility (genome-wide genetic association studies) might be pursued in future developments/continuation of the project, using the unique collection of DNA samples recruited by the GEHA consortium.

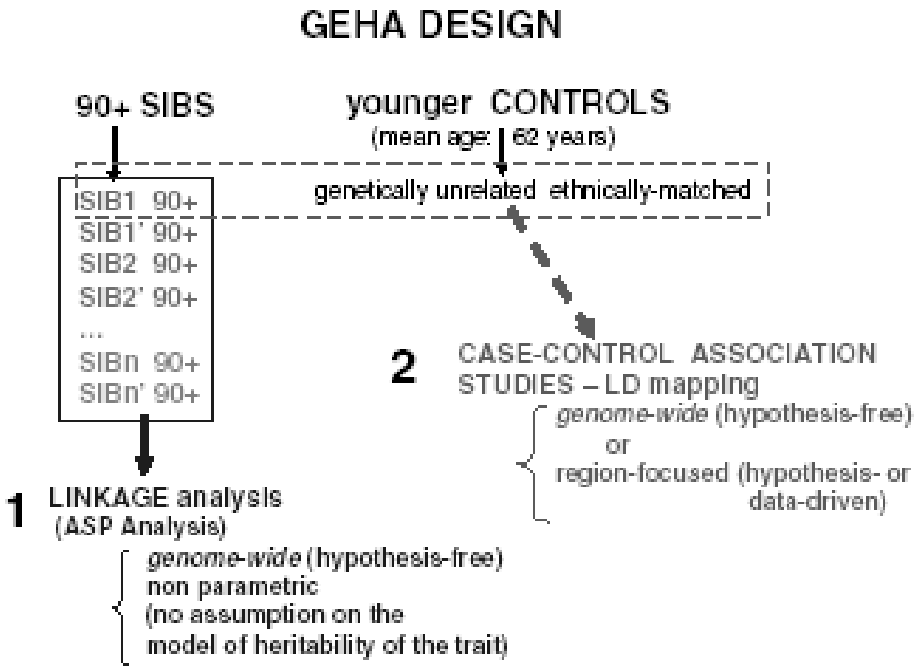


Figure 1.4 - The design of the GEHA project allows to perform either **genome-wide linkage studies**, using the DNA collected from the 2650 90+ sibpairs, or **association studies** using the DNA collected from one (or both) member of each sibship and the DNA collected from the unrelated, ethnically matched younger control. The association studies can be either genome-wide or focused on specific chromosal region(s) or loci.

Indeed, linkage studies on large samples of extreme long-lived siblings may be among the best approaches to identify longevity genes. Linkage analysis looks for coinheritance of chromosomal regions with the trait in families, and it is more powerful than association analysis for identifying rare high-risk disease alleles. Association is an approach to gene mapping that looks for associations between a particular phenotype and allelic variation, that is, for differences in the frequency of genetic variants between unrelated affected individuals and controls, with the expectation that the risk-conferring allele (haplotype) will be more common in cases (the long-living people) than in controls (the younger subjects). Association analysis nowadays can be performed genome-wide and it is expected to be more powerful for the detection of common alleles that confer modest disease risks. The advantage of linkage studies is that they are less influenced by population admixture than the association approach, while the advantage of association case–control studies is that they require much less genotyping to obtain equivalent power. Within an evolutionary and Systems Biology perspective longevity likely results from the interaction and cross-talk between two genomes: (*a*) the Nuclear genome; and (*b*) the Mitochondrial genome (mtDNA). Accordingly a major aim of GEHA is to ascertain the role of

mtDNA inherited as well as epigenetic variability in human longevity taking advantage of the unprecedented number of very old sibpairs recruited by GEHA, belonging to different European populations.

Analysis of mtDNA variability

The GEHA consortium has the capacity to provide the largest dataset on mtDNA variation over age in different populations. To this purpose the main activities will be the following :

(1) mtDNA Resequencing

Different approaches were developed by the GEHA consortium in order to obtain complete mtDNA sequencing. A strategy of quality control of the sequences and the design of a database for the storage and analysis of the sequences and their annotation were developed. mtDNA belonging to the specific populations will be resequenced for a total of about a thousand mtDNA sequences. All other GEHA samples will be genotyped for mtDNA haplogroups and subhaplogrops, using a protocol based on polymerase chain reaction (PCR) amplification and sequencing of the mtDNA D-Loop together with some principal restriction sites. An appropriate database for storage and analysis of mtDNA genetic data will be developed.

(2) Analysis of C150T Mutation

A fast and relatively cheap DHPLC technique to screen heteroplasmy in the whole mtDNA molecule was developed. This will allow to analyse possibly identified common “hot spots” of heteroplasmy (including the C150T mutation) in a large group of sibpairs and controls.

1.3.7 Bioethical issues and implications

Ethics is a major and pervasive topic which dominates all the issues of the GEHA project. The superb expertise of the *ESG* was critical to solve a variety of important and complex problems related to recruitment and the planning of genetic studies. Indeed, there is **a large heterogeneity of ethical rules** among the different countries taking part in the GEHA Consortium that must be taken into consideration whenever facing any decision involving ethical issues. In particular, the ESG produced specific suggestions and recommendations regarding:

(1) The key ethical questions about recruitment and informed consent;

(2) The establishment of criteria for privacy and confidentiality of data and their long-term storage;

(3) The establishment of criteria for let the general public appreciate the ethical implications of a genetics study such as GEHA;

(4) The issue of the use of biological samples after the end of the GEHA consortium.

Additionally, ESG performed a thorough investigation of all the literature regarding the genetics of aging and longevity in humans, in order to have a comprehensive view of how the ethical issues and implications of this type of research have been addressed and solved all over the world. Specific papers have been published on this topic (Matthews *et al.*, 2005).

Afterwards, the investigation moved towards **the collection of data on ethical and social aspects of genetic database management and issues around informed consent**. This work led to the production of an official document providing general practical guidance for the GEHA consortium on data storage, confidentiality, access and exchange. Finally, further activities were focussed on the continuation of the activity of previous years addressing the ethical and social aspects of GEHA, monitoring the literature on the key ethical and social aspects related to: recruitment, biological sample collection, the criteria for privacy and confidentiality of personal data handling, biological data handling, long term storage and continued usage of data gathered including third party (i.e. non-original researcher) access to data.

Particular attention was devoted to the ethical problems related to the continuation of GEHA activity and usage of the collected biological material and databases after the official end of the GEHA project.

1.3.8 Training

The long term success of GEHA consortium depends on successful and integrated working and interchange of ideas between people with different expertise such as demographers, epidemiologists, geriatricians, geneticists, molecular biologists, mathematicians, statisticians and bioinformaticians. In particular a strong effort was devoted to the **training of young scientists** in this field at the cutting edge of the above mentioned disciplines. Three different instruments were used to give to young scientists an interdisciplinary education experience in the genetics of healthy aging and longevity. In total, the following training acrivities were organized:

1. **Short period exchanges of young scientists** amongst GEHA partner labs;
2. **A Short Course on Demographic-Statistical Methods**, held on September 12-30, 2005 at the **Max Planck Institute for Demographic Research** (MPIDR, **Partner N. 7**), Rostock, Germany to whom young members of the GEHA consortium participated;
3. A **Mitochondrial Training Workshop** held on March 2007 at University of Calabria (UNICAL, **Partner N.12**);
4. A **Research and Training Day,** held before the Third GEHA Annual Meeting (Warsaw, 28 June 2007);

5. A **Genetic Data Analysis Workshop**, held on November 11-13, 2007 at the **Max Planck Institute for Demographic Research** (MPIDR, **Partner N. 7**), Rostock, Germany;
6. A **Research and Training Day,** held before the Fourth GEHA Annual Meeting (Rome, 1 July 2008).

1.3.9 Dissemination

The following dissemination initiatives were pursued:
1. A **GEHA web site** (www.geha.unibo.it) was set up since June 2004;
2. Many articles devoted to the most advanced scientific projects in Europe in **daily newspapers and weekly magazines** mentioned the GEHA projects as an example of cooperation at the European level to achieve important goal for the health of citizens;
3. **Several TV and radio programs** in UK, Germany, Italy, Finland, France, Polland, Ukraine and Greece devoted to the aging of the population and to the biological basis of aging and longevity mentioned the GEHA project;
4. **Several scientific article** on the genetic determinants of human longevity were published.

2. AIM OF THE STUDY

The present study is part of the Integrated European Project ***"GEHA – Genetics of Healthy Aging"*** (Franceschi *et al.*, 2007a), whose aim is to identify **genes involved in healthy aging and longevity**, which allow individuals to survive to advanced age in good cognitive and physical function and in the absence of major age-related diseases.
Within the frame of the whole GEHA project the specific aims of this thesis are the following:

1. to outline **the recruitment of 90+ Italian siblings and controls performed by the recruiting units of the University of Bologna (UNIBO) and Rome (ISS).** The procedures related to the following items necessary to perform the study will be described and commented: identification of the eligible area for recruitment, demographic aspects related to the need of getting census lists of 90+siblings, mail and phone contact with 90+ subjects and their families, bioethics aspects of the whole procedure, standardization of the recruitment methodology and set-up of a detailed flow chart to be followed by the European recruitment centres (obtainment of the informed consent form, anonimization of data by using a special code, how to perform the interview, how to collect the blood, how to enter data in the GEHA Phenotypic Data Base hosted at Odense).

2. to provide an overview of the **phenotypic characteristics of 90+ Italian siblings recruited by Bologna** (549 90+ siblings, belonging to 258 families) **and Rome** (216 90+ siblings, belonging to 106 families) **recruiting units** for a total of 765 90+ subjects. The following items will be addressed: socio-demographic characteristics, health status, cognitive assessment, physical conditions (handgrip strength test, chair-stand test, physical ability including ADL, vision and hearing ability, movement ability and doing light housework), life-style information (smoking and drinking habits) and subjective well-being (attitude towards life). Moreover, **haematological parameters collected in the 90+ sibpairs** as optional parameters by the Bologna and Rome recruiting units will be used for a more comprehensive evaluation of the results obtained using the above mentioned phenotypic characteristics reported in the GEHA questionnaire.

3. to better **identify healthy aging phenotypes based on cross-sectional data about health and functional status**, which is a major issue for studies aimed at finding the genetic factors of human longevity, such as the GEHA project. To this purpose, three different classification methods were proposed in various studies on centenarians, based on:

1. actual functional capabilities (ADL, SMMSE, visual and hearing abilities) (Gondo *et al.*, 2006);
2. actual functional capabilities and morbidity (ADL, ability to walk, SMMSE, presence of cancer, ictus, renal failure, anaemia, and liver diseases) (Franceschi *et al.*, 2000a);
3. retrospectively collected data about past history of morbidity and age of disease onset (hypertension, heart disease, diabetes, stroke, cancer, osteopororis, neurological diseases, chronic obstructive pulmonary disease and ocular diseases) (Evert *et al.*, 2003).

Firstly these available models to define the health status of long-living subjects will be applied to our sample and, since the classifications by Gondo and Franceschi are both based on the present functional status, they will be compared in order to better recognize the healthy aging phenotype and to identify the best group of 90+ subjects out of the entire studied population.

4. to investigate the **concordance of the health status and of the functional status among 90+ siblings** in order to divide sibpairs in three categories: the best (both sibs are in good shape), the worst (both sibs are in bad shape) and an intermediate group (one sib is in good shape and the other is in bad shape). Moreover, this evaluation will allow us to discover which variables are concordant among siblings; thus, concordant variables could be considered as familiar variables (determined either by the environment or by genetics).

5. to perform a **survival analysis** by using mortality data at 1st January 2009 from the follow-up as the main outcome and selected functional and clinical parameters as explanatory variables.

3. MATERIALS AND METHODS

3.1 THE RECRUITMENT PROCEDURE

3.1.1 Recruitment of 90+ sibpairs

The eligible subjects for the GEHA study should be aged 90 years old or older (90+) and have at least one sibling of the same surname and an age above 90, but below the age of the proband (**"Proband"** = the oldest sibling of the sibship that was recruited). Multiple sibships are also welcome since they could be even more informative. The only **exclusion criteria** indicated by the Ethic Steering Group was the inability of the 90+ subject to give the informed consent; this implies the exclusion of 90+ siblings whit an evident dementia.

3.1.2 Recruitment of younger control subjects

The subjects eligible to be enrolled as younger controls of the study should be aged 50-75 years and ethnically matched with 90+ sibpairs.

The recruitment of young control subjects followed a geographic/ethnic matching strategy. **The spouses of proband's offspring** (approx. 50-75 years) was the first choice, the only criteria of exclusion being that he/she was not of European origin. However, some probands were without offspring, or offspring were not married, or spouses were dead, not available or denied to participate. In this case **subjects were randomly recruited** (from the same geographic area) having a sex and age compatible to that of the missing person.

The recruitment of these people was done concomitantly with the recruitment of sibpairs.

The recruitment of sibpairs and of a corresponding number of young people **took place in 11 Countries, corresponding to 15 geographic areas** and it started after **a specific Recruitment Course** organized in Bologna in October 2004 (**Figure 3.1**).

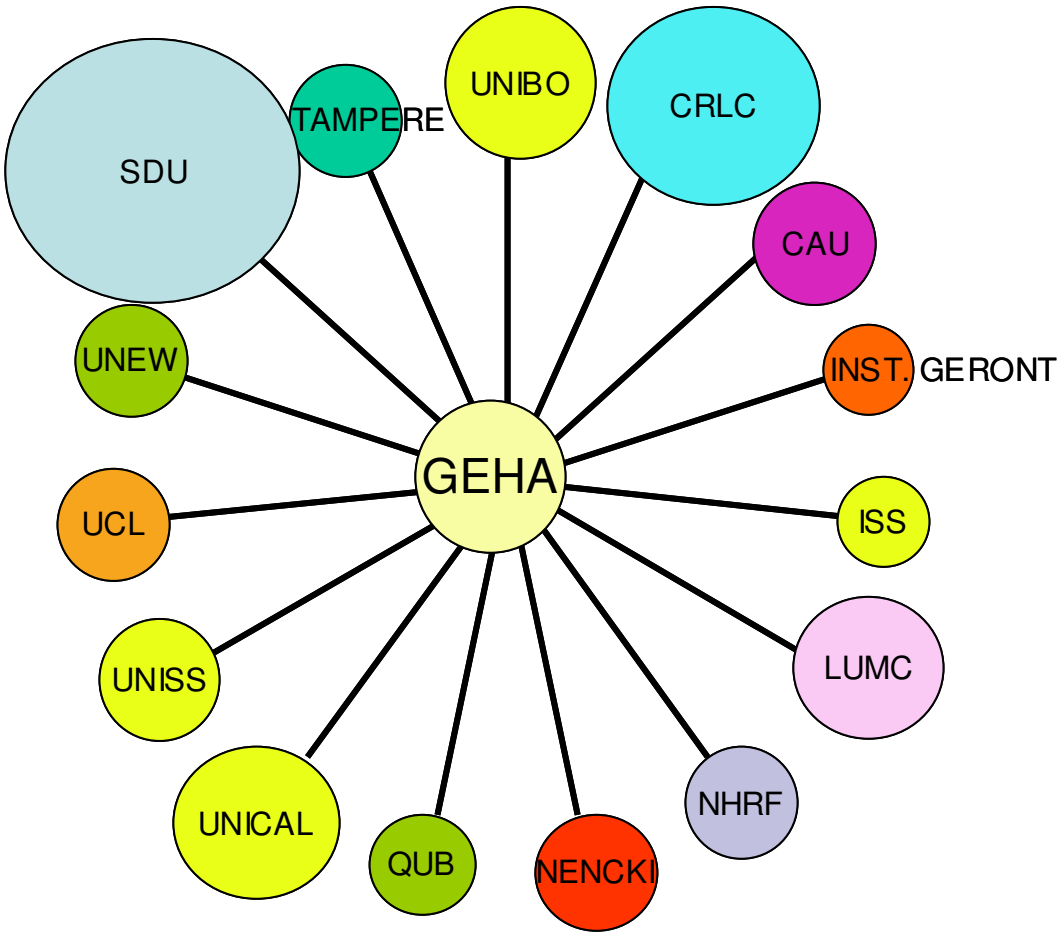


Figure 3.1 - The GEHA recruitment plan - The area of the circles indicates the amount of recruitment burden within GEHA. The same colour identifies units which will recruit sibpairs in the same countries. Recruitment period: May 1st 2004 - August 31st 2008 (it ended 4 months after the original deadline).

Useful definition to enter the logic of the GEHA study:

- **"Proband"**: the oldest sibling of the sibship that was recruited;
- **"TRIOS"**: a sibpair composed by at least two 90+ sibs, or more when available, plus 1 younger ethnically-matched control subject;
- **"COMPLETE TRIOS"**: a trios where at least 2 sibs and the control donated whole blood or a mix of whole blood and cheek-swab samples;
- **"CHEEK-SWAB TRIOS"**: a trios where at least 1 sib or the control donated only cheek-swab samples (these trios are not counted in the total amount of trios recruited by each recruiting unit);
- **"NEVER COMPLETED TRIOS"**: trios that will be never be completed, for example because one sib died in the meantime or refused to take part in the study or after the interview refused to donate biological samples.

3.1.3 Preliminary and preparatory activities to the recruitment

To start the recruitment the following preliminary and preparatory activities were performed:
(1) **Set up of a preliminary demographic survey for recruitment feasibility**, exploring the demographic data of each specific area where recruitment is performed, in order to exactly know the dimension of the geographic areas in which the recruitment takes place, as well as the outnumbering of people to sample. The geographic area suitable for the recruitment of 90+ sibpairs should contain a number of candidate sibpairs larger than the number eventually recruited, because of the expected withdrawals, as a consequence of refusal or impossibility of enrolment of the proband or of the other sibling for personal or medical reasons (severe diseases), the death of the proband or of the other sibling during the recruiting period or presence of unreachable sibpairs or isonimia. **It was estimated that overall there will be about a 50% of refusal or impossibility to recruit the sibpair.**

(2) **Access to demographic data**: the Census data to be acquired during the preliminary survey should comprise all the 88+ people present in the geographic area which will become eligible during the recruitment period. In order to minimize the bias related to the death of the most frail member of the pair, a random sampling on the oldest old in the list was suggested. In this way the principia of random sampling and the economic criteria of taking into account the turnover of the 88+ were matched and combined. The time predicted for overall recruitment had the disadvantage of being spanned over a relatively long period but at the same time had the advantage of peeking up new entries which can be estimated about 20% a year.

3.1.4 Set up of a standardized protocol for the collection of the subjects' data

(1) Set up and standardization of two **Informed Consent Forms**, the first for 90+sibpairs, and the second for the younger controls (in all the collecting country National languages and in English), following the recommendation of the Ethical Steering Group and taking into account the local legislation in the different European countries where recruitment took place. These documents were necessary for obtaining the **approval of the local ethical committees**.

(2) Set up of **a common introductory letter** to be presented to the people asked to participate in the GEHA study, in connection with the common informed consent form in order to give the participants a qualified basis for decision of participation. This introductory letter, written in a clear and understandable way for the recipients, explained the purpose and background of the

study and that participating to the GEHA study would include for 90+ people a visit of approximately 90 minutes by a research nurse or interviewer and a blood sample to be taken. It also underlined that participation did not involve any risk for the participants, that participation was completely voluntary, and that the participant could resign from the study at any time.

(3) Set up and standardization of **three Questionnaires**, one for 90+sibpairs, one for younger controls, and the last for the family of the 90+ sibpair (in all the collecting countries National language and in English), for the clinical assessment of the old sibpairs as well as the younger controls. They contain: 1. Socio-demographic information (including ethnic origin and education); 2. Clinical and anamnestic data; 3. Functional activity (ADL); 4. Life style habits; 5. Physical performance (handgrip-function, walking, stand/sitting-test, etc); 6. Cognitive function (SMMSE); 7. Self-reported health.

How the GEHA Questionnaires were set up?

The aim of the questionnaires is to obtain information making it possible **to classify the long-lived participants in three main groups** based on their functional capabilities: those with an **exceptionally good** health status, those with a **poor** health status, and the group **in between**. This classification will subsequently be the basis for the analyses of the relation between healthy aging and genetic factors.

The GEHA questionnaire for 90+ sibpairs was built on **several years of direct experience that many members of the GEHA Consortium have in the assessment of the health status, interviewing and recruiting very old people in the course of a variety of studies performed on nonagenarians and centenarians in several European countries,** including EU ECHA project, which included interviews and health status assessment of extremely long-lived people in Italy, France and Denmark. Some of the members of the GEHA Consortium were indeed **the first to propose a classification of centenarians based on their health status** assessed on the basis of objective and quantitative criteria (Franceschi *et al.*, 2000a). A starting point of the discussion on the type of questionnaire to be adopted was **a critical evaluation of all the available questionnaires adopted until 2004 in studies on the oldest old**. This critical evaluation arrived to the conclusion that most of the questions posed to very old people in the various questionnaires were apparently useless and they have never been used later on because they refer to poorly quantifiable trait or ambiguous questions. Moreover, **a trade-off likely occurs between the number of questions or items in the questionnaire and the reliability of the responses obtained**. Last but not least, all the GEHA partners involved in

the recruitment agreed that for practical reasons (fatigue of the 90+ people; rate of acceptance of the blood donation) **it was unacceptable an interview which would last more than 90 minutes** maximum. Thus a particular effort was devoted to include in the questionnaire for 90+ sibpairs only critical items suitable to help defining the health status of the oldest old, and to eliminate any ambiguous, poorly quantifiable or likely unreliable item, which most probably would result useless in the final merging of phenotypic data with the genetic ones. The **questionnaire for the 90+ sibpairs** includes questions on **family composition, marital status, education** (according to the ISCED classification), **occupation** (according to ISCO-88(COM) classification), and **housing conditions**. **Functional capability** is assessed by Katz's Activity of Daily Living (ADL) (Katz *et al.*, 1963) and by questions about functional limitations from the Nagi-scheme (reading ability, hearing ability, 500 metres walking ability without aids, going up and down the stairs without anyone's help, doing any kind of exercise and going outside with or without anyone's help) (Nagi SZ, 1976). **Cognitive function** is assessed by the standardized Mini Mental State Examination (Molloy *et al.*, 1991). **Health status** is assessed by a series of questions concerning present and past diseases, and a single question regarding self-perceived health. Also included are a few questions about tobacco and alcohol use. Finally **physical performance** is tested by two simple tests: measurement of handgrip strength and five time chair stand. Height and weight are mostly self-reported, and in some labs directly measured.

The **questionnaire for the younger controls** was a subset of the questionnaire to the old siblings. The most important part of this questionnaire is **a part illuminating the genetic background of the younger controls**: they should comprise a group with a similar genetic composition as the old siblings. Apart from this a few questions about health and life style factors were included, but no assessment of physical, functional or cognitive function is performed.

The information for the old siblings is at two levels: the **individual** level, and the **family** level, information common to siblings. This last level contains information about **parents and grandparents**, and about **other siblings**.

A separate questionnaire for obtaining family information was prepared, including questions about the parents and their origin, and about the other siblings.

The preliminary version of the sibling questionnaire was tested in three centres by interviewing 4 sibling pairs at each centre. Based on this experience minor corrections were made, before the questionnaire was presented to all centres. In parallel, **Partner N. 18 (A. Skytthe, B. Jeune)** prepared **a manual with instructions** for the different parts of the questionnaires.

3.1.5 Visit to the proband and collection of personal data and of biological samples

Once the Census data have been obtained on the basis of the list of eligible people, the interviewer team **contacted the proband, his/her sibpair and the younger control subject and fully explained them the type of research envisaged by the project**, its aim and scope, and the role of the subjects in it. Particular attention was paid to illustrate the type of genetic studies performed and the storage and use of the biological material derived from his/her blood donation (plasma, cells, DNA). After obtaining the informed consent, the interviewer (a MD, preferably a geriatrician, or a specifically trained biologist, biotechnologist or nurse) **administered the questionnaire,** collected the clinical history using the standardized case sheet, performed a clinical and functional examination, collected **blood sample** and stored the biological material (plasma, serum, DNA and blood cells).

In summary, for each person the Partners of the GEHA consortium had to collect:

- informed consent form;
- data and documents for age validation;
- clinical data by the standardized case sheet;
- blood samples and/or cheek-swab following standardized procedures.

3.1.6 Sample identification

The **personal information was kept separated from the genetic information** by creating two identifiers for each patient. In GEHA this was implemented by having a central laboratory unit handling all biological samples from the project, **Partner N. 9 (Dr. M. Perola, KTL, Helsinki)** as well as having separate databases for phenotypic data, **Partner N. 18 (Professor K Christensen SDU, Odense),** and genotypic data, **Partner N. 3 (Professor S. Schreiber, CAU, Kiel)**, as deeply described in the introduction.

Sample identifiers used for GEHA were named as **PID** for the personal identification and GID for the genetic information. The PID numbers were retrieved from a web page created for GEHA by Partner N. 18. **All participating centres** had to retrieve the PID codes for their purpose and **create bar coded labels** for each GEHA patient. The PID was designed to be used during all phases of recruitment and for the material used by the recruiting teams. **Questionnaires, tubes and work sheets were labelled with the PID.** Only PID was decided to be known to the recruiting teams. The data from the questionnaires were entered into the phenotype database using the PID as the identifier.

The PID was designed to contain the following identifiers:

-Center ID (3 digits)

-Family ID (4 digits): for the sibs:1001-4999, for the controls: 70001-99999

-Individual within a family (1 digit)

-Check digit (1 digit)

The **GID** was decided to be **generated by the central laboratory, Partner N. 9,** which was also responsible in handling the biological samples in Helsinki (**extraction and quality control of DNA** for GEHA) and furthermore in operating as a biological **storage and distribution** centre of samples to be analysed in other laboratories for genetic studies. GID was decided to contain 7 digits and to be generated independently from the PID. **The link between two identification numbers (PID and GID) are established and stored by Partner N. 9** and let known to Partner N. 7 responsible for GEHA data analysis. No significant phenotypic nor genotypic information were stored together with the two identifiers.

3.1.7 Sample collection, processing and storing in the recruitment centres

A) Whole Blood

For each patient **3 tubes of EDTA whole blood (7,5 ml each)** had to be collected in plastic tubes by Sarstedt (Sarstedt cod. 01.1605.001). The tubes were handled in each recruiting centre as describe in **Figure 3.2**.

The first whole blood tube was kept untouched and stored locally to -20°C. This tube was sent **to Partner N.9 for DNA extraction.**

The second blood tube was sampled for the removal of **plasma** after centrifugation (15 min, 1000 G). This procedure was followed by all recruiting labs except for Partners N. 1, 11, 12 , 19 involved in collecting lymphocytes and granulocytes for mtDNA analysis. These labs process tube 2 as tube 3. Tube type for plasma was Sarandt cod. 72694106 or Criotube cod. 345418. At least 0,5ml of plasma was placed in each tube and in case of lesser volume than 2,5 ml, the total number of plasma tubes were reduced accordingly. **The plasma tubes as well as the remaining cell fraction tube were stored at the local lab**. Plasma tubes were stored at -80°C or liquid nitrogen and the cell fraction at -20°C for later DNA extraction (backup).

The third whole blood tube was **stored at -20°C in the local lab** (backup) if the recruiting centre was not a partner involved in mtDNA analysis. **For the partners involved in mtDNA study the blood tube was sampled for the separation of different cell lines**; lymphocytes and granulocytes according to specific protocol. The tubes containing lymphocytes and granulocytes were stored in the local lab at -20°C and sent later to **Partner N. 9 for DNA extraction**.

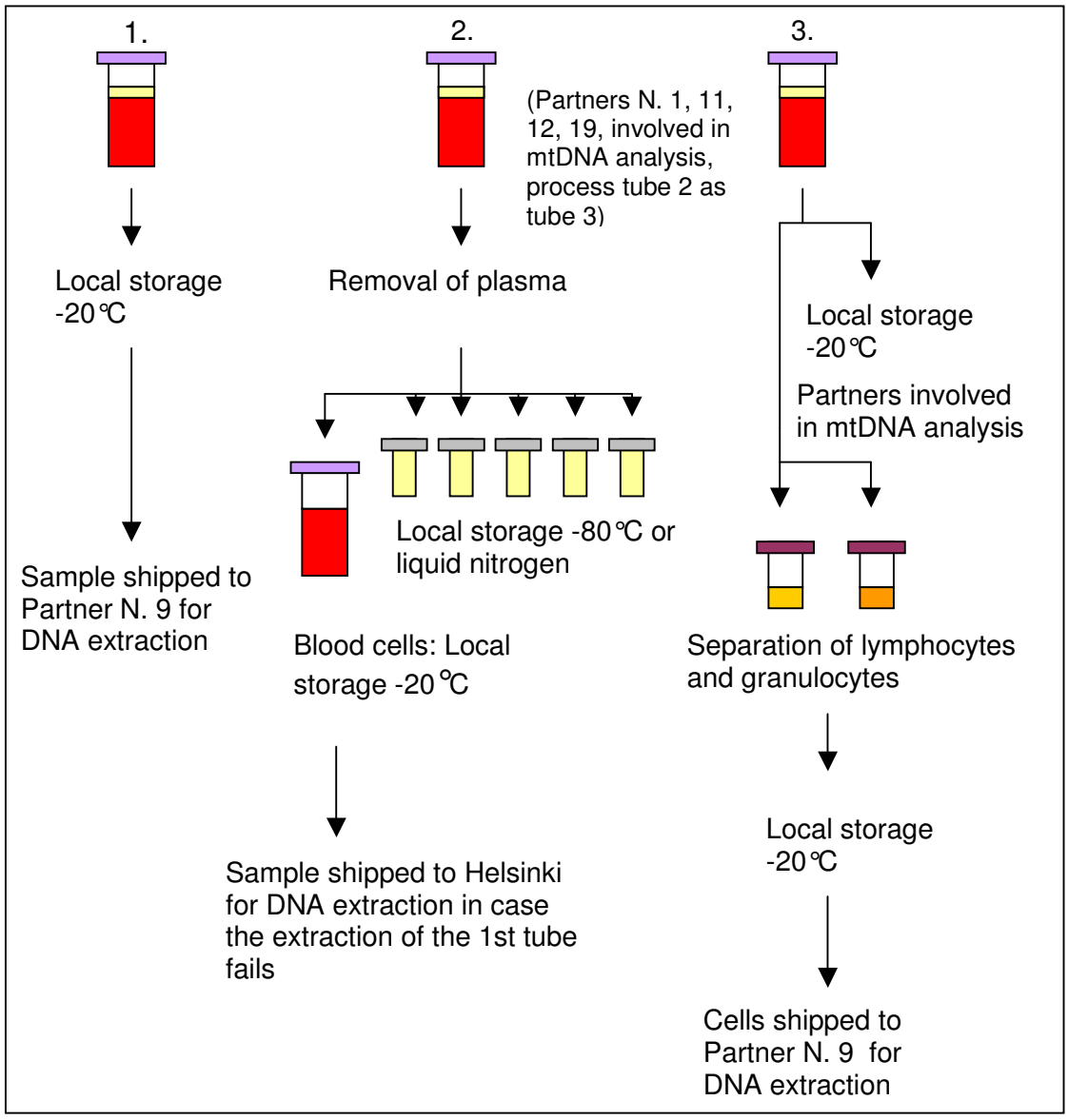


Figure 3.2 - Collection and processing of blood samples

B) Cheek swabs

At the beginning of the project it was established that, in case of unsuccessful withdrawal of blood or refusal, the **cheek swabs could be collected** instead (rule not valid for Partner N. 3, which cannot take any biological sample but blood because of local ethical restrictions). So, **the cheek swabs were stored in the local lab at -20°C and shipped to Partner N. 9 for DNA extraction** with the other blood tubes collected. However, the results of the first extraction of DNA from cheek-swabs, which was performed by Partner N.9 at KTL, indicated that the DNA yield from cheek-swabs was insufficient to perform the genetics analysis established by the project. Thus, **from November 2006 the collection of cheek-swabs samples was no more allowed** and the trios where at least one subject (one of the 90+sibling or the control) donated

cheek-swabs were eliminated from the number of "complete trios" collected by each recruiting centre.

C) Samples shipment

All the samples were to be sent to **GEHA Centralized DNA Logistics Centre (GC-DNA-LC) (Partner N. 9)**. Only complete trio sets ("trios" = 1 sib pair, or more sibs when available, + 1 control) were shipped to Partner N. 9 for DNA extraction and quality control. For shipment, the main guideline was to use properly insulated transport boxes to avoid the samples from thawing during the transport.

The necessary information required by the DNA logistics centre included:

-PID

-Blood volume

-Date of blood drawing

-Date of freezing

-Storage temperature

-Sex (used only for quality control purposes)

-The link between the controls and the sibs

D) Sample processing and storing in the GEHA Centralized DNA Logistics Centre (GC-DNA-LC)

All GEHA samples were extracted and stored at GC-DNA-LC. The process of sample handling is described in the **Figure 3.3**.

All the GEHA samples sent to **(GC-DNA-LC) Partner N. 9** were **labelled with GID** and put into **Partner N. 9 data base**. The whole blood and cell fraction samples were extracted by **Gentra's automated extraction instrument Autopure LS**. Salt precipitation was the method of choice. The cheek swabs were extracted manually by using Gentra's Puregene Salt Precipitation Kit.

After the extraction, all the DNA samples went through **a preliminary concentration measurement** (UV-spectrometric measurement) **and quality control** (visual inspection and UV-measured purity value inspection). Successfully extracted samples were **stored** and **the extraction data and storage locations were documented in the data base.** In case of failed extraction the recruiting centre was contacted and the 2nd tube (cell fraction) was asked to be sent to KTL.

For the analysis of the samples, **DNA was divided into plates and quality control processes were applied** (Pico Green measurements, ID-PCR). **The plates were constructed in such way that the same format can be used in different genotyping centres**. It was decided that the sibs and the control samples were placed in separate plates and that one set of three plates contained sibs from the same family and their corresponsive control. **The samples were sent to the genotyping centres in trios (2 sib plates + 1 control plate).** Each genotyping centre received the DNA samples diluted in **50ng/µl** concentration and each plate contained **2 blind duplicates and 4 empty wells**. The samples lower in concentration were placed to plates and, if needed, whole genome amplification techniques were applied by the genotyping centres.

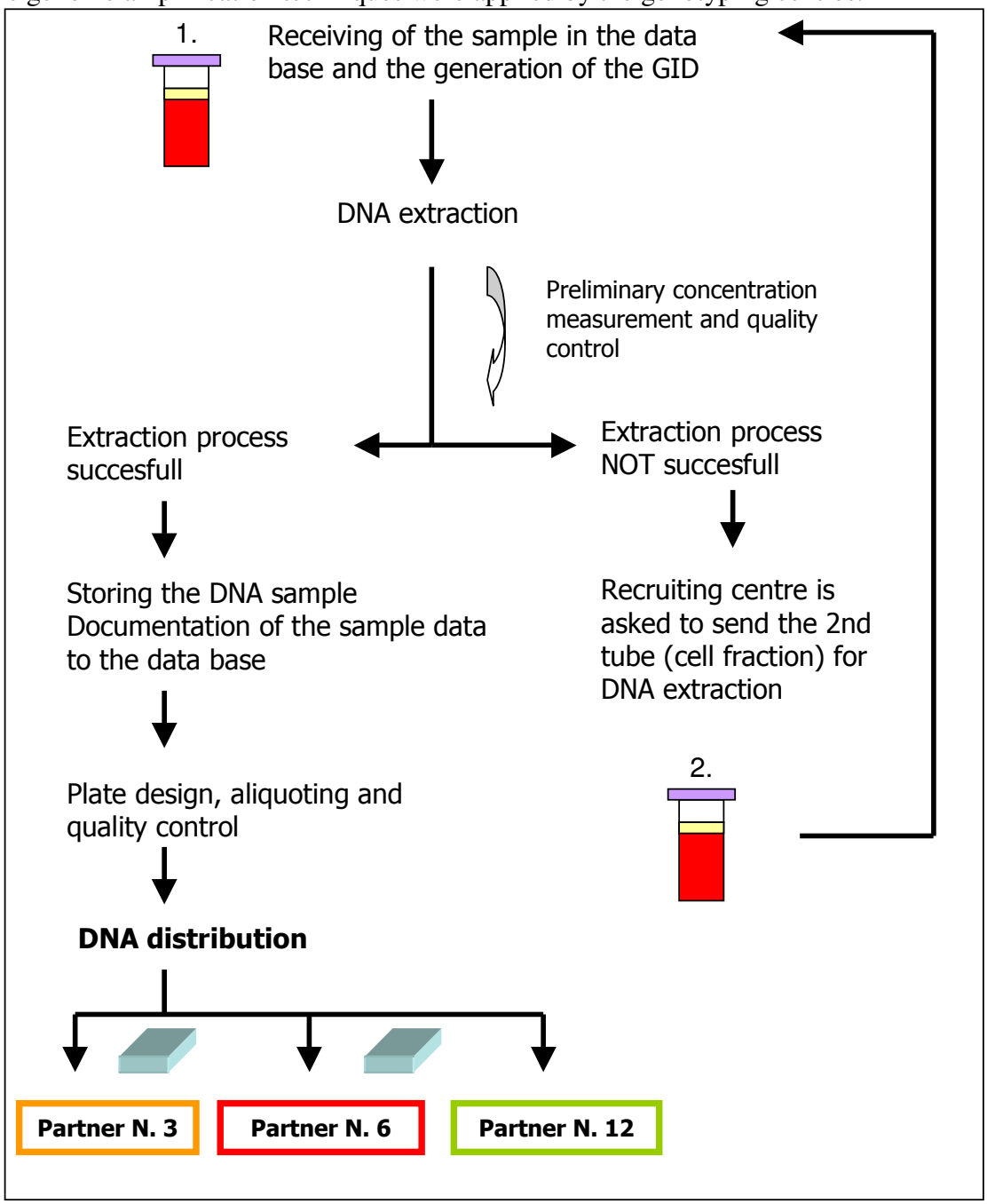


Figure 3.3 - Sample handling and management at Partner N. 9 in Helsinki

3.1.8 Standardized procedure for data entry

To use the data entry system, each Partner collecting phenotype data must have a VPN connection to the phenotype database server at Partner n.18. To ensure standardization of the data, **the data entry system was based on EpiData** so that data were evaluated immediately during data entry. Data were coded exactly as in the common questionnaires used in the GEHA project to further help standardization. Each centre had access to a set of EpiData files using remote desktop. Each centre can only access their own set of files on the server.

In summary, **Figure 3.4** visually describes the whole procedure of the GEHA project, from the recruitment to the genetics analysis, underlining the timing of the different steps.

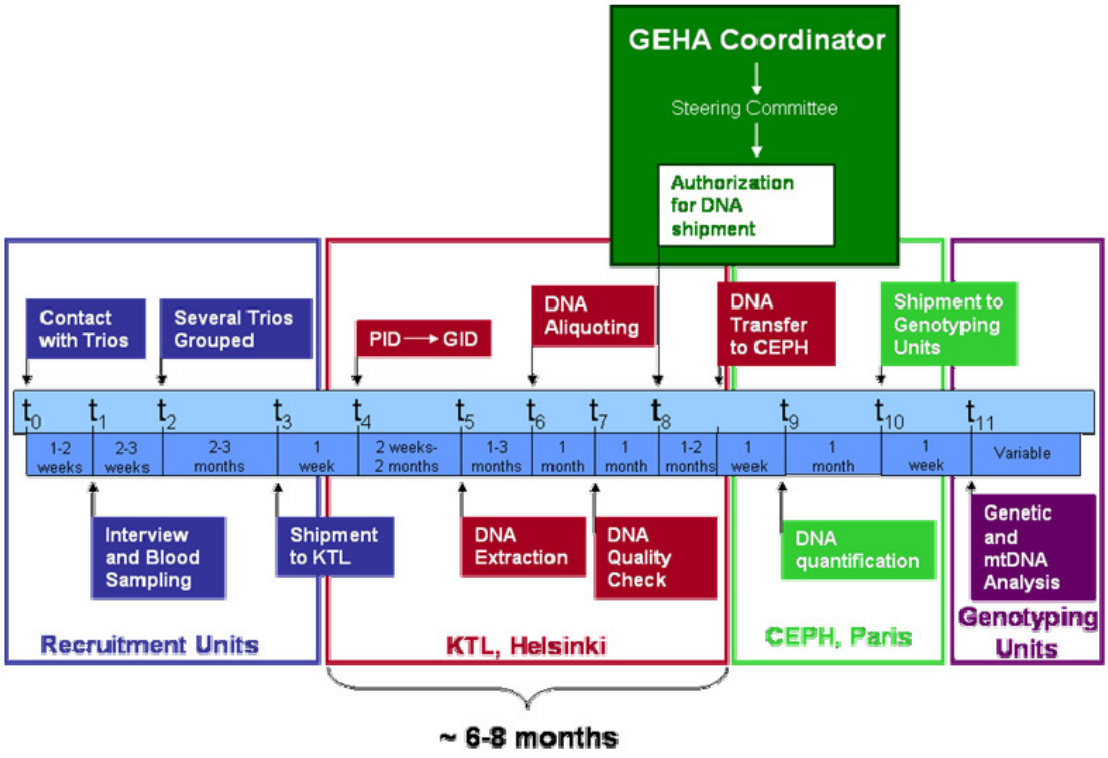


Figure 3.4 - Timing of the phases from recruitment to DNA shipment to genotyping partners

3.2 POPULATION OF THE STUDY AND RECRUITMENT PROCEDURE FOLLOWED BY UNIBO AND ISS

A total of **765 90+ Italian subjects** recruited by **UNIBO** (549 90+ siblings, belonging to 258 families) **and ISS** (216 90+ siblings, belonging to 106 families) **recruiting units** within the Integrated European Project GEHA are included in the analysis. This population contains all 90+ siblings that were interviewed and whose phenotype data were entered in the GEHA Phenotypic Database (localised in Odense, Denmark); thus, it is composed of 90+ siblings belonging to "complete trios", to "cheek-swab trios" and also to "never completed trios".

All 90+ sibpairs who accepted to take part in the study were recruited, except for those who were unable to give informed consent, as established by the Ethics Steering Group.

As regards **UNIBO**, census data for the eligible cohort of 90+ sibpairs were obtained by the Registry Office of the geographical areas elected for the recruitment: each subject constitutes a record containing information on name, date and place of birth and residence. Before providing demographic data, the Register Office checked the effectiveness that individuals with the same surname were actually siblings, allowing reserchers to contact the subjects in the list without performing further controls. However, sometimes it was difficult to find out the phone number to contact the 90+ siblings, since sometimes they move to their offspring house or to nursing-home. Moreover, UNIBO used also an advertisement-based strategy for recruitment: articles on the GEHA project containing the specific characteristics of the eligible subjects were published on local newspapers or on popular magazines, moreover the PI of UNIBO recruiting unit participated to TV scientific programs and asked to 90+ sibpairs from Northern Italy to contact the recruiting centre to be enrolled in the project. Therefore, also volunteer sibpairs who spontaneously offered to take part in the study were recruited by UNIBO recruiting unit.

As regards **ISS**, census data for the eligible cohort of 90+ were obtained by the Registry Office of the municipality of Rome, along with names and surnames of their parents, which allowed researchers to reconstruct the families.

The **local Ethical Committees**, Comitato Etico Indipendente Policlinico S.Orsola Malpighi (UNIBO) and Comitato Etico Istituto Superiore di Sanità (ISS), approved the study.

Interviewers from UNIBO and ISS (medical doctors -geriatricians/epidemiologists- or medical biotechnologists), who visited the participants at their residence, conducted the GEHA study. As defined by the GEHA guidelines, both in UNIBO and in ISS recruiting units, the informative letter was sent to all the 90+ sibpairs with at least one member having a telephone contact available. Two weeks after the letter was sent, a trained person contacted the 90+ sibpairs to explain the study, to obtain consent to participate and possibly fixed the date for the home

interview. Sometimes the interviewers managed to speak directly to nonagenarians, but very often they preferred to explain the aim and the protocol of the study to someone who takes care of the old siblings, such as their offspring, caregivers or nurses in case the participant lived in a residential care. A proxy-responder was encouraged to participate in the interview if the nonagenarian was unable to participate due to mental or physical handicaps. The interviewer and the family made the decision as to whether to use a proxy upon initial contact to obtain consent to participate in the survey. The study consisted of an interview and testing of mental and physical functioning. In addition, participants were asked to give a biological sample (blood or cheek swab) from which DNA could be extracted.

For the survival analysis, the vital status of the recruited 90+ sibpairs and younger controls was checked at **January 1st, 2009** and an official certificate of the vital status was collected from the Register Office of the Municipalities of residence of the 90+ sibpairs. As regards UNIBO recruiting unit, a total of 180 Municipalities were contacted to collect data on vital status of 90+ siblings and younger controls. As regards ISS, a direct access to the Rome municipality database was available. This permitted to check on line the vital status of the all participants.

3.3 VARIABLES ASSESSED BY GEHA QUESTIONNAIRE FOR 90+ SIBPAIRS AND INCLUDED IN THE ANALYSIS

3.3.1 Sociodemographic Factors

Questions about marital status, years of schooling and level of education, occupation, type of residence, cohabitation.

3.3.2 Lifestyle Factors

Participants were classified as smokers, former smokers, or never smokers. Moreover, the cases of consumption of alcohol every day were recorded, but not the quantity of alcohol intake.

3.3.3 Disability

Questions in this area covered the Katz Index of activities of daily living (ADL) - bathing, dressing, toileting, transfer, feeding and continence – and two different categorizations were performed:

(1) **Five-item ADL scale** (where continence was not included in accordance with the recommendations in the literature) (Fillenbaum GG, 1996); it was used to construct a three-level five-item ADL scale: "not disabled" was defined as independent in all items (ADL = 5), "moderately disabled" as dependent in one or two items (ADL = 3-4), and "severely disabled" as dependent in three or more items (ADL = 0-2) in accordance with the definitions given in Katz' paper (Katz *et al.*, 1970). These categories defined three sizable groups, which ranged from a group capable of doing the most basic activities independently to a group that was dependent in the majority of the five basic activities (Nybo *et al.*, 2001a).

(2) **Six-item ADL scale** (including continence) in accordance with a classification proposed by Franceschi *et al.*; it was used to construct a three-level six-item ADL scale; "not disabled" was defined as independent in at least 4 items out of 6 (ADL = 4-6), "moderately disabled" as dependent in three or four items (ADL = 2-3), and "severely disabled" as dependent in five or more items (ADL = 0-1) (Franceschi *et al.*, 2000a).

Furthermore, some questions about the functional limitations from the **Nagi-scheme** (Nagi SZ, 1976) were also added: reading newspaper without glasses, recognize someone 4 metres away without glasses, hearing ability without aids, 500 metres walking without aids, going up and down the stairs without anyone's help, doing any kind of exercise, going outside with or without anyone's help.

3.3.4 Measures of Physical Performance

Handgrip strength and ability to perform a five times chair stand test were included in the study (Nybo *et al.*, 2001b). **Handgrip strength** was measured using a hand-held dynamometer (SMEDLYS' dynamometer, Scandidact, Kvistgaard, Denmark) for two performances with each hand. The best performance of these four was used for the analysis (Nybo *et al.*, 2001a; Jeune *et al.*, 2006). For the analysis of handgrip strength, the participants were divided into separate quartiles for men and women. The first quartile consisted of the best-performing participants. In the **chair stand** test, participants were divided in two groups (able to complete the test and unable to complete the test).

3.3.5 Health

Participants were presented with a list of 14 diseases and asked whether a physician had ever told them that they *at the moment* suffered from any of them. The number of present diseases was divided into three groups (0, 1–2, and $\geq$ 3). Furthermore, subjective health was assessed using the question: "How do you consider your health in general?" with five response categories (excellent, good, acceptable, poor, and very poor).

3.3.6 Body Mass Index

Body mass index (Kg/m^2) was calculated using data on height and weight. The height data used for the analysis were measured by the interviewers from UNIBO and ISS using a common metre, and they were available only for the 82% of subjects (631 out of 765). The weight data were measured using a common balance (SECA Mod. 761) for the 83% of subjects (635 out of 765), while they were self-reported for the 12% of subjects (in these cases the persons did not answer the question themselves and an estimate was then made by the interviewer or reported by the proxy) and they were not available for the 5% of the subjects; on the whole, weight data were available for 95% of subjects (727 out of 765). Since no difference was found between measured and self-reported weight in each recruiting centre (data not shown), they were put together to calculate BMI values. Participants were divided into three groups ($\leq$ 21, 22–27, $\geq$ 28).

3.3.7 Cognitive Function

Cognitive function was measured using the Standardized Mini-Mental State Examination (**SMMSE**) (Molloy *et al.*, 1991). Two different categorizations where performed to assess the cognitive status:

(1) "severe cognitive impairment" (0–17 points), "mild" (18–23 points) and "not present" (24–30 points) (Nybo *et al.*, 2003);

(2) “severe cognitive impairment” (0–12 points), “mild” (13-19 points) and “not present” (20–30 points) (Franceschi *et al.*, 2000a).

The results of the SMMSE were corrected by education according to the reference given by Magni *et al.* in a study on Italian population up to 89 years of age (Magni *et al.*, 1996), as reported in **Table 3.1**. Since no validated adjustment coefficients are available for subjects aged more than 90 years, we included 90+ subjects in the last category proposed by Magni *et al.* (85-89 years).

Age-range	65-69	70-74	75-79	80-84	85-89
Education					
0-4 years	+0,4	+0,7	+1,0	+1,5	+2,2
5-7 years	-1,1	-0,7	-0,3	+0,4	+1,4
8-12 years	-2,0	-1,6	-1,0	-0,3	+0,8
13-17 years	-2,8	-2,3	-1,7	-0,9	+0,3

Coefficients are to be added (or subtracted) to the raw SMMSE score to obtain the adjusted SMMSE score

Table 3.1 - SMMSE adjustment coefficients for age-groups and education levels for Italian population (Magni *et al.*, 1996)

3.3.8 Concordance of the health and the functional status among 90+ siblings

For the evaluation of the concordance of the **Health Status**, we checked the following items: self-reported health, self reported number of diseases, past myocardial infarction, past cancer, past hip fracture, some haematological and biochemical parameters (such as haemoglobin, creatinine, PCR) and the classifications used to identify the health status.

For the evaluation of the concordance of the **Functional Status**, we checked the following items: ADL, ability to read newspaper without glasses, ability to face someone at 4 metres without glasses, ability to walk 500 metres without aids, Hand Grip, Chair Stand, SMMSE.

3.3.9 Survival Analysis

Vital status for the total cohort was assessed at **January 1st 2009**. This means that 90+ siblings were followed for different periods of time on the basis of their recruitment time: the first sibpairs who entered the study (interviewed in November 2004) were followed for about 4 years (49 month), while the last sibpairs who participated in the study (interviewed in April 2008) were followed only for 8 months. This discrepancy among 90+siblings was taken into account when the survival analysis was performed.

On the whole, differences in the number of cases are due to the presence of missing values.

3.4 CLASSIFICATION METHODS FOR THE ASSESSMENT OF HEALTH STATUS OF 90+ SIBLINGS

A major aim of GEHA is to identify gene(s) and gene variant(s) related to successful/healthy and unsuccessful aging. To this purpose the recruited sibpairs must be carefully assessed as far as their health status is concerned, in order to correctly classify all of them.
To this aim, firstly **a methodological work** was performed in order **to asses the health status of 90+ siblings** recruited by UNIBO and ISS recruiting units, which consisted in the application of the three different classification methods available in literature for the assessment of the health status of long-living subjects. They were proposed in three studies on centenarians and they are based on:

(1) functional capabilities (ADL, MMSE, visual and hearing abilities; Japanese Study) (Gondo *et al.*, 2006), as reported in **Table 3.2**.

(2) functional status, current pathologies and few haematological parameters (ADL, MMSE, presence of ictus, cancer, renal failure, liver disease, levels of creatinine, haemoglobin; Italian Centenarian Study) (Franceschi *et al.*, 2000a), as reported in **Table 3.3**.

(3) morbidity history and age of disease onset (hypertension, heart disease, diabetes, stroke, non-skin cancer, skin cancer, osteopororis, thyroid condition, Parkinson's disease, chronic obstructive pulmonary disease and cataracts; New England Centenarian Study) (Evert *et al.*, 2003), as reported in **Table 3.4**.

As reported in Table 3.2-3.3-3.4, we used as a starting point the inclusion and exclusion criteria of the classification methods and we adapted them on the items available in the GEHA questionnaire. For example, the classification by Gondo used the Barthel Index as a measure of the physical condition, therefore, since it was not present in the GEHA questionnaire, we calculated a score analogous to the Barthel index starting from the items of the GEHA questionnaire.

Classification by Gondo		
Exceptional (subjects having all the reported characteristics)		
	Gondo et al. *J Gerontol, 61A (3): 305-310. 2006*	**Our analysis**
Intact vision and hearing functions	"No problem" in the questionnaire	Reading newspaper without glasses (m22); Recognize someone 4 metres away without glasses (m23); Hearing ability without aids (m24)
Fully Independent	Barthel index = 100	Barthel index = 100
Excellent cognitive functions	CDR = 0; MMSE ≥ 21	SMMSE ≥ 21
Normal (subjects having all the reported characteristics)		
	Gondo et al. *J Gerontol, 61A (3): 305-310. 2006*	**Our analysis**
Somewhat Independent	Barthel index ≥ 80	Barthel index ≥ 80
Good cognitive functions	CDR ≤ 0.5	SMMSE ≥ 21
Frail (subjects having at least one of the reported characteristics)		
	Gondo et al. *J Gerontol, 61A (3): 305-310. 2006*	**Our analysis**
Somewhat Dependent	Barthel index ≤ 79	Barthel index: 20-79
Cognitive impairment	CDR ≥ 1	SMMSE: 11-20
Fragile (subjects having all the reported characteristics)		
	Gondo et al. *J Gerontol, 61A (3): 305-310. 2006*	**Our analysis**
Totally Dependent	Barthel index < 20	Barthel index < 20
Severe cognitive impairment	CDR ≥ 3	SMMSE < 11

Table 3.2 – Inclusion and exclusion criteria used to classify centenarians according to their health status proposed by Gondo *et al.* J Gerontol, 61A (3):305-310. 2006

Classification by Franceschi		
Category A **-** ***Good Health Status*** (subjects having all the reported characteristics)		
	Franceschi at al. ***Aging Clin Exp Res, 12: 77-84. 2000***	**Our analysis**
Absence of physical disabilities and ability to walk without help	ADL ≥ 4; IADL = 4 in males; IADL = 6 in females	ADL ≥ 4; Walking ability for 500 m without aids (m25)
Absence of severe cognitive impairment	MMSE ≥ 20	SMMSE ≥ 20
Absence of clinically evident cancer		Absence of self-reported cancer (m53g)
Absence of ictus in the previous 6 months		Absence of self-reported ictus in the previous 12 months (m55c)
Absence of severe renal failure	Creatinine < 2 mg/dl, BuN < 0,80	Creatinine < 2 mg/dl
Absence of severe anaemia	Haemoglobin > 10 g/dl	Haemoglobin > 10 g/dl
Absence of severe liver diseases		ALT < 40 U/l
Absence of other severe diseases		
Inclusion in this category is compatible with the presence of sensory loss (hypoacusia, vision reduction) which does not severely affect overall physical and cognitive capacity		
Category B **-** ***Intermediate Health Status***		
Subjects who cannot be included in category A or C		
Category C **-** ***Bad Health Status*** (subjects having at least one of the reported characteristics)		
	Franceschi at al. ***Aging Clin Exp Res, 12: 77-84. 2000***	**Our analysis**
Presence of severe physical impairment	ADL = 0-1; IADL = 0	ADL = 0-1
Presence of severe cognitive impairment	MMSE < 12	SMMSE < 12
Presence of overt cancer		Presence of self-reported cancer (m53g)
Presence of ictus within 6 months		Presence of self-reported ictus in the previous 12 months (m55c)
Presence of renal insufficiency		Presence of self-reported renal insufficiency (m53i)
Presence of severe anaemia	Haemoglobin < 7 g/dl	Haemoglobin < 7 g/dl
Presence of severe liver diseases		ALT ≥ 40 U/l

Table 3.3 – Inclusion and exclusion criteria used to classify centenarians according to their health status proposed by Franceschi *et al.* Aging Clin Exp Res, 12:77-84. 2000

Classification by Evert	
Categories are based on reports of the age of onset of the major age-associated illnesses	
Evert at al. *J Gerontol, 58A (3): 232-237. 2003*	**Our analysis**
Illnesses: Hypertension, Heart Diseases, Diabetes, Stroke, Non Skin Cancer, Skin camcer, Osteoporosis, Thyroid condition, Parkinson's disease, COPD (chronic obstructive pulmonary disease), Cataracts	Illnesses: Hypertension (m53e), Heart Diseases (m53d), Diabetes (m53j), Stroke (m55c), Non Skin Cancer (m53g), Osteoporosis (m53l), Neurological Diseases (suc as Parkinson's disease) (m53c), Chronic respiratory tract diseases (such as COPD, asthma) (m53h), Sight diseases (m53a), Chronic renal insufficiency (m53i)
Escapers Subjects who attained their age without the diagnosis of illnesses	
Delayers Subjects who delayed the onset of illnesses until at least the age of 80	
Survivors Subjects who had a diagnosis of an illness prior to the age of 80	

Table 3.4 – Inclusion and exclusion criteria used to classify centenarians according to their health status proposed by Evert *et al.* J Gerontol, 58A (3): 232-237. 2003

3.5 STATISTICAL ANALYSIS

Univariate analysis.

i) **Chi-square test** or **Fisher's Exact test** (on the basis of the number of observations) were used to analyse categorical variables (gender, type of interview, place of birth, marital status, level of education, type of occupation, type of residency, cohabitation, confination to bed, ADL scale categories, number of self-reported diseases, self-reported health status, use of drugs, falls within the last year, hospitalisation within the last year, loss of weight within the last year, classifications for the health status).

ii) **Student *t* test** or **Wilcoxon rank-sum test** (on the basis of the number of observations) were used to compare scores of continuous variables (SMMSE, knee height, total height, measured weight, BMI, Hand Grip, hemocytometric results, clinical chemistry results).

iii) **Odds Ratio and 95% Confidence Intervals** were calculated in order to compare groups.

Multivariate analysis

i) **Logistic regression** was performed in order to evaluate differences in age, gender, self reported health, attitude towards life, number of disease, handgrip, chair stand test, falls, hospitalization and loss of weight within the last year on the health status of 90+ siblings. Odds ratios and 95% confidence intervals were calculated adjusting for family cluster, in order to take into account the non independence of observations within the family.

ii) Techniques of **Data Mining** were used to select the most informative variables for reclassify our study population according to health status.

Concordance analysis

To evaluate the concordance for functional status and for health status between siblings, two different and complementary approaches were used:

i) **Probandwise Concordance test**: for a group of siblings in which at least one member of each pair is "affected", probandwise concordance is a measure of the proportion of families where siblings are concordant for a specific item out of the families where at least one sibling is able to perform the item; it can be calculated with the formula of 2C/(2C+D), in which C is the number of concordant pairs and D is the number of discordant pairs. This analysis allowed us to measure the percentages of families

where the oldest and the second siblings obtained the same positive result in a specific item.

ii) **Conditional Logistic Regression test**: it was used to examine the prediction of the youngest sibling by the oldest sibling. The binary outcome for the conditional logistic regression analysis was whether or not the youngest sibling was positive to a test. The predictor was an indicator variable of whether or not the oldest sibling was positive the test. Categorical variables with binary outcomes were evaluated.

Survival Analysis

i) Kaplan-Meyer methods were used to test the equality of the survival functions across various groups and to give a pictorial representation of the observed survival experience.

ii) Cox regression model was used to calculate the effect of potential risk factors on mortality. Hazard ratios (HRs) were computed for all variables using data on all possible subjects and were adjusted for family cluster. **Cox regression-based test** for equality of survival curves was used to compare the mortality in different groups of subjects.

All the analysis were performed using Stata version 9.0 (Stata Corp., College Station, TX). Data Mining was performed by SIPINA Software by R. Rakotomalala, University of Lyon.

4. RESULTS

4.1 GEHA ACHIEVEMENTS: DATA ON ALL EUROPEAN RECRUITING UNITS

4.1.1 Recruitment of GEHA trios

The objective of GEHA recruitment activity was to recruit, within August 31st, 2008 (4 months after the original deadline), 2650 90+ sibapairs and 2650 younger controls (for a total of 7950 subjects) from 15 European areas in 11 European countries.

The total number of trios (each trio is composed by at least two 90+ sibs, or more when available, plus 1 younger ethnically-matched control subject) **collected until August 31st, 2008 by all the European recruiting units is 2311 out of the expected 2650 trios, representing the 87% of the number of trios to be recruited for the study**. On the whole, these results are remarkable, taking into account the time needed to obtain the approval by the local ethics committees of all the recruiting units and the complex procedure to identify, contact and interview **both** members of each sibpair **plus an unrelated ethnically-matched younger control** from the same geographical region. Moreover, a particular attention was paid by the GEHA project in recruiting **trios with additional (more than 2) 90+ members**, according to the hypothesis that such families are enriched in longevity genes and should be more informative for the genetic analysis. Indeed, **188 trios are composed by three 90+ sibs**, **21 trios are composed by four 90+ sibs and 4 trios are composed by five 90+ sibs,** accounting for about 9% of the total trios. Thus, **4622 90+ sibs** (i.e. 2311 x 2) + **455 additional sibs** and **2311 younger controls for a total of 7388 subjects** were recruited by the GEHA consortium within the four years activity.

Moreover, until the beginning of the third year of activity, when the DNA extraction results revealed that the cheek swab yield was not enough for the whole genetics analysis envisaged by the project workplan, the recruitment involved even trios whose members donated only cheek swab instead of whole blood. Adding these subjects to those quoted above, **the whole number of subjects enrolled by the GEHA consortium at the end of the recruitment period is 5353 90+sibs and 2447 younger controls accounting for total 7800 recruited subjects, i.e. 98% of 7950 expected subjects to be recruited by the end of the recruitment task**. I remind here that the collected cheek swab trios, i.e. those trios in which less than two siblings plus a control donated whole blood, were not considered any more appropriate as trios available for the planned genetics analysis because of the low yield of the cheek swab samples, giving rise to disrupted trios; however phenotypic data related to these subjects are available in the phenotype database.

Data related to "complete trios" recruited Partner by Partner within August 31st, 2008 are reported in **Table 4.1**.

Recruitment Data at August 31th 2008

Partner N.	PI	Geographic area	Expected trios	Complete trios	% Total trios / Expected trios
1	UNIBO	Northern Italy	220	223	100
2	CRLC	Langue d'Oc and Savoye	300	281	94
3	CAU	Kiel area	100	100	100
5	ISS	Central Italy	100	81	81
6	LUMC	Leiden area	200	170	85
8	NHRF	Athens area	130	100	77
10	NENCKI	Warsaw	150	138	92
11	QUB	Belfast area	100	65	65
12	UNICAL	Southern Italy	200	200	100
14	UNISS	Sardinia (Italy)	150	85	57
15	UCL	Wallonia	100	85	85
17	UNEW	Newcastle upon Tyne area	150	106	71
18	SDU	Denmark	450	451	100
19	UTA	Tampere/Helsinki area	180	170	94
26	UKRAINE	Kiev area	120	56	47
Total			2650	2311	87

Table 4.1 – Recruitment of "complete trios" at August 31st, 2008

4.1.2 Collection of biological samples

The collection of samples was carried out for all 7800 recruited subjects, but from now on the analysis will be focussed only on those trios in which at least two sibs plus a control donated whole blood (named "*complete trios*"). At the end of the recruitment period, the GEHA collection of biological samples is composed as follows:

- 90+ subjects: 99% of whole blood, 0.3% of cheek swab, and 0.7% of a mix of whole blood and check swab;
- younger controls: 99.8% of whole blood, 0% of check swab and 0.2% of a mix of whole blood and check swab.

It is important to note that: **1. each recruiting lab usually has a second tube of blood as a backup in case of failure of shipment or low yield of DNA extraction** (such tubes have been requested by GEHA Biobank, Partner N. 9, in 15% of subjects) and **2.** most of the recruiting partners also collected plasma and some of them serum (Partners N. 1, 8, and 11) from all recruited people.

4.1.3. Data entry in the phenotype database

At the end of the recruitment period the percentage of entered data was 98%. **Phenotypic data** related to **2257 trios** (out of 2311 recruited trios) were entered in the GEHA centralized **Phenotypic Database**. 13 Partners (Partners N. 1, 2, 3, 5, 6, 8, 10, 12, 15, 17, 18, 19 and 26) entered data related to all the recruited trios, while 2 Partners (Partners N. 11 and 14) entered almost all the collected data.

4.1.4. Sample shipment to GEHA Biobank

The shipment of the collected biological material to Partner N. 9 for DNA extraction was **successful** and at the end of the recruitment period accounted for the **98% of all collected samples**. In particular, the collected **biological material** (whole blood, cheek swab, mix of blood sample and cheek swab) related to **2282 complete trios** (out of 2311 recruited trios) **was sent to Partner N. 9 for centralized DNA extraction**. In particular, all Partners managed to send the biological material related to almost all the recruited trios.

4.2 PREPARATORY ACTIVITIES TO THE RECRUITMENT: DATA FROM UNIBO AND ISS RECRUITING UNITS

4.2.1 Obtainment of the authorization of the local Ethics Committee for recruitment procedure

UNIBO recruiting unit obtained a first approval on month 3 and a second approval on month 10 after submitting a new request of approval upon completion of the final version of the recruitment protocol agreed during the Recruitment Training Course held in Bologna on October 2004. ISS recruiting unit submitted only one request to the local Ethics Committee in order to acquire the authorization for the recruitment procedure and obtained the approval on month 8.

4.2.2 Preliminary demographic survey and identification of geographic areas suitable for 90+ sibpairs recruitment

UNIBO recruiting unit elected two geographic areas for the recruitment at the far beginning of the project:

- Area 1: Bologna City and Bologna Province (649.540 inhabitants, 3.702 Km^2)
- Area 2: Town of Varese Ligure (SP) (2.255 inhabitants, 136.63 Km^2)

for a total of 651.795 inhabitants

Then, since the eligible sibpairs identified at the beginning of the project were not sufficient to reach the expected number of trios (220 trios), the recruitment was extended to additional areas in Emilia-Romagna region:

-- Area 3: Town of Forlì (114.683 inhabitants, 228.19 Km^2)

- Area 4: Town of Faenza (Ravenna Province) (56.641 inhabitants, 215.72 Km^2)
- Area 5: Modena City (175.502 inhabitants, 182.74 Km^2)

Finally, a further area was identified for the recruitment since there was a somewhat high proportion of 90+ sibpairs:

- Area 6: Livorno City (148.143 inhabitants, 104.1 Km^2)

ISS recruiting unit elected a single geographic areas for the recruitment:

- Area 1: Rome (2.500.000 inhabitants, 129 Km^2).

4.2.3 Obtainment of demographic data on 90+ sibpairs and young controls

Firstly, UNIBO and ISS recruiting units obtained the authorization to have access to the census data by month 5 (a month of delay respect to the project deliverable), then demographic data related to the eligible sibpairs and controls were obtained.

Data on 90+ sibpairs

UNIBO recruiting unit: at month 6 data on 642 sibpairs were obtained from Registry Office of Bologna Province and Town of Varese Ligure. Then, as the recruitment proceeded, data on 70 sibpairs were obtained by the Registry Office of Forlì, on 41 sibpairs by the Registry Office of Faenza, 72 sibpairs by the Registry Office of Modena and 53 sibpairs by the Registry Office of Livorno.

ISS recruiting unit: at month 11 a total of 33 90+ twins were identified in the elected area (data from Twin Registry). Since the initial target for ISS was to enrol 50 twin pairs, considering a response rate of 50%, it was decided to recruit also non-twin siblings, favouring the enrolment of very old sib-pairs and sib-trios. Then, the final target for ISS was to recruit a total of 100 trios and data on all 90+ sibpairs living in Rome were obtained by the Registry Office of the municipality of Rome.

Data on young people

For the recruitment of younger controls, the suggested criterion "spouse of the proband children" was followed. For UNIBO, no demographic information about 55-75 years old people was asked before starting the recruitment task, considering that the ethnic origin of younger controls should match that of the enrolled 90+ sibpairs and that these data cannot be known before visiting the sibs.

4.3 PARTECIPATION OF 90+ SIBLINGS IN THE GEHA STUDY: DATA FROM UNIBO AND ISS RECRUITING UNITS

On the whole, the **total number of families** (the word *family* should be intended as 2 sibs or more when available) **contacted** by mail and phone (the first contact) by UNIBO and ISS recruiting units is **1427**. This number includes also people that spontaneously contacted UNIBO recruiting team after having known about the GEHA project by advertising.

The percentage of **families that gave a positive response** is **25.5%**: this percentage is higher in UNIBO recruiting unit (32.1%) in comparison with ISS (17.0%), probably because UNIBO used the advertising as a further recruiting strategy. In fact, if the demographic lists are considered as the only mean of contact of 90+ sibpairs, the recruitment success for UNIBO recruiting unit decreases to 22%.

The percentage of families that after a first contact **did not entered the study** is **69.2%** in good agreement with the initial theoretical assumption of GEHA consortium and with previously reported data. In ISS recruiting unit this percentage is higher (83.0%) in comparison with UNIBO (58.6%) and probably the explanation is related to the different recruitment strategy. During the recruitment, the following **causes of exclusion** were assessed: **death** of one or more members of the sibpair: 20.2%, **dementia or severe functional impairment**: 11.5%, **immediate refusal (largely unexplained)**: 51.9%, and **untraceable** subjects (missing address of one sib or unreachable living area): 16.3%. No differences are present between centres in the causes of exclusion and these results are the same as the ones obtained when all the recruiting units are considered (data not shown).

Finally, the percentage of **families in a stand-by state**, i.e. for which is still not possible to decide whether they will enter the study, is **5.3%**; considering the recruiting unit, this data is higher in UNIBO in comparison to ISS.

	Recruiting Centre				Total	
	UNIBO		ISS			
	N	%	N	%	N	%
Subject responsiveness to the GEHA Project						
Families that gave positive response	258	32,1	106	17,0	364	25,5
Families that did not enter the study	471	58,6	517	83,0	988	69,2
Families in stand-by after a first contact	75	9,3	0	0,0	75	5,3
Causes of 90+ siblings exclusion						
Immediate Refusal of one or more members	217	46,1	296	57,3	513	51,9
Dementia or Functional Impairments of one or more members	46	9,8	68	13,2	114	11,5
Death of one or more members	147	31,2	53	10,3	200	20,2
No traceability	61	13,0	100	19,3	161	16,3

Table 4.2 – Partecipation of 90+ siblings in the GEHA Study

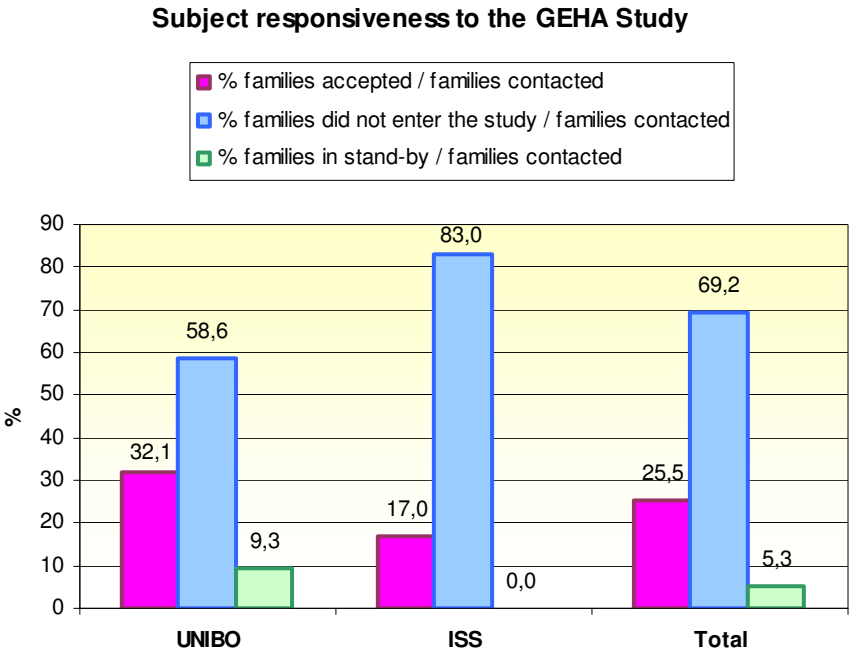


Figure 4.1 – Subject responsiveness to the GEHA Study

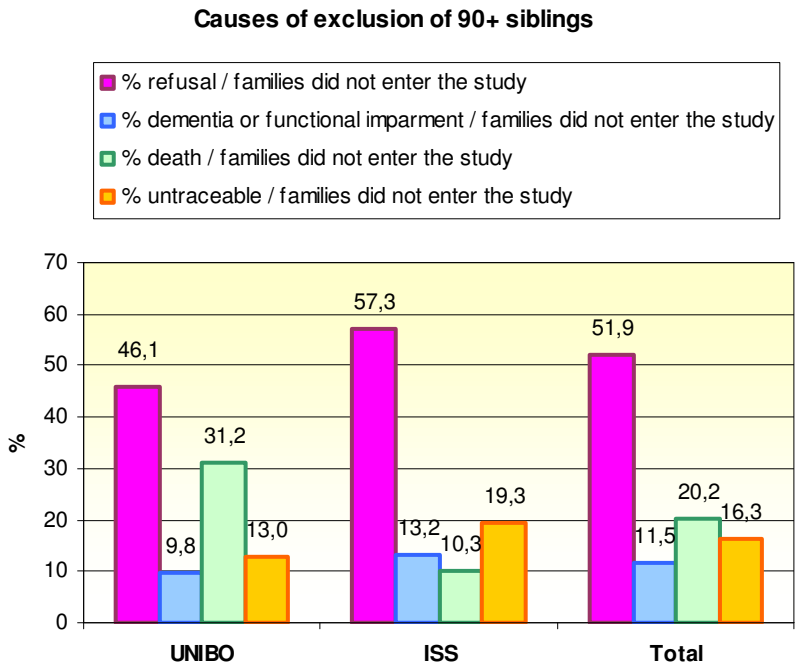


Figure 4.2 – Causes of 90+ siblings exclusion (immediate refusal, dementia, death and no traceability)

4.4 CHARACTERISTICS OF GEHA FAMILIES RECRUITED BY UNIBO AND ISS RECRUITING UNITS

The characteristic of the 364 families enrolled in Bologna and Rome recruiting units are shown in **Table 4.3**. The proportion of deceased males among 90+ siblings is higher than the proportion of deceased females. There were 9 living male spouses of long-living females, while there were 83 living female spouses of long-living males. These results are comparable to those obtained by Schoenmaker M *et al.* on the families enrolled within the Leiden Longevity Study (Schoenmaker *et al.*, 2006).

	Males		Females	
	N	**Age[a]**	**N**	**Age[a]**
Parents of nonagenarian subjects				
Deceased	317	77 (65-84)	329	82 (70-90)
Total sibship [b]				
Alive	327	91 (87-94)	697	92 (90-94)
Deceased	390	72 (53-83)	350	81 (65-89)
Spouses of nonagenarian subjects				
Alive	9	95 (93-96)	83	85 (83-88)
Deceased	428	76 (64-85)	115	80 (69-86)

[a] Age displayed as median (interquartile range)

[b] Total sibship includes interviewed nonagenarian subjects and all the siblings

Table 4.3– Characteristics of 364 GEHA families (258 recruited in Bologna and 106 in Rome)

This analysis could be extended by evaluating the mortality characteristics of parents, siblings and spouses of GEHA nonagenarian sibpairs and by comparing these data with the general Italian population in order to evaluate whether the GEHA study has resulted in a population genetically enriched for longevity and extreme survival, as performed by Schoenmaker M *et al.* on the families enrolled within the Leiden Longevity Study (Schoenmaker *et al.*, 2006).

4.5 DETAILED OVERVIEW OF THE PHENOTYPIC CHARACTERISTICS OF GEHA 90+ SIBLINGS RECRUITED BY UNIBO AND ISS RECRUITING UNITS

A detailed overview of the **phenotypic characteristics of 90+ siblings recruited by Bologna** (549 90+ siblings, belonging to 258 families) **and Rome** (216 90+ siblings, belonging to 106 families) **recruiting units** for a total of 765 90+ subjects was performed. As described in "Materials and Methods", the population of this study contains all 90+ siblings who agreed to participate in the study and were interviewed and whose phenotype data were entered in the GEHA Phenotypic Database; thus, it is composed of 90+ siblings belonging to "complete trios", to "cheek-swab trios" and also to "never completed trios", as shown in **Table 4.4**.

	Recruiting Centre					
	UNIBO (258 sib pairs)		**ISS (106 sib pairs)**		**Total (364 families)**	
	N	**%**	**N**	**%**	**N**	**%**
Complete trios	223	86,4	81	76,4	304	83,5
Cheek-swab trios	21	8,1	25	23,6	46	12,6
Never Completed trios	14	5,4	0	0,0	14	3,8

Table 4.4

On average, the time necessary to complete the interview (including physical tests and antrophometric measures) was **52 minutes**; it was 5 minutes higher in UNIBO (53 ± 18 minutes) than in ISS recruiting unit (48 ± 17 minutes).

All the items assessed by the GEHA questionnaire were analysed and results are reported in the following sections.

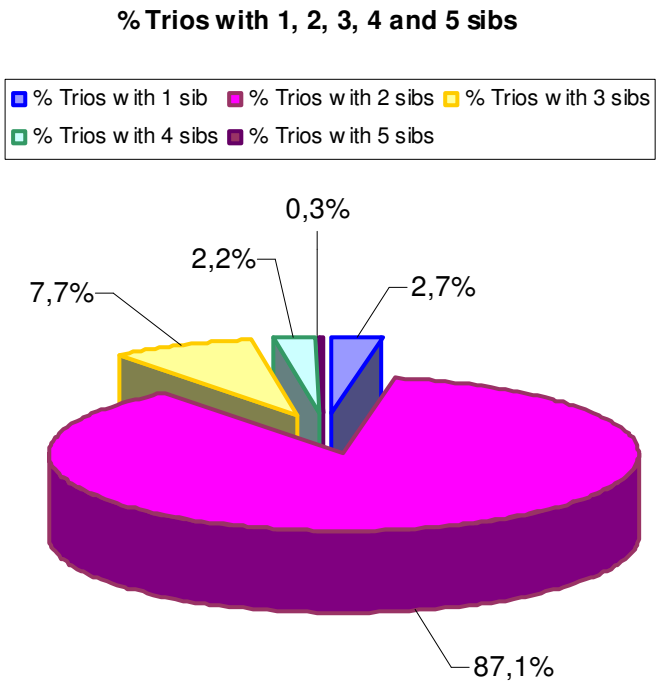
4.5.1 Basic characteristics of the GEHA Study Population and Collection of Biological Samples

A total of **364 families** were recruited by UNIBO and ISS recruiting units and they are composed as follow: 2.7% with only one 90+ sibling (these are the *"never completed trios"* where it was not possible to recruit the second sibling because he/she died before the interview or changed his/her mind and refused to participate), 87.1% with two 90+ siblings, 7.7% with three 90+ siblings, 2.2% with four 90+ sibling and 0.3% with five 90+ sibling. In the whole project only UNIBO, CRLC (France) and TAMPERE (Finland) managed to recruit families with five 90+ siblings. This result is noteworthy because it implies a greater effort in terms of economical and human resources to complete trios without neglecting any sibling in the families, allowing for insight on the human longevity in large families.

As regards the **gender composition of families**, females represent the 72.5% of probands and the 70.1% of the second siblings, indicating that the sample is enriched in females, in a similar manner for UNIBO and ISS.

As regards the **collection of biological material**, 90% of 90+ siblings who were interviewed donated blood sample and 10% donated cheek-swabs. A 7.5ml tube of whole blood was stored locally at –20°C to be sent to Partner N. 9 for DNA extraction, according to the standard procedures agreed among the consortium members. Then, aliquots of granulocytes and PBMCs were separated and collected for 78.9% of 90+ subjects recruited by UNIBO and for 2.8% of 90+ subjects recruited by ISS. These aliquots were required samples collected by UNIBO because they were necessary to perform mtDNA C150T mutation analysis.

	Recruiting Centre						
	UNIBO n = 258 families		ISS n = 106		Total n = 364		
	N	%	N	%	N	%	*p* Value
FAMILY SIZE							
1 90+ sibling	10	3,9	0	0,0	10	2,7	
2 90+ siblings	215	83,3	102	96,2	317	87,1	
3 90+ siblings	24	9,3	4	3,8	28	7,7	
4 90+ siblings	8	3,1	0	0,0	8	2,2	
5 90+ siblings	1	0,4	0	0,0	1	0,3	
SIBLING 1							
Males	70	27,1	30	28,3	100	27,5	0,820
Females	188	72,9	76	71,7	264	72,5	
SIBLING 2							
Males	79	31,9	27	25,5	106	29,9	0,230
Females	169	68,1	79	74,5	248	70,1	



	Recruiting Centre					
	UNIBO n = 549		ISS n = 216		Total n = 765	
	N	%	N	%	N	%
BIOLOGICAL SAMPLES						
Blood	508	92,5	182	84,3	690	90,2
Cheek Swabs	38	6,9	43	19,9	81	10,6
Granulocytes	433	78,9	6	2,8	439	57,4
Lymphocytes plus Monocytes	432	78,7	6	2,8	438	57,3

Table 4.5 – Basic characteristics of the Study Population and Biological Samples

4.5.2 Socio-demographic characteristics of the GEHA Study Population

A total of 765 90+ subjects were recruited by UNIBO and ISS: 29% were males (mean-age: 93.4 years) and 71% were females (mean-age: 93.8 years). In **Table 4.6** their main socio-demographic characteristics by recruiting unit are shown.

Among all 765 subjects, 91.6% were interviewed personally while for 8.4% the interview was performed by a proxy, meaning that data on SMMSE, self-reported health ("How is your health in general?") and attitude towards life ("How is your attitude towards life?") are missing in the questionnaire.

The distribution of 90+ siblings according to their **place of birth** points out two main districts: 68% of 90+ siblings was born in Northern Italy (the elected recruitment area for UNIBO) and 28% was born in Central and Southern Italy (the elected recruitment area for ISS); the rest 4% of the population was born in Italian islands.

As regards the **marital status**, most of the population was widow/widower (74.8%), the same percentage (12%) of 90+ subjects was still married or never married and no differences were found between recruiting centres.

The **level of literacy** was higher in 90+ siblings recruited by ISS (8.2 mean years of education) in comparison with UNIBO (4.9 mean years of education): indeed, about 50% of UNIBO subjects did not finish primary school, while 27.8% of ISS subjects finished primary school and 19.4% reached the second stage of secondary level education. This discrepancy is probably related to the different social contest from which the two populations came from: almost all UNIBO subjects had lived and still live in the countryside, while ISS subjects live in a city such as Rome, where it was easier to have access to school.

A difference between UNIBO and ISS is also present as regards the **type of occupation**: for UNIBO subjects the main lifetime jobs were being a farmer (19.1%), a craftsmen (19.3%) or a farm-labourer (20.8%), perfectly in accordance with a population who lived in a rural an agriculture-based contest. For ISS subjects the situation is different because the main lifetime jobs were being a clerk (24.1%) or a tradesman (12.55), in accordance with a population who lived in an urban contest, and a surprisingly high percentage of subjects (11.1%) belonged to the category of legislators, senior officials and managers. Moreover, a much higher percentage of housekeepers in present among ISS females (28.2%) in comparison to UNIBO (9,8%), probably because UNIBO woman living in the countryside worked as farmers together with the rest of the family.

The different social background between UNIBO and ISS subjects was also reflected on the **type of residence**: even if most of the subjects lived in apartment both in UNIBO (66.3%) and in ISS (89.8%) population, an higher percentage of UNIBO subjects lived in a house (25%) in comparison with ISS (5.1%), while the percentage of subjects living in institution was similar (8.7% in UNIBO versus 5.1% in ISS).

Excluding the institutionalised subjects, the most frequent **living condition** of 90+ siblings was the cohabitation with their sons, daughters or siblings. Few subjects had a paid cohabiting person and the 25.8% of UNIBO subjects lived alone versus 18.3% of ISS subjects.

	Recruiting Centre						
	UNIBO n = 549 subjects		ISS n = 216 subjects		Total n = 765 subjects		
	N	%	N	%	N	%	*p* Value
Male	164	29,9	58	26,9	222	29,0	0,407
Female	385	70,1	158	73,1	543	71,0	
AGE: mean (SD)							
Males	93,5 (2,9)		93,1 (2,4)		93,4 (2,7)		0,069
Females	94,1 (3,0)		93,6 (2,9)		93,8 (2,9)		
INTERVIEW TYPE							
In person	498	90,7	203	94,0	701	91,6	0,141
By Proxy	51	9,3	13	6,0	64	8,4	
PLACE OF BIRTH							
ITC: North-West Italy	42	7,7	4	1,9	46	6,0	0,000
ITD: North-East Italy	449	81,8	22	10,2	471	61,6	
ITE: Centre Italy	35	6,4	111	51,4	146	19,1	
ITF: South Italy	14	2,6	58	26,9	72	9,4	
ITG: Italian Islands	5	0,9	16	7,4	21	2,7	
Other	4	0,7	5	2,3	9	1,2	
MARITAL STATUS							
Never Married	69	12,6	26	12,0	95	12,4	0,777
Married	66	12,0	28	13,0	94	12,3	
Divorced, Separated	2	0,4	2	0,9	4	0,5	
Widow/Widowerer	412	75,0	160	74,1	572	74,8	
EDUCATION							
Years at school: mean (SD)	4,9 (3,0)		8,2 (5,1)		5,8 (4,0)		0,000
Never went to school	10	1,8	8	3,7	18	2,4	0,000
Did not finish primary school	274	49,9	44	20,4	318	41,6	
Finished primary school	195	35,5	60	27,8	255	33,3	
First Stage of Secondary Level Education	32	5,8	31	14,4	63	8,2	
Second Stage of Secondary Level Education	20	3,6	42	19,4	62	8,1	
Third Level: Other than University Degree	2	0,4	0	0,0	2	0,3	
Third Level: Initial University Degree	13	2,4	28	13,0	41	5,4	
Third Level: Higher University Degree or Post-graduate	1	0,2	2	0,9	3	0,4	
Unknown	2	0,4	0	0,0	2	0,3	
TYPE OF OCCUPATION							
Legislators, senior officials and managers	11	2,0	24	11,1	35	4,6	0,000
Professionals	20	3,6	9	4,2	29	3,8	
Technicians and associate professionals	4	0,7	0	0,0	4	0,5	
Clerks	28	5,1	52	24,1	80	10,5	
Service workers and shop and market sales workers	44	8,0	15	6,9	59	7,7	
Skilled agricultural and fishery workers	105	19,1	9	4,2	114	14,9	
Craft and related trades workers	106	19,3	27	12,5	133	17,4	
Plant and machine operators and assemblers	62	11,3	10	4,6	72	9,4	
Elementary occupation	114	20,8	3	1,4	117	15,3	
Military	1	0,2	6	2,8	7	0,9	
Not applicable (Housekeeper)	54	9,8	61	28,2	115	15,0	
TYPE OF RESIDENCY							
House	137	25,0	11	5,1	148	19,3	0,000
Apartment	364	66,3	194	89,8	558	72,9	
Sheltered housing/nursing home	48	8,7	11	5,1	57	7,5	
COHABITATION							
Subjects living alone	130	25,8	38	18,3	168	23,6	0,032
Subjects living with others	374	74,2	170	81,7	544	76,4	

Table 4.6 – Type of Interview and SocioDemographic Characteristics of the Recruited Subjects

4.5.3 Cognitive Status of the GEHA Study Population

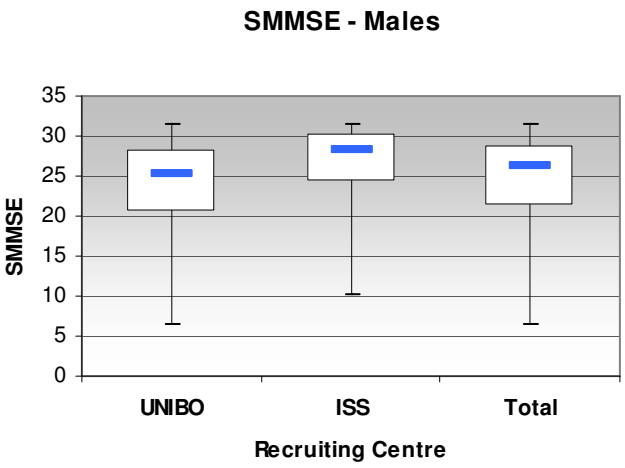
The **SMMSE** was used as measure of the cognitive status of 90+ siblings and it was calculated in a total of 699 subjects out of 765 as a consequence of proxy interviews. The raw SMMSE score was adjusted for age and years of education according to the reference given by Magni *et al.* 1996 in a study on Italian population. The results (**Table 4.7**) indicate that males (mean score = 24.8; SD = 5.1) were generally more cognitively intact than females (mean score = 23.1; SD = 6.3) both in UNIBO and in ISS recruiting units, but ISS subjects (both males and females) performed higher scores in comparison to UNIBO subjects. Cognitive function was classified into three levels according to two different categorizations:

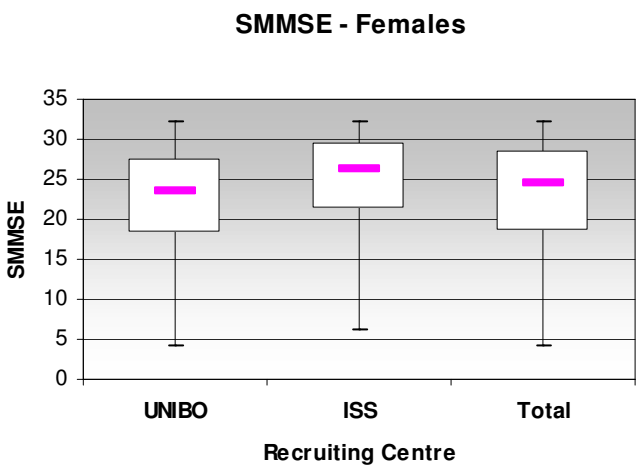
(1) if we use the stricter cut-off point "Cognitive Unimpairment" (24-30), "Mild Cognitive Impairment" (18-23) and "Severe Cognitive Impairment" (0-17), 56.2% of 90+ siblings is classified as "not impaired", 27.1% as "mildly impaired" and 16.6% as "severely impaired";

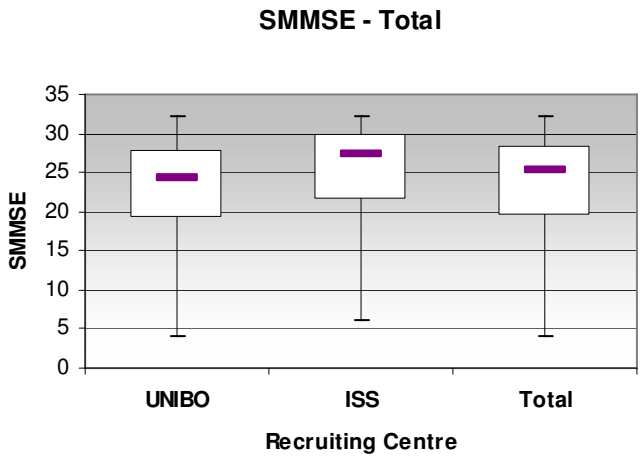
(2) if we use the wider cut-off point "Cognitive Unimpairment" (20-30), "Mild Cognitive Impairment" (13-19) and "Severe Cognitive Impairment" (0-12), 74.2% of 90+ siblings were classified as "not impaired", 19.9% as "mildly impaired" and only 5.9% as "severely impaired".

	Recruiting Centre						
	UNIBO n = 496 subjects		**ISS n = 203 subjects**		**Total n = 699 subjects**		
COGNITIVE STATUS	**N**	**%**	**N**	**%**	**N**	**%**	***p* Value**
1. SMMSE							
Males	151	30,4	55	27,1	206	29,5	
mean (SD)	22,7 (5,2)		25,5 (5,0)		23,4 (5,3)		0,001
Females	345	69,6	148	72,9	493	70,5	
mean (SD)	20,7 (6,3)		23,5 (6,0)		21,5 (6,3)		0,000
Total	496	100,0	203	100,0	699	100,0	
mean (SD)	21,3 (6,0)		24,0 (5,8)		22,1 (6,1)		0,000
2. SMMSE corrected for age and years of education (Magni et al., 1996)							
Males	150	30,3	54	26,7	204	29,3	
mean (SD)	24,2 (5,1)		26,5 (4,7)		24,8 (5,1)		0,004
Females	345	69,7	148	73,3	493	70,7	
mean (SD)	22,4 (6,2)		24,8 (5,7)		23,1 (6,2)		0,000
Total	495	100,0	202	100,0	697	100,0	
mean (SD)	23,0 (6,0)		25,2 (5,5)		23,6 (5,9)		0,000
3. SMMSE corrected categories (Nybo H et al., 2003)							
Cognitive Unimpairment (24-30)	259	52,3	133	65,8	392	56,2	
Mild Cognitive Impairment (18-23)	141	28,5	48	23,8	189	27,1	0,002
Severe Cognitive Impairment (0-17)	95	19,2	21	10,4	116	16,6	
4. SMMSE corrected categories (Franceschi et al., 2000)							
Cognitive Unimpairment (20-30)	348	70,3	169	83,7	517	74,2	
Mild Cognitive Impairment (13-19)	113	22,8	26	12,9	139	19,9	0,001
Severe Cognitive Impairment (0-12)	34	6,9	7	3,5	41	5,9	

Table 4.7 – Cognitive Status of the Recruited Subjects (Proxy interviews are not included)


SMMSE - Males
SMMSE
35
30
25
20
15
10
5
0
UNIBO
ISS
Total
Recruiting Centre


SMMSE - Females
SMMSE
35
30
25
20
15
10
5
0
UNIBO
ISS
Total
Recruiting Centre


SMMSE - Total
SMMSE
35
30
25
20
15
10
5
0
UNIBO
ISS
Total
Recruiting Centre

4.5.4 Anthropometric characteristics of the GEHA Study Population

In **Table 4.8** the results concerning the anthropometric characteristics of 90+ siblings are shown by recruiting unit. As recruitment procedure, UNIBO and ISS units measured the total height and the weight whenever it was possible. The height data, available for the 82% of subjects (631 out of 765), were always measured. The weight data were measured for the 83% of subjects (635 out of 765), they were self-reported for the 12% of subjects (92 out of 765) and they were not available for the 5% of subjects (38 out of 765). Since no difference was found between measured and self-reported weight in each recruiting centre (data not shown), they were put together to calculate BMI. Differences in the number of cases are due to the presence of missing values. As regards the **total height**, results indicate that males (mean height = 164.3 cm; SD = 6.8) are taller than females (mean height = 151,1 cm; SD = 8.3) in both recruiting units, but both males and females recruited by ISS are taller than subjects recruited by UNIBO. As regards the **weight**, it is higher in males (mean weight = 69.2 Kg; SD = 11.7) than females (mean weight = 56.6 Kg; SD = 10.8) and no differences were found between centres. Finally, as regards the **BMI** values it results that for males the mean BMI value is 25.8 and no differences are present between UNIBO and ISS, while for females the mean BMI is 24.8 and it is higher in 90+ siblings recruited by UNIBO in comparison to those recruited by ISS. This result indicates that 90+ females show a much more complex and heterogeneous phenotype than males.

	Recruiting Centre						
	UNIBO		ISS		Total		
	N	%	N	%	N	%	*p* Value
TOTAL HEIGHT							
Males	142	34,1	57	26,6	199	31,5	
mean (SD)	163,6 (6,8)		166,1 (6,4)		164,3 (6,8)		0,021
Females	275	65,9	157	73,4	432	68,5	
mean (SD)	148,9 (8,3)		155,0 (6,8)		151,1 (8,3)		0,000
Total	417	100,0	214	100,0	631	100,0	
mean (SD)	153,9 (10,5)		157,9 (8,3)		155,3 (10,0)		0,000
MEASURED WEIGHT							
Males	150	31,6	42	26,1	192	30,2	
mean (SD)	69,5 (12,1)		68,4 (10,3)		69,2 (11,7)		0,617
Females	324	68,4	119	73,9	443	69,8	
mean (SD)	56,8 (11,2)		56,1 (9,7)		56,6 (10,8)		0,575
Total	474	100,0	161	100,0	635	100,0	
mean (SD)	60,8 (13,0)		59,3 (11,3)		60,4 (12,6)		0,203
BMI (Body Mass Index)							
Males	140	34,3	57	26,6	197	31,7	
mean (SD)	26,1 (4,0)		25,0 (3,5)		25,8 (3,9)		0,077
Females	268	65,7	157	73,4	425	68,3	
mean (SD)	25,6 (4,5)		23,3 (4,0)		24,8 (4,5)		0,000
Total	408	100,0	214	100,0	622	100,0	
mean (SD)	25,8 (4,3)		23,8 (4,0)		25,1 (4,3)		0,000

Table 4.8 – Antrophometric characteristics of the Recruited Subjects

4.5.5 Functional Status of the GEHA Study Population

In **Table 4.9** the results of the items concerning the functional status of 90+ siblings are shown by recruiting unit.

The proportion of **bedridden subjects** was higher among UNIBO subjects (9.3%) in comparison with ISS subjects (5.6%).

The **ADL** was used as the main measure of the functional status. The results indicate that feeding is the most conserved ability (in 92.2% of 90+ subjects), followed by toileting (in 71.9%), transfer from/to bed (in 70.2%), dressing (in 66.3%) and finally bathing, which is conserved only in 52.4% of subjects, as well as the urine continence (still present in 52.5% of subjects). A **five-items ADL scale** (without continence) was calculated and the functional status was classified into three levels according to original cut-off point (Nybo H *et al.*, 2001): 50.8% of 90+ siblings was classified as "not disabled" (ADL = 5), 19.2% as "moderately disabled" (ADL = 3-4), 29.9% as "severely disabled" (ADL = 0-2). Moreover, a **six-items ADL scale** (including continence) was calculated and the functional status was classified into three levels according to wider cut-off point (Franceschi *et al.*, 2000a): 67.7% of 90+ siblings was classified as "not disabled" (ADL = 4-6), 10.5% as "moderately disabled" (ADL = 2-3), 21.8% as "severely disabled" (ADL = 0-1). No difference between centres was reported in relation to ADL score.

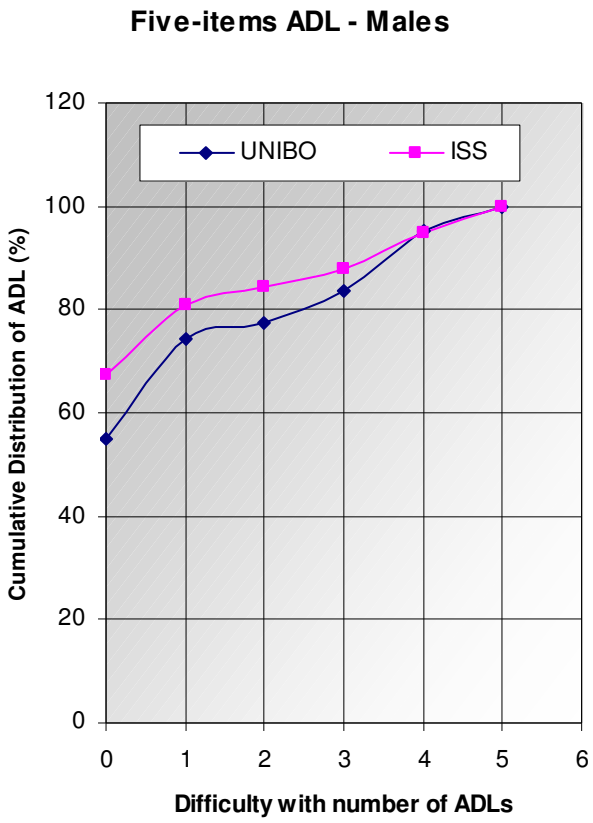
The questions about functional limitations taken from the **Nagi-scheme** indicate that the vision ability is intact in 33.3% of subjects (still able to read without glasses) while the hearing ability in 68.5% of subjects (still able to hear without aids). In addition, the ability of going up and down the stairs without anyone's help was maintained in 63% of subjects, similarly to the ability of doing any kind of exercise, maintained in 57.5%. Interestingly, the ability to walk 500 metres without aids seems to be the most difficult task, as it was conserved only in 37.1% of subjects and different results were obtained by recruiting unit (34.6% of UNIBO subject versus 43.5% of ISS subjects).

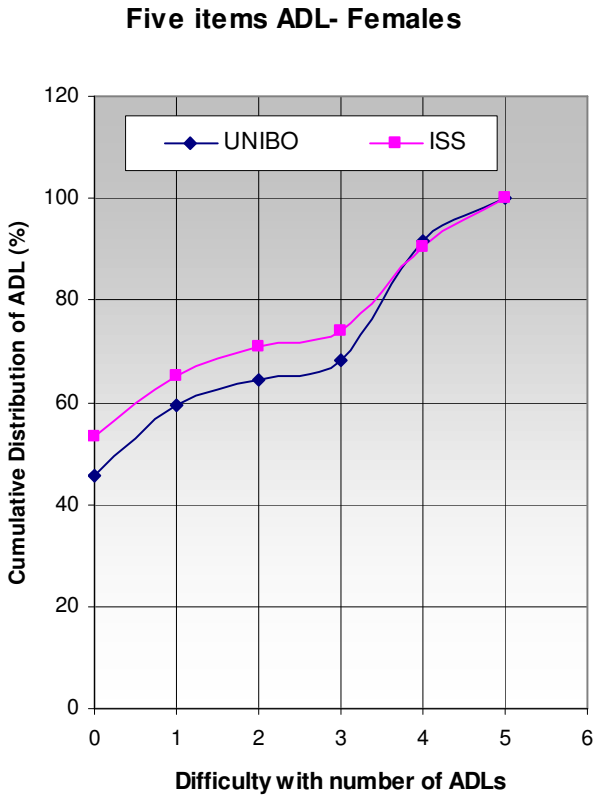
The **Hand Grip** strength was measured in 91% of the total subjects, with a similar proportion in the two recruiting units. The results indicate that measured hand grip strength was significantly higher for males (mean score = 23.7; SD = 7.1) than for females (mean score = 14.4; SD = 5.7) both in UNIBO and in ISS recruiting units and no differences were reported between centres.

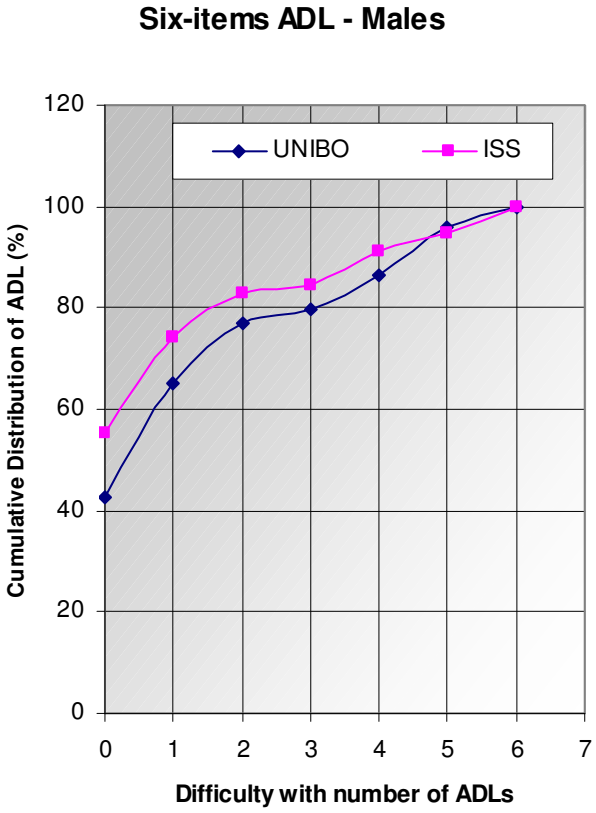
The **Chair Stand test** was performed by 43.1% of subjects, with a similar proportion in the two recruiting units.

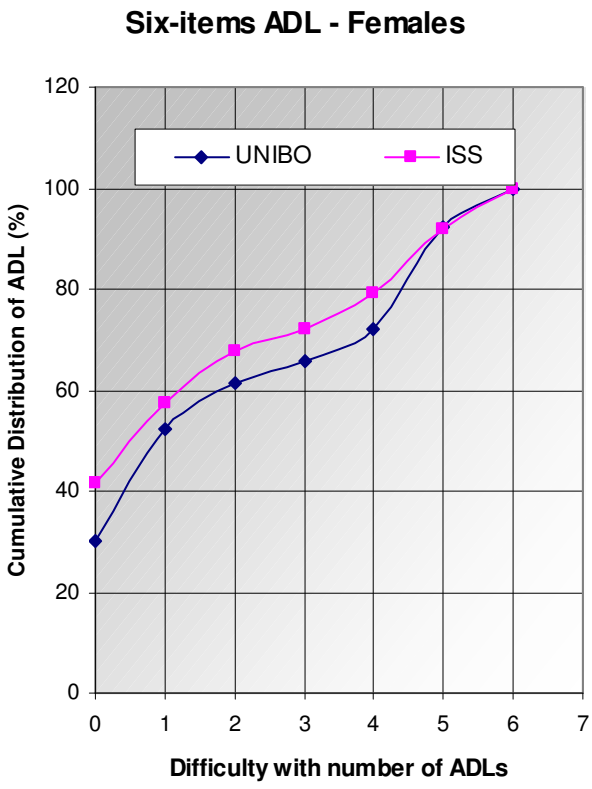
	Recruiting Centre						
	UNIBO n = 549 subjects		ISS n = 216 subjects		Total n = 765 subjects		
FUNCTIONAL CAPACITY	**N**	**%**	**N**	**%**	**N**	**%**	***p* Value**
1. Confination to bed							
Bedridden	51	9,3	12	5,6	63	8,2	0,004
Not Bedridden	498	90,7	204	94,4	702	91,8	
2. Five-item ADL scale							
a. Feeding ability	507	92,3	198	91,7	705	92,2	0,752
b. Transfer from/to bed ability	372	67,8	165	76,4	537	70,2	0,014
c. Dressing ability	358	65,2	149	69,0	507	66,3	0,321
d. Going to the toilet ability	391	71,2	159	73,6	550	71,9	0,508
e. Bathing ability	272	49,5	129	59,7	401	52,4	0,035
Five-item ADL scale categories (Nybo H et al., 2001)							
Not disabled (ADL=5)	266	48,5	123	56,9	389	50,8	0,099
Moderately disabled (ADL=3-4)	109	19,9	38	17,6	147	19,2	
Severely disabled (ADL=0-1-2)	174	31,7	55	25,5	229	29,9	
3. Six-item ADL scale							
a. Feeding ability	507	92,3	198	91,7	705	92,2	0,752
b. Transfer from/to bed ability	372	67,8	165	76,4	537	70,2	0,014
c. Dressing ability	358	65,2	149	69,0	507	66,3	0,321
d. Going to the toilet ability	391	71,2	159	73,6	550	71,9	0,508
e. Bathing ability	272	49,5	129	59,7	401	52,4	0,035
f. No urine incontinence	274	49,9	128	59,3	402	52,5	0,020
Six-item ADL scale categories (Franceschi et al., 2000)							
Not disabled (ADL=4-5-6)	363	66,1	155	71,8	518	67,7	0,200
Moderately disabled (ADL=2-3)	57	10,4	23	10,6	80	10,5	
Severely disabled (ADL=0-1)	129	23,5	38	17,6	167	21,8	
4. NAGI-scheme (Nagi SZ, 1976)							
a. Reading newspaper without glasses	182	33,2	73	33,8	255	33,3	0,293
b. Recognize someone 4 metres away	402	73,2	138	63,9	540	70,6	0,028
c. Hearing ability without aids	377	68,7	147	68,1	524	68,5	0,869
d. 500 metres walking ability without aids	190	34,6	94	43,5	284	37,1	0,022
e. Going up and down the stairs without anyone's help	349	63,6	133	61,6	482	63,0	0,607
f. Doing any kind of exercise	333	60,7	107	49,5	440	57,5	0,005
g. Going outside with or without anyone's help (from every day to once a month)	417	76,1	162	76,1	579	75,7	0,991
5. Hand grip (Kg)							
Males	154	31,4	56	27,9	210	30,4	
mean (SD)	24,0 (7,1)		22,8 (7,1)		23,7 (7,1)		0,273
Females	336	68,6	145	72,1	481	69,6	
mean (SD)	14,4 (5,7)		14,4 (5,6)		14,4 (5,7)		0,989
6. Ability to perform Chair Stand Test	240	43,7	90	41,7	330	43,1	0,606

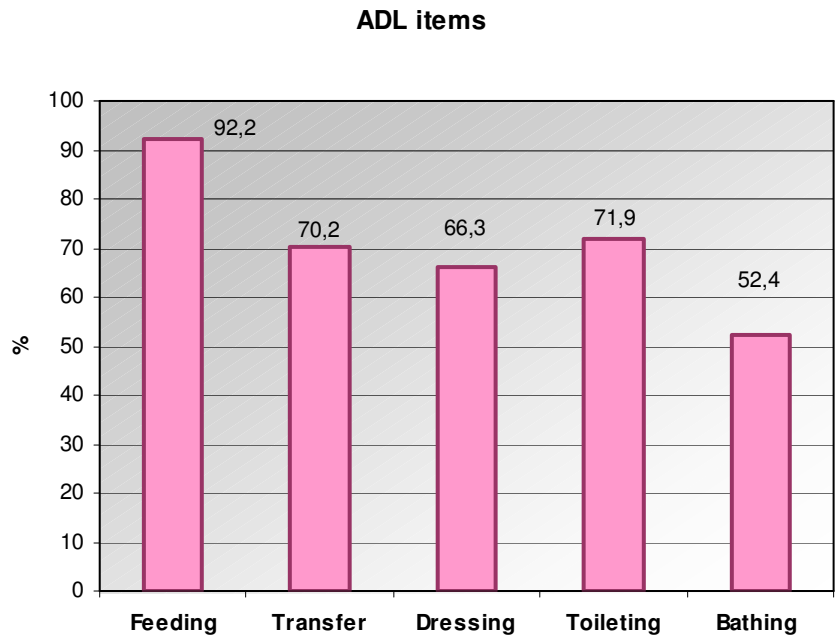
Table 4.9 – Functional Status of the Recruited Subjects

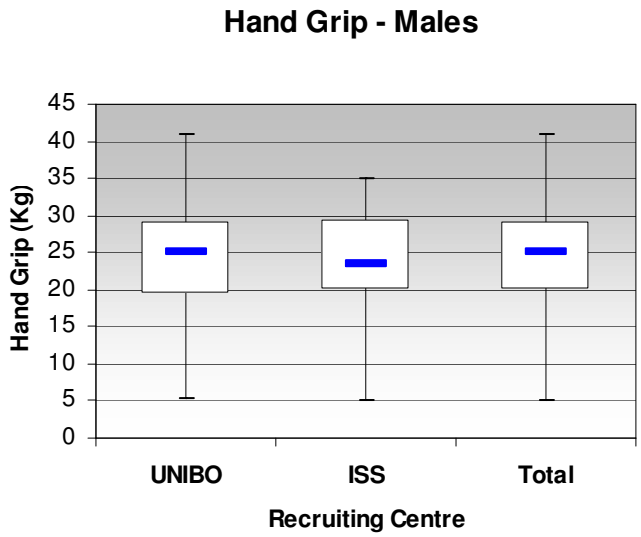

Five-items ADL - Males
UNIBO
ISS
Cumulative Distribution of ADL (%)
Difficulty with number of ADLs
0
20
40
60
80
100
120
0
1
2
3
4
5
6

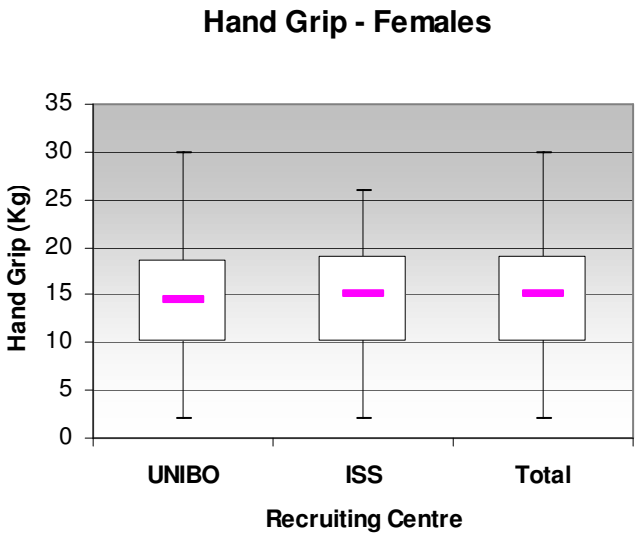

Five items ADL- Females
UNIBO
ISS
Cumulative Distribution of ADL (%)
Difficulty with number of ADLs
0
20
40
60
80
100
120
0
1
2
3
4
5
6

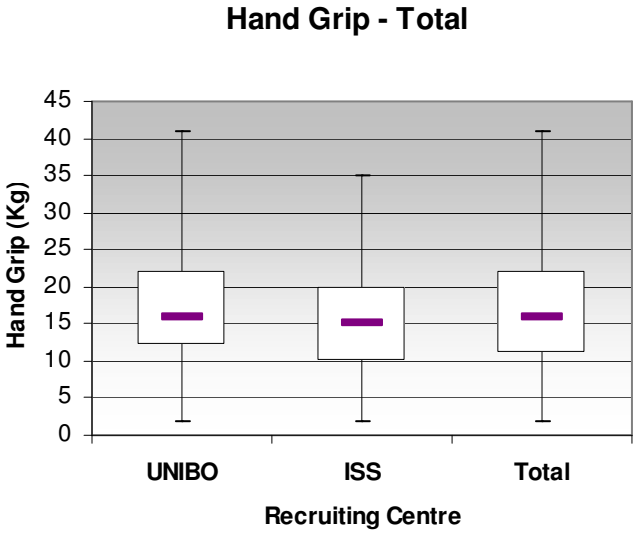

Six-items ADL - Males
UNIBO
ISS
Cumulative Distribution of ADL (%)
Difficulty with number of ADLs
0
20
40
60
80
100
120
0
1
2
3
4
5
6
7


Six-items ADL - Females
UNIBO
ISS
Cumulative Distribution of ADL (%)
Difficulty with number of ADLs
0
20
40
60
80
100
120
0
1
2
3
4
5
6
7


ADL items
100
90
80
70
60
50
40
30
20
10
0
%
92,2
70,2
66,3
71,9
52,4
Feeding
Transfer
Dressing
Toileting
Bathing


Hand Grip - Males
45
40
35
30
25
20
15
10
5
0
Hand Grip (Kg)
UNIBO
ISS
Total
Recruiting Centre


Hand Grip - Females
35
30
25
20
15
10
5
0
Hand Grip (Kg)
UNIBO
ISS
Total
Recruiting Centre


Hand Grip - Total
45
40
35
30
25
20
15
10
5
0
Hand Grip (Kg)
UNIBO
ISS
Total
Recruiting Centre

4.5.6 Life-Style and Health Status of the GEHA Study Population

In **Table 4.10** the **smoking status** and **alcohol intake** among 90+ siblings are shown by recruiting unit.

Most of 90+ siblings never smoked (74.8%), with a significantly higher proportion in UNIBO than in ISS: 77.7% versus 67.4%. Only 2.5% of 90+ siblings were currently smoking every day or some days, with on the contrary an higher proportion in ISS than in UNIBO: 4.7% versus 1.6%.

Moreover, 56% of 90+ siblings reported alcohol use every day, but in low quantity.

	Recruiting Centre						
	UNIBO n = 549 subjects		ISS n = 216 subjects		Total n = 765 subjects		
	N	%	N	%	N	%	*p* Value
SMOKING							
Never Smoker	426	77,7	145	67,4	571	74,8	
Former Smoker	113	20,6	60	27,9	173	22,7	0,003
Smokers	9	1,6	10	4,7	19	2,5	
ALCOHOL INTAKE							
Use of alcohol every day	317	57,8	110	51,2	427	56,0	0,094

Table 4.10 – Lifestyle characteristics of the Recruited Subjects

In **Table 4.11** the characteristics about the **health status** of 90+ siblings are shown by recruiting unit.

Most of 90+ siblings declared to have 3 or more **diseases** at the recruitment time (61.7%), with a significantly higher proportion in UNIBO than in ISS: 67.7% versus 46.5%. Only 4.3% of 90+ siblings did not report any disease, with on the contrary an higher proportion in ISS than in UNIBO: 6.5% versus 3.5%.

As regards the **self reported health** ("How is your health in general?"), 50% of 90+ siblings answered "Good", 24.1% "Fair", 13.2% "Poor/Very Poor" and 12.9% "Very Good", with a slightly more positive pattern in ISS than in UNIBO. As regards the **attitude towards life** (How is your attitude towards life?"), 53.7% of 90+ siblings is "optimistic" (analogous data in UNIBO and ISS), 13.3% is "pessimistic" with a significantly higher proportion in UNIBO than in ISS (15.4% versus 8%) and 33% declared to be "neither optimistic nor pessimistic" with on the contrary a significantly higher proportion in ISS than in UNIBO (41.8% versus 29.4%).

Most of 90+ siblings made **use of drugs** (91.1%), with analogous data in UNIBO and in ISS.

Most of 90+ siblings **did not fall down** within the last year (65.8%), with a significantly higher proportion in ISS than in ISS: 72.6% versus 63.2%.

Most of 90+ siblings were **not hospitalised** within the last year (77.2%), with analogous data in UNIBO and in ISS.

Most of 90+ siblings **did not lose weight** within the last year (80.7%), with analogous data in UNIBO and in ISS.

	Recruiting Centre						
	UNIBO n = 549 subjects		ISS n = 216 subjects		Total n = 765 subjects		
	N	%	N	%	N	%	*p* Value
NUMBER OF DISEASES							
0	19	3,5	14	6,5	33	4,3	
1-2	158	28,8	101	47,0	259	33,9	0,000
≥ 3	371	67,7	100	46,5	471	61,7	
"HOW IS YOUR HEALTH IN GENERAL?"							
Very good	69	13,9	21	10,4	90	12,9	
Good	236	47,7	110	54,7	346	49,7	0,047
Fair	114	23,0	54	26,9	168	24,1	
Poor/Very poor	76	15,4	16	8,0	92	13,2	
ATTITUDE TOWARDS LIFE							
Optimistic	272	55,2	101	50,2	373	53,7	
Neither optimistic nor pessimistic	145	29,4	84	41,8	229	33,0	0,004
Pessimistic	76	15,4	16	8,0	92	13,3	
USE OF DRUGS	499	90,9	196	91,6	695	91,1	0,075
NO FALLS WITHIN THE LAST YEAR	347	63,2	156	72,6	503	65,8	0,014
NO HOSPITALIZATION WITHIN THE LAST YEAR	418	76,1	172	80,0	590	77,2	0,252
NO LOSS OF WEIGHT WITHIN THE LAST YEAR	433	79,2	182	84,7	615	80,7	0,084

Table 4.11 – Health Status of the Recruited Subjects

4.5.7 Haematological and Biochemical parameters of the GEHA Study Population

In **Table 4.12** the main blood parameters of 604 nonagenarian subjects are shown. Most of the parameters fell within the standard ranges valid for the healthy adult population, with only few exceptions: the red cell count in males from ISS and the hematocrit in males both from UNIBO and ISS were a bit lower. No differences were found between recruiting unit. The sex-dependent difference in red blood cell counts seen usually in younger adults in favour of males was not present in nonagenarians, which may find its explanation in the postmenopausal increase of haemoglobin levels in females.

	Recruiting Centre							
	UNIBO n = 448 subjects		ISS n = 156 subjects		Total n = 604 subjects			
HEMOCYTOMETRIC RESULTS	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	***p* Value**	**Reference Values**
Males - Red cells count (10^6/ml)	4,5	0,5	4,4	0,6	4,5	0,6	0,143	M: 4,50-6,10
Females - Red cells count (10^6/ml)	4,4	0,5	4,3	0,6	4,4	0,6	0,283	F: 4,20-5,40
Males - Haemoglobin (g/dl)	13,6	1,6	13,4	1,7	13,5	1,6	0,46	M: 13,0-16,5
Females - Haemoglobin (g/dl)	12,8	1,5	13,1	1,4	12,9	1,5	0,066	F: 12,0-15,0
Males - Hematocrit (%)	40,8	4,8	40,2	5,2	40,7	4,9	0,450	M: 42,0-52,0
Females - Hematocrit (%)	38,9	4,3	39,1	4,5	38,9	4,4	0,770	F: 37,0-47,0
MCV (fl)	89,5	5,7	89,9	6,2	89,6	5,8	0,392	80,0-96,0
Leukocytes (10^3/ml)	6,5	2,8	6,8	2,6	6,5	2,7	0,204	4,20-9,0
Lymphocytes (%)	27,4	9,1	29,1	10,0	27,8	9,3	0,041	19,0-48,0
Monocytes (%)	5,9	1,5	8,4	2,6	6,5	2,2	0,000	3,0-9,0
Neutrofiles (%)	61,2	9,9	58,3	10,2	60,5	10,0	0,002	40,0-74,0
Eosinofiles (%)	3,1	2,1	3,4	2,4	3,1	2,2	0,134	0,0-6,0
Basofiles (%)	0,5	0,3	0,7	0,6	0,6	0,4	0,000	0,0-1,5
Platelets (10^3/μl)	243,1	77,4	234,4	86,4	240,8	79,8	0,245	150-380

Table 4.12 – Hemocytometric results of the Recruited Subjects

In **Table 4.13** the chemical parameters and the lipoprotein profiles of 598 nonagenarian subjects are shown. Also these parameters fell within the normal ranges of the healthy adult population, with only one exception: the level of total cholesterol slightly exceeded the normal range in 90+ siblings from ISS (214.8 mg/dl). Moreover, even if the levels of the measured parameters fell within the normal ranges of the healthy adult population, some significant differences between UNIBO and ISS were discovered: creatinine level is higher in UNIBO than in ISS (1.2 mg/dl versus 1.1 mg/dl), glucose level is higher in ISS than in UNIBO (95.2 mg/dl versus 86.8 mg/dl), GPT level is higher in UNIBO than in ISS (13.6 U/l versus 11.8 U/l) and total cholesterol is higher in ISS than in UNIBO (214.8 mg/dl versus 197.3 mg/dl).

	Recruiting Centre							
	UNIBO n = 440 subjects		ISS n = 158 subjects		Total n = 598 subjects			
CLINICAL CHEMISTRY RESULTS	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	***p* Value**	**Reference Values**
Creatinine (mg/dl)	1,2	0,4	1,1	0,4	1,2	0,4	0,024	0,5-1,2
Glucose (mg/dl)	86,8	31,2	95,2	24,3	89,0	29,8	0,002	60-110
Males - ALT (GPT) (U/l)	15,9	13,8	12,3	5,7	15	12,4	0,094	M: < 41
Females - ALT (GPT) (U/l)	13,6	7,8	11,8	7,4	13,1	7,7	0,037	F: < 31
LIPID PROFILE								
Total Cholesterol (mg/dl)	197,3	40,6	214,8	45,4	202,0	42,6	0,000	< 200
Males - HDL-C (mg/dl)	56,2	14,4	53,7	14,4	55,6	14,4	0,317	M: > 35
Females - HDL-C (mg/dl)	64,4	15,8	61,4	16,5	63,5	16,1	0,100	F: > 45
Triglycerides (mg/dl)	117,8	51,1	121,5	55,4	118,8	52,3	0,443	< 180

Table 4.13 – Clinical chemistry results of the Recruited Subjects

Finally, in **Table 4.14** all the items assessed by the GEHA questionnaire were reported, pointing out if their specific results were homogeneous or different in UNIBO and ISS recruiting units.

Are UNIBO and ISS population homogeneous?		
=	≠	*In what they differ?*
Gender Composition of trios	Families that gave **positive response to participate in the study**	Higher in UNIBO
Gender	Families **did not enter the study**	Higher in ISS
Age	**Place of Birth**	UNIBO: Northern Italy; ISS: Central-Southern Italy
Marital Status	**Education**	Higher in ISS
ADL	**Occupation**	Higher in ISS
Hand Grip test	**Type of Residency**	
Chair Stand test	**Cohabitation**	Higher in UNIBO
Alcohol Intake	**SMMSE**	Higher in ISS
Use of drugs	**Total Height**	Higher in ISS
Self-reported Health	**BMI** (Females)	Higher in UNIBO
No hospitalization within last year	**Confination to Bed**	Higher in UNIBO
No loss of weight within last year	**Smoking**	Higher in ISS
Red Cells Count (Males and Females)	**Number of Diseases**	Higher in UNIBO
Hemoglobin (Males and females)	**Attitude towards life**	Higher "neither optimistic nor optimistic" in ISS
Hematocrit (Males and Females)	**No falls within last year**	Higher in ISS
MCV	**Creatinine**	Higher in UNIBO
Leukocytes	**Glucose**	Higher in ISS
Platelets	**GPT** (Females)	Higher in UNIBO
GPT (Males)	**Total Cholesterol**	Higher in ISS
HDL (Males and females)		
Triglycerides		

Table 4.14 – Summary of the Homogeneity and Differences between UNIBO and ISS population

4.6 ASSESMENT OF THE HEALTH AND THE FUNCTIONAL STATUS OF GEHA 90+ SIBLINGS RECRUITED BY UNIBO AND ISS RECRUITING UNITS

4.6.1 Application of the classifications for the health status available in literature

The identification of the determinants which contribute to survive to old age and the definition of a precise healthy aging phenotype are a major issue for studies aimed at finding the genetic factors of human longevity, such as the GEHA project. To this purpose, three different classification methods were proposed in various studies on centenarians, based on:

1. actual functional capabilities (ADL, SMMSE visual and hearing abilities) (Gondo *et al.*, 2006);

2. actual functional capabilities and morbidity (ADL, ability to walk, SMMSE, presence of cancer, ictus, renal failure, anaemia, and liver diseases) (Franceschi *et al.*, 2000a);

3. retrospectively collected data about past history of morbidity and age of disease onset (hypertension, heart disease, diabetes, stroke, cancer, osteopororis, neurological diseases, chronic obstructive pulmonary disease and ocular diseases) (Evert *et al.*, 2003).

These available models to define the health status of long-living subjects were applied to our sample and the results are reported in **Table 4.15**.

	Recruiting Centre						
	UNIBO n = 549 subjects		ISS n = 216 subjects		Total n = 765 subjects		
	N	%	N	%	N	%	*p* Value
GONDO et al., 2006							
Exceptional	31	5,6	18	8,3	49	6,4	0,170
Normal	188	34,2	86	39,8	274	35,8	
Frail and Fragile *	279	50,8	99	45,8	378	49,4	
Proxy	51	9,3	13	6,0	64	8,4	
FRANCESCHI et al., 2000							
A	110	20,0	56	25,9	166	21,7	0,060
B	154	28,1	46	21,3	200	26,1	
C	119	21,7	37	17,1	156	20,4	
Proxy	51	9,3	13	6,0	64	8,4	
Not applicable	115	20,9	64	29,6	179	23,4	
EVERT et al., 2003							
Escapers	49	8,9	21	9,7	70	9,2	0,637
Delayers	390	71,0	145	67,1	535	69,9	
Survivors	89	16,2	38	17,6	127	16,6	
Not applicable	21	3,8	12	5,6	33	4,3	

Table 4.15 – Health Status of the Recruited

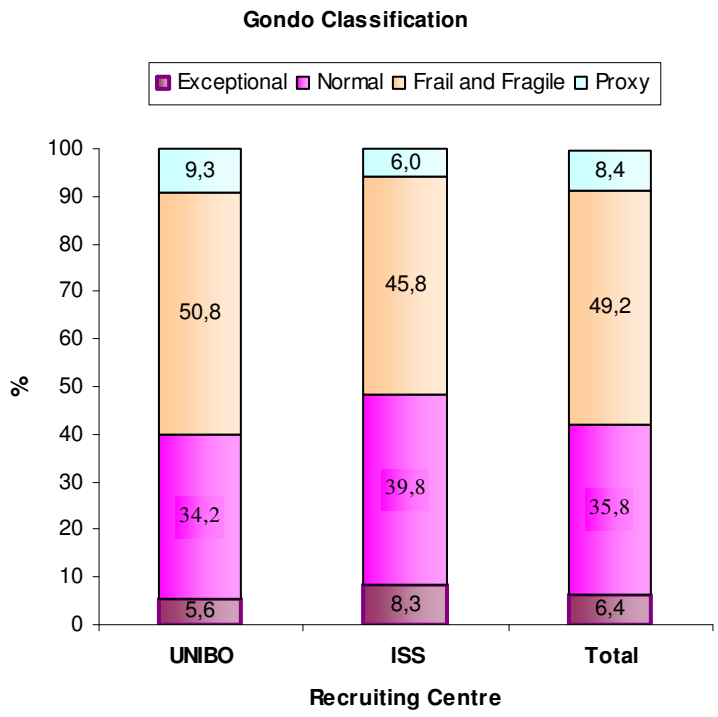
* Frail and Fragile categories were united since only 2 subjects (1 subject from UNIBO and 1 from ISS) were classified as Fragile

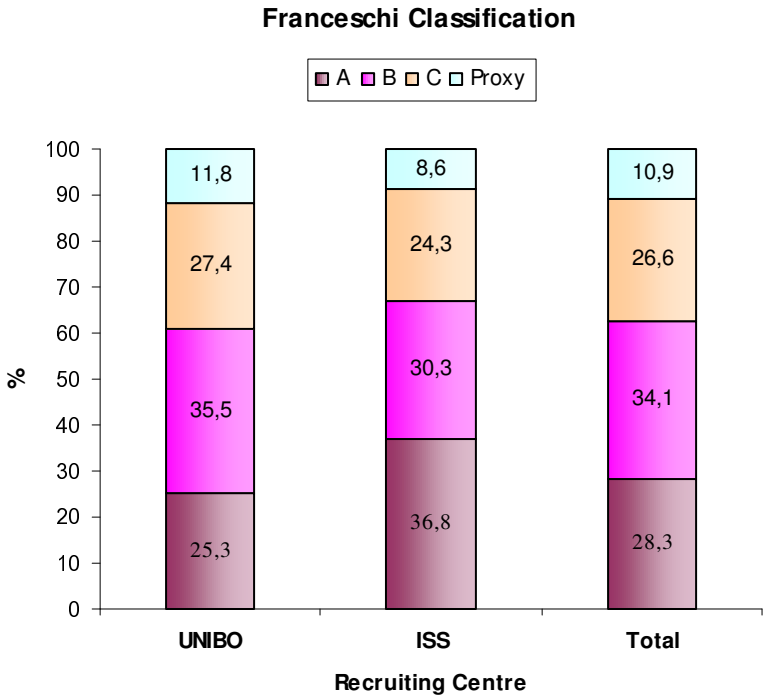
Subjects for which a proxy interview was performed were not included in the analysis because of the lack of SMMSE score.

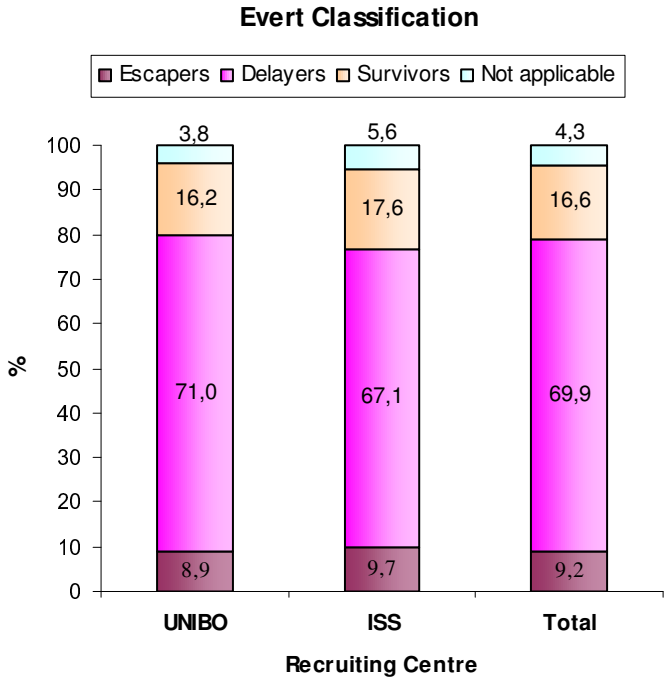
According to the classification by **Gondo**, only 6.4% of 90+ siblings were categorised as "Exceptional", 35.8% as "Normal" and most of 90+ siblings were categorised as "Frail" (49.4%). Since only 2 subjects (one from UNIBO and one from ISS) were categorised as "Fragile", in the analysis they were added to the "Frail" group. Moreover, this classification method was applicable for all subjects. No differences among health status categories were found between UNIBO and ISS.

According to the classification by **Franceschi**, 21.7% of 90+ siblings belonged to category "A" (good mental and physical conditions), 26.1% to category "B" (intermediate health status) and 20.4% to category "C" (bad health status). Moreover, this classification method was not applicable for 23.4% of subjects, where haematological and biochemical parameters were missing. No differences among health status categories were found between UNIBO and ISS.

According to the classification by **Evert**, 9.2% of 90+ siblings were categorised as "Escapers", 69.9% as "Delayers" and 16.6% as "Survivors". Moreover, it was not applicable for 4.3% of subjects, where data on diseases history were missing. No differences among health status categories were found between UNIBO and ISS.




Franceschi Classification
A
B
C
Proxy
100
90
80
70
60
50
40
30
20
10
0
%
11,8
27,4
35,5
25,3
8,6
24,3
30,3
36,8
10,9
26,6
34,1
28,3
UNIBO
ISS
Total
Recruiting Centre


Evert Classification
Escapers
Delayers
Survivors
Not applicable
100
90
80
70
60
50
40
30
20
10
0
%
3,8
16,2
71,0
8,9
5,6
17,6
67,1
9,7
4,3
16,6
69,9
9,2
UNIBO
ISS
Total
Recruiting Centre

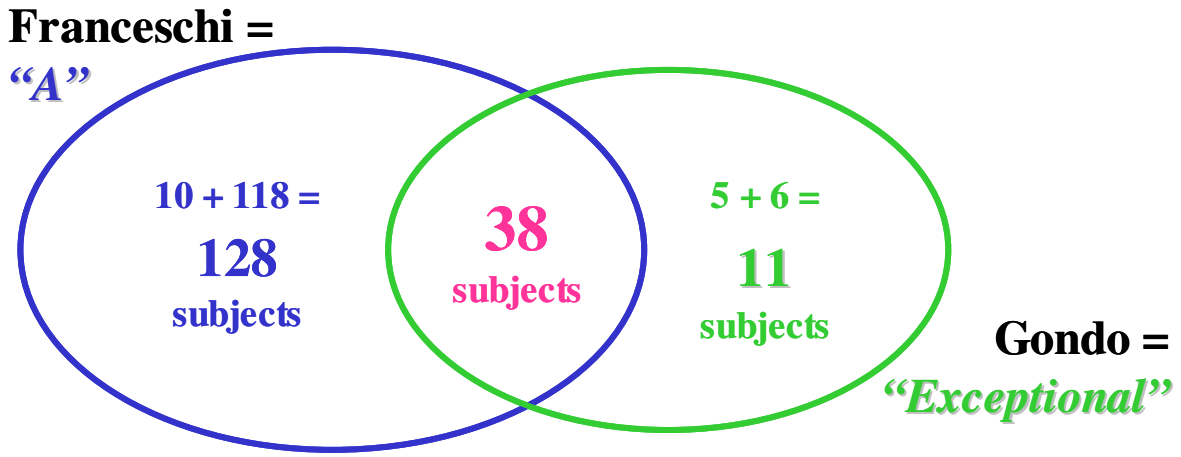
4.6.2 Comparison between the classifications for the health status proposed by Gondo and Franceschi and identification of "The Best" group of 90+ siblings

Since the classifications by Gondo and Franceschi are both based on the present functional status, they were compared (**Table 4.16**).

		GONDO et al., 2006				
		Proxy	*Frail and Fragile*	*Normal*	*Exceptional*	**Total**
FRANCESCHI et al., 2000	***Not applicable***	0	115	58	**6**	179
	Proxy	64	0	0	**0**	64
	C	0	113	38	**5**	156
	B	0	140	60	**0**	200
	A	**0**	**10**	**118**	**38**	166
	Total	64	378	274	49	765

Table 4.16 – Comparison between Gondo and Franceschi classifications

In order to better recognize the healthy aging phenotype and to identify the best group of 90+ subjects out of the entire studied population, our focus was on the subjects classified as "A" by Franceschi and on the subjects classified as "Exceptional" by Gondo. Are they the same subjects? What are the phenotype differences between subjects classified as "A" by Franceschi but not as "Exceptional" by Gondo and viceversa between subjects classified as "Exceptional" by Gondo but not as "A" by Franceschi? Do they represent an homogeneous group of subjects in terms of health status?



To answer these questions we performed a series of comparison:

a) Comparison between subjects "Franceschi=A and Gondo=Exceptional" (n=38) versus "Franceschi=A and Gondo ≠ Exceptional" (n=128), divided by gender (Table 4.17)

In addition to the parameters inside the definition of the classifications (as confirmatory measure), we considered other variables related to the present status of nonagenarian subjects (such as age, smoking habit, use of alcohol, self-reported health, attitude towards life, cohabitation, comorbidity, 500 metres walking ability without aids, going up and down the stairs without help, ability to perform Hand Grip strength test and Chair Standing test) and also variables related to event occurred in the last year (such as falls, hospitalisation and loss of weight).

We discovered that the differences between the two group of subjects only depend on the parameters that define the classifications: the subjects classified as "A" by Franceschi but not as "Exceptional" by Gondo differ only for not having perfectly intact self-reported vision and hearing abilities.

b) Comparison between "Franceschi=A and Gondo=Exceptional" (n=38) versus "Franceschi ≠ A and Gondo=Exceptional" (n=11), divided by gender (Table 4.18).

Also in this case, the differences between the two group of subjects are intrinsic in the parameters that define the classifications: 6 subjects classified as "Exceptional" by Gondo are not classified by Franceschi because of the lack of haematological and biochemical parameters, while 5 subjects classified as "Exceptional" by Gondo are not classified as "A" by Franceschi because of past diseases and/or wrong haematological and biochemical results.

	Males											Females										
	Franceschi=A and Gondo≠Exceptional n=47					*Franceschi=A and Gondo=Exceptional* n=14						*Franceschi=A and Gondo≠Exceptional* n=81					*Franceschi=A and Gondo=Exceptional* n=24					
	N	%	mean	SD	median	N	%	mean	SD	median	*p* Value	N	%	mean	SD	median	N	%	mean	SD	median	*p* Value
PARAMETERS INSIDE CLASSIFICATIONS																						
SMMSE corrected for age and education			27,4	2,8	27,8			27,4	2,9	27,3	0,993			27,2	3,4	28,2			28,5	2,0	29,2	0,203
Six-items ADL			5,6	0,7	6,0			6	0	6,0	**0,017**			5,6	0,6	6,0			6	0,0	6,0	**0,000**
Barthel ADL Index			93,9	9	100			100	0	100	**0,005**			94,7	8,2	100			100	0,0	100	**0,000**
Creatinine			1,2	0,2	1,2			1,2	0,2	1,1	0,382			1	0,2	1,0			0,9	0,2	0,9	0,287
Hemoglobin			14,2	1,4	14,3			14,3	1,1	14,4	0,770			13,2	1,1	13,1			13,3	1,1	13,3	0,606
GPT (ALT)			14	5,8	13			14,4	3,2	13	0,326			12,7	4,6	13			14,7	5,9	14	0,142
Reading newspaper without glasses	13	27,7				14	100,0				**0,000**	25	30,9				24	100,0				**0,000**
Recognize someone 4 metres away without glasses	35	76,1				14	100,0				**0,053**	63	77,8				24	100,0				**0,011**
Hearing ability without aids	36	76,6				14	100,0				**0,054**	62	76,5				24	100,0				**0,006**
Absence of ictus in the previous 6 months	47	100,0				14	100,0				n.a.	81	100,0				24	100,0				n.a.
Absence of cancer	47	100,0				14	100,0				n.a.	81	100,0				24	100,0				n.a.
Absence of severe renal failure	46	97,9				13	92,9				0,409	81	100,0				24	100,0				n.a.
PRESENT PARAMETERS																						
Age			92,3	2,1	92			92,4	2,2	91,5	0,951			92,3	2,1	92			91,9	1,9	91,5	0,432
Smoking																						
Never Smoker	14	29,8				7	50,0					70	86,4				22	91,7				
Former Smoker	30	63,8				7	50,0				0,362	8	9,9				1	4,2				0,863
Smokers	3	6,4				0	0,0					3	3,7				1	4,2				
Use of alcohol every day	35	74,5				12	85,7				0,488	44	54,3				14	58,3				0,817
"How is your health in general?"																						
Very good	15	31,9				3	21,4					9	11,1				8	33,3				
Good	22	46,8				10	71,4				0,510	55	67,9				11	45,8				**0,046**
Fair	8	17,0				1	7,1					13	16,0				5	20,8				
Poor/Very poor	2	4,3				0	0,0					4	4,9				0	0,0				
Attitude towards life																						
Optimistic	31	66,0				10	71,4					48	59,3				15	62,5				
Neither optimistic nor pessimistic	10	21,3				4	28,6				0,402	22	27,2				8	33,3				0,463
Pessimistic	6	12,8				0	0,0					11	13,6				1	4,2				
Cohabitation																						
Subjects living alone	11	23,4				2	14,3				0,713	46	57,5				9	39,1				0,156
Subjects living with others	36	76,6				12	85,7					34	42,5				14	60,9				
Comorbidity (number of current diseases)			2,1	1,5	2			2,5	1,2	3	0,240			2,2	1,3	2			2,1	0,9	2	0,856
500 metres walking ability without aids	47	100,0				14	100,0				n.a.	81	100,0				24	100,0				n.a.
Going up and down the stairs without help	46	97,9				14	100,0				0,770	80	98,8				24	100,0				0,770
Ability to perform Hand Grip test	47	100,0				14	100,0				n.a.	81	100,0				24	100,0				n.a.
Hand grip (Kg)			27,7	6,6	28			26,6	6,3	27,5	0,547			18,1	4,8	18			19	4,5	20	0,356
Ability to perform Chair Stand test	41	87,2				10	71,4				0,120	71	87,7				21	87,5				0,102
PAST PARAMETERS																						
No Fall within the last year	39	83,0				14	100,0				0,180	62	76,5				20	83,3				0,583
No Hospitalization within the last year	42	89,4				13	92,9				1,000	72	88,9				21	87,5				1,000
No Loss of weight within the last year	42	91,3				13	92,9				1,000	72	88,9				22	91,7				1,000

	Total										
	Franceschi=A and Gondo≠Exceptional n=128					*Franceschi=A and Gondo=Exceptional* n=38					
	N	%	mean	SD	median	N	%	mean	SD	median	*p* Value
PARAMETERS INSIDE CLASSIFICATIONS											
SMMSE corrected for age and education			27,3	3,2	28,2			28,1	2,4	28,7	0,282
Six-items ADL			5,6	0,6	6,0			6	0	6,0	**0,000**
Barthel ADL Index			94,4	8,5	100			100	0	100	**0,000**
Creatinine			1,1	0,2	1,0			1,0	0,2	1,0	0,296
Hemoglobin			13,6	1,3	13,5			13,7	1,2	13,4	0,481
GPT (ALT)			13,2	5,1	13			14,6	5,0	13,0	0,094
Reading newspaper without glasses	38	29,7				38	100				**0,000**
Recognize someone 4 metres away without glasses	98	77,2				38	100				**0,000**
Hearing ability without aids	98	76,6				38	100				**0,000**
Absence of ictus in the previous 6 months	128	100,0				38	100				n.a.
Absence of cancer	128	100,0				38	100				n.a.
Absence of severe renal failure	127	99,2				37	97,4				0,406
PRESENT PARAMETERS											
Age			92,3	2,1	92			92,1	2,0	91,5	0,521
Smoking											
Never Smoker	84	65,6				29	76,3				
Former Smoker	38	29,7				8	21,1				0,549
Smokers	6	4,7				1	2,6				
Use of alcohol every day	79	61,7				26	68,4				0,566
"How is your health in general?"											
Very good	24	18,8				11	28,9				
Good	77	60,2				21	55,3				0,400
Fair	21	16,4				6	15,8				
Poor/Very poor	6	4,7				0	0,0				
Attitude towards life											
Optimistic	79	61,7				25	65,8				
Neither optimistic nor pessimistic	32	25,0				12	31,6				0,145
Pessimistic	17	13,3				1	2,6				
Cohabitation											
Subjects living alone	57	44,9				11	29,7				0,129
Subjects living with others	70	55,1				26	70,3				
Comorbidity (number of current diseases)			2,1	1,4	2			2,3	1,0	2	0,362
500 metres walking ability without aids	128	100,0				38	100,0				n.a.
Going up and down the stairs without help	126	98,4				38	100,0				0,594
Ability to perform Hand Grip test	128	100,0				38	100,0				n.a.
Hand grip (Kg)			21,6	7,2	20			21,8	6,4	21,5	0,703
Ability to perform Chair Stand test	112	87,5				31	81,6				**0,015**
PAST PARAMETERS											
No Fall within the last year	101	78,9				34	89,5				0,163
No Hospitalization within the last year	114	89,1				34	89,5				1,000
No Loss of weight within the last year	114	89,8				35	92,1				1,000

Table 4.17 - Comparison between Franceschi=A and Gondo=Exceptional (n=38) versus Franceschi=A and Gondo ≠ Exceptional (n=128)

	Males											Females										
	Franceschi≠A and Gondo=Exceptional n=7					*Franceschi=A and Gondo=Exceptional* n=14						*Franceschi≠A and Gondo=Exceptional* n=4					*Franceschi=A and Gondo=Exceptional* n=24					
	N	%	mean	SD	median	N	%	mean	SD	median	*p* Value	N	%	mean	SD	median	N	%	mean	SD	median	*p* Value
PARAMETERS INSIDE CLASSIFICATIONS																						
SMMSE corrected for age and			26,6	3,2	26,8			27,4	2,9	27,3	0,601			25,4	2,1	24,9			28,5	2	29,2	**0,027**
Six-items ADL			6	0	6			6	0	6	n.a.			6	0	6			6	0	6	n.a.
Barthel ADL Index			100	0	100			100	0	100	n.a.			100	0	100			100	0	100	n.a.
Creatinine			2,0	1,1	1,7			1,2	0,2	1,1	0,242			0,9	0	0,9			0,9	0,2	0,9	0,781
Hemoglobin			12,1	1,6	11,8			14,3	1,1	14,4	**0,020**			13,5	0	13,5			13,3	1,1	13,3	0,627
GPT (ALT)			40,2	59,8	11			14,4	3,2	13	0,211			11	0	11			14,7	5,9	14	0,576
Reading newspaper without glasses	7	100				14	100				n.a.	4	100				24	100				n.a.
Recognize someone 4 metres away without glasses	7	100				14	100				n.a.	4	100				24	100				n.a.
Hearing ability without aids	7	100				14	100				n.a.	4	100				24	100				n.a.
Absence of ictus in the previous 6 months	7	100				14	100				n.a.	4	100				24	100				n.a.
Absence of cancer	5	71,4				14	100				0,100	3	75				24	100				0,143
Absence of severe renal failure	6	85,7				13	92,9				1,000	4	100				24	100				n.a.
PRESENT PARAMETERS																						
Age			93,4	2,3	93			92,4	2,2	91,5	0,285			92	2,2	91,5			91,9	1,9	91,5	0,920
Smoking																						
Never Smoker	3	42,9				7	50,0					3	75				22	91,7				
Former Smoker	4	57,1				7	50,0				1,000	1	25				1	4,2				0,382
Smokers	0	0,0				0	0,0					0	0				1	4,2				
Use of alcohol every day	6	85,7				12	85,7				1,000	3	75				14	58,3				1,000
"How is your health in general?"																						
Very good	3	42,9				3	21,4					1	25				8	33,3				
Good	2	28,6				10	71,4				0,176	3	75				11	45,8				0,800
Fair	2	28,6				1	7,1					0	0				5	20,8				
Poor/Very poor	0	0,0				0	0,0					0	0				0	0,0				
Attitude towards life																						
Optimistic	5	71,4				10	71,4					4	100				15	62,5				
Neither optimistic nor	1	14,3				4	28,6				0,527	0	0				8	33,3				0,388
Pessimistic	1	14,3				0	0,0					0	0				1	4,2				
Cohabitation																						
Subjects living alone	0	0,0				2	14,3				0,533	4	100				9	39,1				**0,041**
Subjects living with others	7	100,0				12	85,7					0	0				14	60,9				
Comorbidity (number of current diseases)			1,7	1,4	2			2,5	1,2	3	0,216			2,5	1,3	2,5			2,1	0,9	2	0,537
500 metres walking ability without aids	7	100,0				14	100,0				n.a.	4	100				24	100,0				n.a.
Going up and down the stairs without help	7	100,0				14	100,0				n.a.	4	100				24	100,0				n.a.
Ability to perform Hand Grip test	7	100,0				14	100,0				n.a.	4	100				24	100,0				n.a.
Hand grip (Kg)			28,3	4,2	28			26,6	6,3	27,5	0,431			19	1,8	19			19	4,5	20	0,716
Ability to perform Chair Stand test	6	85,7				10	71,4					4	100				21	87,5				1,000
PAST PARAMETERS																						
No Fall within the last year	6	85,7				14	100,0				0,333	3	75				20	83,3				1,000
No Hospitalization within the last year	5	71,4				13	92,9				0,247	4	100				21	87,5				1,000
No Loss of weight within the last year	6	85,7				13	92,9				1,000	3	75				22	91,7				0,382

	Total										
	Franceschi≠A and Gondo=Exceptional n=11					Franceschi=A and Gondo=Exceptional n=38					
	N	%	mean	SD	median	N	%	mean	SD	median	*p* Value
PARAMETERS INSIDE CLASSIFICATIONS											
SMMSE corrected for age and education			26,2	2,8	25,4			28,1	2,4	28,7	**0,053**
Six-items ADL			6	0	6			6	0	6	n.a.
Barthel ADL Index			100	0	100			100	0	100	n.a.
Creatinine			1,8	1,1	1,3			1,0	0,2	1,0	0,088
Hemoglobin			12,4	1,5	12,3			13,7	1,2	13,4	**0,057**
GPT (ALT)			34,4	53,4	11			14,6	5,0	13	0,269
Reading newspaper without glasses	11	100				38	100				n.a.
Recognize someone 4 metres away without glasses	11	100				38	100				n.a.
Hearing ability without aids	11	100				38	100				n.a.
Absence of ictus in the previous 6 months	11	100				38	100				n.a.
Absence of cancer	8	72,7				38	100				**0,009**
Absence of severe renal failure	10	90,9				37	97,4				0,402
PRESENT PARAMETERS											
Age			92,9	2,2	92			92,1	2,0	91,5	0,247
Smoking											
Never Smoker	6	54,5				29	76,3				
Former Smoker	5	45,5				8	21,1				0,333
Smokers	0	0,0				1	2,6				
Use of alcohol every day	9	81,8				26	68,4				0,475
"How is your health in general?"											
Very good	4	36,4				11	28,9				
Good	5	45,5				21	55,3				0,899
Fair	2	18,2				6	15,8				
Poor/Very poor	0	0,0				0	0,0				
Attitude towards life											
Optimistic	9	81,8				25	65,8				
Neither optimistic nor pessimistic	1	9,1				12	31,6				0,163
Pessimistic	1	9,1				1	2,6				
Cohabitation											
Subjects living alone	4	36,4				11	29,7				0,720
Subjects living with others	7	63,6				26	70,3				
Comorbidity (number of current diseases)			2	1,3	2			2,3	1,0	2	0,629
500 metres walking ability without aids	11	100,0				38	100,0				n.a.
Going up and down the stairs without help	11	100,0				38	100,0				n.a.
Ability to perform Hand Grip test	11	100,0				38	100,0				n.a.
Hand grip (Kg)			24,9	5,8	27,5			21,8	6,4	21,5	0,190
Ability to perform Chair Stand test	10	90,9				31	81,6				0,416
PAST PARAMETERS											
No Fall within the last year	9	81,8				34	89,5				0,605
No Hospitalization within the last year	9	81,8				34	89,5				0,605
No Loss of weight within the last year	9	81,8				35	92,1				0,311

Table 4.18 - Comparison between Franceschi=A and Gondo=Exceptional (n=38) versus Franceschi ≠ A and Gondo=Exceptional (n=11)

4.6.3 Model N.1 for the identification of "The Best 1" group of 90+ siblings *(Franceschi category "A" or Gondo "Exceptional")*

The previous analysis revealed that 90+ subjects classified as "A" by Franceschi and subjects classified as "Exceptional" by Gondo do not differ for all the variables of the GEHA questionnaire that were not included in the classification criteria. Therefore, this result drove us to a first definition of **the best group of 90+ siblings, i.e. subjects classified as "A" by Franceschi plus subjects classified as "Exceptional" by Gondo for a total of 177 individuals**. Now, the question is: which characteristics should be respected in order to be part of this group of best subjects? Using techniques of Data Mining we were able to reclassify our study population using a smaller and meaningful set of variables; a synthetic criterion able to include almost all these 177 subjects is based on the following conditions: **SMMSE ≥ 20, Ability to walk for 500 meters without aids and Haemoglobin ≥ 10 g/dl** (**Table 4.19**).

	SMMSE ≥ 20, walking ability for 500 m, Hgb ≥ 10 g/dl		
	No	**Yes**	Total
The Best 1	5	**172**	177
The Others 1	559	29	588
Total	564	201	765

Table 4.19 – Model N.1: synthetic criteria for the identification of "The Best 1" subjects

According to this new classification, hereafter identified as "Model N.1", 90+ siblings were divided in two groups: the "best subjects", hereafter identified as "The Best 1" (n = 177, 23% of the sample), and the rest of the sample, included "proxy subjects", hereafter identified as "The Others 1" (n = 588, 77% of the sample) (**Table 4.20**). Considering the gender composition, it results that males are classified as healthier than females (30.6% of males are included in "The Best 1" category versus 20.1% of females). Moreover, OR value shows that being females reduces the probability of being classified as "The Best 1".

	The Best 1 **(Franceschi=A or Gondo=Exceptional) n=177**		***The Others 1*** **n=588**			
	N	**%**	**N**	**%**	***p* Value**	**OR (95% CI)**
Males	68	30,6	154	69,4	0,001	1
Females	109	20,1	434	79,9		0,57 (0,40-0,81)

Table 4.20 – Model N.1: "The Best 1" versus "The Others 1"

To confirm the validity of the "Model N.1", we performed an univariate analysis between "The Best 1" subjects and "The Others 1":

(1) we assessed age, marital status, cohabitation, education, smoking status, alcohol intake, as examples to explore the influences of the social and environment factors on the health status and we compared these values among the groups;

(2) we also compared self-reported health, attitude towards life and comorbidity, as further parameters of health status (since Franceschi classification included only four age-related diseases, cancer, ictus, renal failure and liver disease);

(3) we also compared hand grip, chair stand test, 500 metres walking ability and going up and down the stairs without help as measured functional parameters;

(4) finally we compared the absence of falls, hospitalisation and loss of weight within the last year together with the vital status at January 1st, 2009 as main external criteria.

OR were calculated to evaluate the association between single variables and the health status. The results, reported in **Table 4.21-4.22-4.23**, are divided by gender and the unadjusted OR were corrected for family cluster because the population is composed of 90+ siblings and not of 90+ singletons.

On the whole, data indicate that the "The Best 1" subjects are actually different from "The Others 1" for all the variables considered in the analysis, even if the overall picture is different between males and females. In particular, males are in better shape than females and their phenotype is less complex than females because a restricted number of factors is associated with health status. On the contrary, females present a more heterogeneous phenotype, where much more factors contribute to the definition of the health status. Results indicate that:

(1) parameters related to the social and environmental field do not explain the health status both in males and in females, even if the fact of being married is protective for males but not for females; however, they became significant in the total population;

(2) self-reported health, attitude towards life and comorbidity are associated with the health status both in males and in females, even if in females they play a stronger role;

(3) parameters related to the physical performance (hand grip and chair stand) and functional limitations (the Nagi items) are strongly associated with the health status both in males and in females, with higher scores in females;

(4) finally the absence of falls, hospitalisation and loss of weight within the last are all positively associated with a good health status both in males and in females and, interestingly, the vital status is protective only for females, suggesting that for males the fact of being classified in "The Best 1" category is less protective than for females.

	Males									
	"The Best 1" (Franceschi=A or Gondo=Exceptional) n=68				*"The Others 1"* n=154					
	N	%	mean	SD	N	%	mean	SD	*p* Value	OR (95% CI)
Age			92,4	2,1			93,8	2,9	**0,000**	0,80 (0,70-0,91)
Centre										
UNIBO	46	67,6			118	76,6			0,161	1
ISS	22	32,4			36	23,4				1,56 (0,82-2,99)
Marital status										
Never Married	5	7,4			13	8,4				1
Married	32	47,1			53	34,4			0,201	1,57 (0,53-4,64)
Widow/Widowerer , Divorced, Separated	31	45,6			88	57,1				0,91 (0,30-2,77)
Cohabitation										
Subjects living alone	13	19,1			23	14,9			0,436	1
Subjects living with others	55	80,9			131	85,1				0,74 (0,34-1,60)
Education										
Years at school			7,6	5,4			6,8	4,9	0,306	1,0 (0,97-1,09)
Smoking										
Never Smoker	24	35,3			64	41,8				1
Former Smoker	41	60,3			85	55,6			0,557	2,0 (0,42-79,59)
Smokers	3	4,4			4	2,6				1,28 (0,71-2,31)
Use of alcohol every day	53	77,9			104	68,0			0,132	1,66 (0,84-3,3)
"How is your health in general?"										
Very good/Good	55	80,9			88	57,1				1
Fair/Poor/Very poor	13	19,1			49	31,8			**0,001**	0,42 (0,20-0,87)
Proxy and Missing	0	0,0			17	11,0				not assessable
Attitude towards life										
Optimistic	46	67,6			77	50,0				1
Neither optimistic nor pessimistic/Pessimistic	22	32,4			59	38,3			**0,004**	0,62 (0,33-1,18)
Proxy and Missing	0	0,0			18	11,7				not assessable
Number of diseases										
0-2	40	58,8			63	40,9			**0,014**	1
≥ 3	28	41,2			91	59,1				0,48 (0,26-0,90)
BMI (Body Mass Index)										
≤ 21	7	10,6			16	12,2				1
22-27	37	56,1			71	54,2			0,939	1,19 (0,45-3,14)
≥ 28	22	33,3			44	33,6				1,14 (0,41-3,17)
500 metres walking ability without aids	68	100,0			39	25,3			**0,000**	not assessable
Going up and down the stairs without help	67	98,5			102	66,2			**0,000**	34,1 (4,55-256,6)
Hand Grip (Kg) *										
First quartile	8	11,8			44	28,6				1
Second quartile	9	13,2			41	26,6				1,20 (0,43-3,35)
Third quartile	21	30,9			31	20,1			**0,000**	3,72 (1,52-9,10)
Fourth quartile	30	44,1			26	16,9				6,3 (2,5-15,9)
Could not complete	0	0,0			12	7,8				not assessable
Ability to perform Chair Stand test	57	83,8			57	37,0			**0,000**	8,8 (4,26-18,24)
No Fall within the last year	59	86,8			107	69,5			**0,006**	2,88 (1,31-6,31)
No Hospitalization within the last year	60	88,2			114	74,0			**0,018**	2,63 (1,16-5,97)
No Loss of weight within the last year	62	91,2			126	81,8			0,074	2,30 (0,89-5,95)
Vital Status										
Not alive	16	23,5			54	35,1			0,088	1
Alive	52	76,5			100	64,9				1,75 (0,91-3,38)

Table 4.21 – Model N.1: Univariate Analysis on *males*

(OR are adjusted for family cluster)

	Males
Hand Grip (Kg)	
First quartile	0-19
Second quartile	20-24
Third quartile	25-29
Fourth quartile	≥ 29

	Females									
	"The Best 1" (Franceschi=A or Gondo=Exceptional) n=109				*"The Others 1"* n=434					
	N	%	mean	SD	N	%	mean	SD	*p* Value	OR (95% CI)
Age			92,2	2,1			94,4	3,0	**0,000**	0,71 (0,64-0,80)
Centre										
UNIBO	70	64,2			315	72,6			0,086	1
ISS	39	35,8			119	27,4				1,47 (0,91-2,38)
Marital status										
Never Married	17	15,6			60	13,8				1
Married	1	0,9			8	1,8			0,722	0,44 (0,05-3,7)
Widow/Widowerer , Divorced, Separated	91	83,5			366	84,3				0,88 (0,48-1,60)
Cohabitation										
Subjects living alone	59	54,1			73	16,8			**0,000**	1
Subjects living with others	50	45,9			361	83,2				0,17 (0,11-0,27)
Education										
Years at school			6,1	3,5			5,1	3,3	**0,003**	1,1 (1,02-1,16)
Smoking										
Never Smoker	95	87,2			389	89,6				1
Former Smoker	10	9,2			37	8,5			0,492	2,1 (0,57-7,3)
Smokers	4	3,7			8	1,8				1,1 (0,55-2,2)
Use of alcohol every day	61	56,0			209	48,2			0,145	1,37 (0,89-2,1)
"How is your health in general?"										
Very good/Good	87	79,8			205	47,2				1
Fair/Poor/Very poor	22	20,2			176	40,6			**0,000**	0,29 (0,17-0,49)
Proxy and Missing	0	0,0			53	12,2				not assessable
Attitude towards life										
Optimistic	67	61,5			183	42,2				1
Neither optimistic nor pessimistic/Pessimistic	42	38,5			198	45,6			**0,000**	0,58 (0,38-0,88)
Proxy and Missing	0	0,0			53	12,2				not assessable
Number of diseases										
0-2	72	66,1			117	27,0			**0,000**	1
≥ 3	37	33,9			317	73,0				0,20 (0,12-0,29)
BMI (Body Mass Index)										
≤ 21	19	20,0			81	24,5				1
22-27	58	61,1			160	48,5			0,091	1,54 (0,86-2,8)
≥ 28	18	18,9			89	27,0				0,86 (0,41-1,79)
500 metres walking ability without aids	109	100,0			68	15,7			**0,000**	not assessable
Going up and down the stairs without help	108	99,1			205	47,2			**0,000**	120,64 (16,7-867,6)
Hand Grip (Kg) *										
First quartile	3	2,8			93	21,4				1
Second quartile	16	14,7			119	27,4				4,2 (1,2-14,7)
Third quartile	36	33,0			91	21,0			**0,000**	12,3 (3,7-40,8)
Fourth quartile	54	49,5			69	15,9				24,3 (7,3-80,6)
Could not complete	0	0,0			62	14,3				not assessable
Ability to perform Chair Stand test	96	88,1			120	27,6			**0,000**	19,3 (10,6-35,2)
No Fall within the last year	85	78,0			253	58,3			**0,000**	2,53 (1,56-4,1)
No Hospitalization within the last year	97	89,0			320	73,7			**0,001**	2,88 (1,54-5,4)
No Loss of weight within the last year	97	89,0			333	76,7			**0,005**	2,45 (1,30-4,61)
Vital Status										
Not alive	14	12,8			172	39,6			**0,000**	1
Alive	95	87,2			262	60,4				4,45 (2,46-8,07)

	Females
Hand Grip (Kg)	
First quartile	0-9
Second quartile	10-14
Third quartile	15-18
Fourth quartile	≥ 19

Table 4.22 – Model N.1: Univariate Analysis on f*emales*
(OR are adjusted for family cluster)

	Total									
	"The Best 1" (Franceschi=A or Gondo=Exceptional) n=177				*"The Others 1"* n=588					
	N	**%**	**mean**	**SD**	**N**	**%**	**mean**	**SD**	***p* Value**	**OR (95% CI)**
Age			92,3	2,1			94,2	3	**0,000**	0,74 (0,68-0,80)
Centre										
UNIBO	116	65,5			433	73,6			**0,036**	1
ISS	61	34,5			155	26,4				1,47 (0,99-2,18)
Marital status										
Never Married	22	12,4			73	12,4				1
Married	33	18,6			61	10,4			**0,012**	1,79 (0,96-3,34)
Widow/Widowerer , Divorced, Separated	122	68,9			454	77,2				0,89 (0,52-1,52)
Cohabitation										
Subjects living alone	72	40,7			96	16,3			**0,000**	1
Subjects living with others	105	59,3			492	83,7				0,28 (0,19-0,41)
Education										
Years at school			6,7	4,4			5,5	3,9	**0,001**	1,06 (1,02-1,11)
Smoking										
Never Smoker	119	67,2			453	77,0				1
Former Smoker	51	28,8			122	20,7			**0,022**	2,2 (0,83-5,92)
Smokers	7	4,0			12	2,0				1,59 (1,09-2,33)
Use of alcohol every day	114	64,4			313	53,3			**0,009**	1,58 (1,12-2,25)
"How is your health in general?"										
Very good/Good	142	80,2			293	49,8				1
Fair/Poor/Very poor	35	19,8			225	38,3			**0,000**	0,32 (0,21-0,49)
Proxy and Missing	0	0,0			70	11,9				not assessable
Attitude towards life										
Optimistic	113	63,8			260	44,2				1
Neither optimistic nor pessimistic/Pessimistic	64	36,2			257	43,7			**0,000**	0,57 (0,40-0,82)
Proxy and Missing	0	0,0			71	12,1				not assessable
Number of diseases										
0-2	112	63,3			180	30,6			**0,000**	1
≥ 3	65	36,7			408	69,4				0,26 (0,18-0,37)
BMI (Body Mass Index)										
≤ 21	26	14,7			97	16,5				1
22-27	95	53,7			231	39,3			0,140	1,53 (0,93-2,53)
≥ 28	40	22,6			133	22,6				1,12 (0,63-1,99)
500 metres walking ability without aids	177	100,0			107	18,2			**0,000**	not assessable
Going up and down the stairs without help	175	98,9			307	52,2			**0,000**	80,1 (19,7-325,4)
Hand Grip (Kg) *										
First quartile	11	6,2			137	23,3				1
Second quartile	25	14,1			160	27,2				1,94 (0,93-4,08)
Third quartile	57	32,2			122	20,7			**0,000**	5,81 (2,94-11,50)
Fourth quartile	84	47,5			95	16,2				11,01 (5,61-21,62)
Could not complete	0	0,0			74	12,6				not assessable
Ability to perform Chair Stand test	153	86,4			177	30,1			**0,000**	14,8 (9,25-23,70)
No Fall within the last year	144	81,4			360	61,2			**0,000**	2,76 (1,84-4,15)
No Hospitalization within the last year	157	88,7			434	73,8			**0,000**	2,78 (1,71-4,54)
No Loss of weight within the last year	159	89,8			459	78,1			**0,000**	2,48 (1,48-4,16)
Vital Status										
Not alive	30	16,9			226	38,4			**0,000**	1
Alive	147	83,1			362	61,6				3,06 (1,96-4,76)

Table 4.23 – Model N. 1: Univariate Analysis on all 90+ subjects (OR are adjusted for family cluster)

4.6.4 Model N.1: parameters associated with the health status

On the basis of the "Model N.1" we proposed, we evaluated the possible associations between a series of parameters (gender, age, education, self-reported health, attitude towards life, number of diseases, going up and down the stairs without anyone's help, handgrip, chair stand test, absence of fall within the last year, absence of hospitalisation within the last year and absence of weight loss within the last year) and the health status of 90+ siblings. The analysis was performed in males and females separately and the OR results were adjusted for family cluster (**Table 4.24**).

When the logistic regression model is applied to *males*, the ability of going up and down the stairs without anyone's help (p = 0,031), hand grip (p = 0.047) and chair stand (p = 0.026) show a correlation with the health status. When the multivariate analysis model is applied to *females*, the ability of going up and down the stairs without anyone's help (p = 0,004), hand grip (p = 0.011) and chair stand (p = 0,000) continue to be correlated with the health status, even if more strongly than in males, and new variables such as age (p = 0.001) and comorbidity (p = 0.000) show a correlation with the health status. When the model is applied to the *total population*, additionally to the previous parameters also the absence of falls (p = 0.051) and hospitalisation (p = 0.024) within the last year show a strong correlation with the health status, probably because they are related to comorbidity, which influences the health status only in females.
In summary, **age**, **comorbidity**, **the ability of going up and down the stairs without anyone's help, hand grip**, **chair stand**, **absence of falls and hospitalisation within the last year** show a strong correlation with the health status.

Characteristic	*Males* n=203		*Females* n=487		*Total* n=690	
	OR (95% CI)	***p* Value**	**OR (95% CI)**	***p* Value**	**OR (95% CI)**	***p* Value**
Gender					0,81 (0,50-1,32)	0,400
Age	0,89 (0,77-1,05)	0,181	0,77 (0,66-0,90)	**0,001**	0,83 (0,74-0,92)	**0,000**
Education	1,05 (0,98-1,13)	0,179	1,04 (0,96-1,13)	0,356	1,05 (1,0-1,11)	0,064
Self-reported Health (*Fair/Poor/Very poor*)	1,08 (0,42-2,76)	0,870	0,89 (0,47-1,70)	0,733	0,92 (0,54-1,55)	0,749
Attitude towards life (*Neither optimistic nor pessimistic/Pessimistic*)	0,86 (0,39-1,91)	0,717	0,29 (0,16-0,51)	0,668	0,86 (0,54-1,36)	0,516
Number of diseases ≥ 3	0,76 (0,35-1,68)	0,498	0,29 (0,16-0,51)	**0,000**	0,42 (0,27-0,67)	**0,000**
Going up and down the stairs without help	9,61 (1,22-75,5)	**0,031**	19,1 (2,50-145,7)	**0,004**	15,0 (3,55-63,41)	**0,000**
Hand Grip (Kg)						
Third quartile	1,95 (0,70-5,46)	0,205	2,91 (0,95-8,89)	0,061	2,31 (1,09-4,91)	**0,029**
Fourth quartile	2,91 (1,01-8,38)	**0,047**	4,22 (1,40-12,72)	**0,011**	3,40 (1,62-7,13)	**0,001**
Ability to perform Chair Stand test	2,79 (1,13-6,88)	**0,026**	4,37 (2,19-8,72)	**0,000**	3,60 (2,07-6,26)	**0,000**
No Fall within the last year	1,91 (0,80-4,52)	0,144	1,60 (0,87-2,94)	0,126	1,62 (1,0-2,62)	**0,051**
No Hospitalization within the last year	2,65 (0,92-7,64)	0,070	1,76 (0,79-3,93)	0,169	2,01 (1,10-3,70)	**0,024**
No Loss of weight within the last year	1,40 (0,41-4,76)	0,594	1,07 (0,47-2,45)	0,871	1,14 (0,59-2,19)	0,703

Table 4.24 – Model N.1: multivariate analysis model on the health status of 90+ siblings
(OR are adjusted for family cluster)

4.6.5 Model N.1: family history and health status of GEHA 90+ siblings at the recruitment time

This analysis aimed at finding a possible **relationship between the family history of GEHA 90+ siblings and their health status at the recruitment time**. It was performed on the 354 families with at least 2 nonagenarian siblings (in the families with more than 2 siblings, the proband was compared only with the second sibling according to the birth order) and results are reported in **Table 4.25**. Firstly, we identified the families where the proband and the second sibling shared the health status category, as defined by the "Model N.1" we proposed, and the families where they were discordant for the health category. We found that the proband and the second sibling shared the health status category "The Best 1" in 26 families (7.3%, hereafter identified as ***"Concordant Good Families"*** and representing "The Best 1" families of the study population), they shared the health status category "The Others 1" in 224 families (63.3%, hereafter identified as ***"Concordant Bad Families"***) and they did not share the health status category in 104 families (29.4%, hereafter identified as ***"Discordant Families"***). In summary, the siblings shared the health status in about 70% of the families and they were discordant in about 30% of the families. No difference in **gender composition** was found in the three groups of families, even if in "Concordant Good Families" we found an higher percentage of MM sibpairs (23.1%) in comparison with "Discordant families" (14.4%) and "Concordant Bad Families" (7.6%), indicating that nonagenarian males are healthier than females. On the contrary, a significant difference is present in the **age** of 90+siblings: it progressively increases passing from "Concordant Good Families" (92.4 yrs) to "Discordant Families" (93.2 yrs) and finally to "Concordant Bad Families" (94.4yrs), and concomitantly also the delta age between the proband and the second sibling increases in the three family groups, as expected. Interestingly, we discovered that in "Concordant Good Families" the **parents age of death** is higher in comparison to the other family groups: indeed, the mean value of the father age of death reaches 77.2 years and for the mother 80.4 years. Moreover, we checked if the **dimension of the total sibship** influenced the health status of the recruited 90+ siblings and, reassuringly, we found that the mean number of siblings was about 5-6 in all the three family groups, indicating that the health status of 90+ subjects, as defined by Model N.1, is not biased by the initial sibship of the family to which the belong to. **Useful definitions:**

Concordant Good Families = both siblings are in "The Best 1" category
Concordant Bad Families = only one sibling is in "The Best 1" category
} *Concordant Families*

Discordant Families = both siblings are in "The Others 1" category

	Concordant Good Families (11)				*Discordant Families (10)*				*Concordant Bad Families (00)*						
	N	**%**	**mean**	**SD**	**N**	**%**	**mean**	**SD**	**N**	**%**	**mean**	**SD**	***p* Value**	***p* Value (11 vs 00)**	***p* Value (11 vs 10)**	***p* Value (10 vs 00)**
Families with at least 2 nonagenarian siblings (n=354)	26	7,3			104	29,4			224	63,3						
Siblings Gender Composition																
MM	6	23,1			15	14,4			17	7,6						
MF	3	11,5			19	18,3			46	20,5						
FM	6	23,1			21	20,2			33	14,7			0,063			
FF	11	42,3			49	47,1			128	57,1						
Age																
Siblings Age			92,4	1,5			93,2	1,7			94,4	2,3		0,000	0,039	0,000
Siblings Delta Age			2,1	1			3,2	1,8			3,5	2,2		0,002	0,004	0,258
Parents Age of Death																
Father			77,2	14,6			73,7	16,6			73,1	15,9				
Mother			80,4	18,5			76,4	18,0			78,1	16,1				

	Concordant Good Families (11)				*Discordant Families (10)*				*Concordant Bad Families (00)*						
	N	**%**	**mean**	**SD**	**N**	**%**	**mean**	**SD**	**N**	**%**	**mean**	**SD**	***p* Value (0 vs 11)**	***p* Value (10 vs 11)**	***p* Value (0 vs 10)**
Total Sibship (included 90+ interviewed subjects)			6,1	2,8			5,5	2,3			5,8	2,6	0,643	0,244	0,220
Alive siblings/Total Sibship (at recruitment time)			0,6	0,2			0,6	0,2			0,5	0,2	0,269	0,695	0,008

Table 4.25 – Model N.1: influence of the family history on the health status of 90+ siblings as defined by the "Model N.1"

4.6.6 Model N.2 for the identification of "The Best 2" group of 90+ siblings *(not disabled and cognitively intact, i.e. independent)*

The model N.1 we proposed for the identification of "The Best 1" group of subjects was based on three parameters: one about cognitive status, one about physical ability and one haematological parameters (haemoglobin). On the one hand this model has the advantage to come out from an empirical analysis on phenotypic data and not from *a priori* assumption and it is also able to select the best group of subject from the whole population. However, it should be noted that only Italian recruiting units collected haematological and biochemical parameters on GEHA 90+ siblings, because the clinical check-up was not a compulsory activity of the GEHA project. For this reason, it would have not been possible to apply the model N.1 we proposed to the other GEHA dataset collected by European units and it would have been difficult to compare our results with other studies because the proportion of blood samples varies very much between studies. Therefore, to overcame this limit we suggested a model N. 2 for the identification of "The Best 2" group of subjects, based on **five-item ADL scale and SMMSE**, which represent the most valid functional items that most aging research collect and can be used in the comparisons with results from a lot of studies. "The Best 2" category is thereby defined as **"non-disabled and cognitively intact", i.e. "independent" (SMMSE ≥ 24 and ADL = 5)**.
According to this classification, hereafter identified as "Model N.2", 90+ siblings were divided in two groups: the best subjects, hereafter identified as "The Best 2" (n = 286, 37% of the sample), and the rest of the sample, included "proxy subjects", hereafter identified as "The Others 2" (n = 479, 63% of the sample) (**Table 4.26**). Considering the gender composition, it results that males are classified as healthier than females (45% of males are included in "The Best 2" category versus 34.3% of females).

	The Best 2 *(SMMSE ≥ 24 and ADL = 5)* **n=286**		*The Others 2* **n=479**		
	N	**%**	**N**	**%**	***p* Value**
Males	100	45,0	122	55,0	0,001
Females	186	34,3	357	65,7	

Table 4.26 – Model N.2: "The Best 2" versus "The Others 2"

To confirm the validity of the "Model N.2", we performed an univariate analysis between "The Best 2" subjects and "The Others 2":

(1) we assessed age, marital status, cohabitation, education, smoking status, alcohol intake, as examples to explore the influences of the social and environment factors on the health status and we compared these values among the groups;

(2) we also compared self-reported health, attitude towards life and comorbidity, as further parameters of health status;

(3) we also compared hand grip, chair stand test, 500 metres walking ability and going up and down the stairs without help as measured functional parameters;

(4) finally we compared the absence of falls, hospitalisation and loss of weight within the last year together with the vital status at January 1st, 2009 as main external criteria.

OR were calculated to evaluate the association between single variables and the health status. The results, reported in **Table 4.27-4.28-4.29**, are divided by gender and the unadjusted OR were corrected for family cluster because the population is composed of 90+ siblings and not of 90+ singletons.

On the whole, when the total population is considered, data indicate that the "The Best 2" subjects are actually different from "The Others 2" for all the variables considered in the analysis, but some differences are present between males and females. In particular, results indicate that:

(1) as regards parameters related to the social and environmental field, the marital status (the fact of being married) and education are associated with health in males, while education and the smoking status are protective factors in females;

(2) self-reported health and attitude towards life are associated with the health status both in males and in females, while comorbidity is associated with the health status only in females;

(3) parameters related to the physical performance (hand grip and chair stand) and functional limitations (the Nagi items) are strongly associated with the health status both in males and in females, with higher scores in females;

(4) finally the absence of falls and loss of weight within the last are positively associated with a good health status only in males, while the absence of hospitalisation within the last year was associated to health status only in females; interestingly, the vital status is associated with the health status both in males and in females, with a much higher OR in females than in males, indicating that the health status is more associated with mortality in females than in males.

	Males									
	The Best 2 (SMMSE ≥ 24 and ADL = 5) n=100				*The Others 2 (SMMSE < 24 or ADL < 5)* n=122					
	N	**%**	**mean**	**SD**	**N**	**%**	**mean**	**SD**	***p* Value**	**OR (95% CI)**
Age			92	2,2			94,2	2,9	**0,000**	0,77 (0,67-0,87)
Centre										
UNIBO	66	66,0			98	80,3			**0,000**	1
ISS	34	34,0			24	19,7				2,10 (1,15-3,84)
Marital status										
Never Married	6	6,0			12	9,8				1
Married	48	48,0			37	30,3			**0,024**	2,60 (0,89-7,59)
Widow/Widowerer , Divorced, Separated	46	46,0			73	59,8				1,26 (0,44-3,63)
Cohabitation										
Subjects living alone	22	22,0			14	11,5			**0,034**	1
Subjects living with others	78	78,0			108	88,5				0,46 (0,23-0,94)
Education										
Years at school			8	5,2			6,3	4,8	**0,011**	1,07 (1,01-1,13)
Smoking										
Never Smoker	37	37,0			51	41,8				1
Former Smoker	3	3,0			4	3,3			0,664	1,03 (0,21-4,99)
Smokers	60	60,0			66	54,1				1,25 (0,73-2,16)
Use of alcohol every day	72	72,0			85	69,7			0,619	1,16 (0,64-2,11)
"How is your health in general?"										
Very good/Good	76				67	54,9				1
Fair/Poor/Very poor	24				38	31,1			**0,000**	0,56 (0,30-1,03)
Proxy and Missing	0				17	13,9				not assessable
Attitude towards life										
Optimistic	68	68,0			55	45,1				1
Neither optimistic nor pessimistic/Pessimistic	32	32,0			49	40,2			**0,000**	0,53 (0,30-0,94)
Proxy and Missing	0	0,0			18	14,8				not assessable
Number of diseases										
0-2	53	53,0			50	41,0			0,074	1
≥ 3	47	47,0			72	59,0				0,62 (0,35-1,07)
BMI (Body Mass Index)										
≤ 21	13	13,0			10	8,2				1
22-27	49	49,0			59	48,4			0,469	0,64 (0,26-1,57)
≥ 28	35	35,0			31	25,4				0,86 (0,35-2,18)
500 metres walking ability without aids	68	68,0			39	32,0			**0,000**	4,92 (2,74-8,80)
Going up and down the stairs without help	93	93,0			76	62,3			**0,000**	8,04 (3,44-18,79)
Hand Grip (Kg) *										
First quartile	8	8,0			44	36,1				1
Second quartile	21	21,0			29	23,8				3,99 (1,57-10,09)
Third quartile	30	30,0			22	18,0			**0,000**	7,50 (2,96-19,01)
Fourth quartile	41	41,0			15	12,3				15,03 (5,74-39,39)
Could not complete	0	0,0			12	9,8				not assessable
Ability to perform Chair Stand test	77	77,0			37	30,3			**0,000**	7,69 (4,25-13,90)
No Fall within the last year	83	83,0			83	68,0			**0,011**	2,29 (1,19-4,39)
No Hospitalization within the last year	78	78,0			96	78,7			0,901	0,96 (0,51-1,81)
No Loss of weight within the last year	91	91,0			97	79,5			**0,018**	2,61 (1,15-5,91)
Vital Status										
Not alive	24	24,0			76	62,3			**0,029**	1
Alive	76	76,0			76	62,3				1,91 (1,09-3,38)

Table 4.27 – Model N.2: Univariate Analysis on *males* (OR are adjusted for family cluster)

	Females									
	The Best 2 (SMMSE ≥ 24 and ADL = 5) n=186				*The Others 2 (SMMSE < 24 or ADL < 5)* n=357					
	N	**%**	**mean**	**SD**	**N**	**%**	**mean**	**SD**	***p* Value**	**OR (95% CI)**
Age			92,6	2,1			94,5	3,1	**0,000**	0,74 (0,69-0,80)
Centre										
UNIBO	114	61,3			271	75,9			**0,000**	1
ISS	72	38,7			86	24,1				1,99 (1,30-3,04)
Marital status										
Never Married	28	15,1			49	13,7				1
Married	1	0,5			8	2,2			0,317	0,22 (0,03-1,84)
Widow/Widowerer , Divorced, Separated	157	84,4			300	84,0				0,92 (0,55-1,53)
Cohabitation										
Subjects living alone	89	47,8			43				**0,000**	1
Subjects living with others	97	52,2			314					0,15 (0,09-0,23)
Education										
Years at school			6,1	3,5			4,9	3,3	**0,001**	1,1 (1,04-1,17)
Smoking										
Never Smoker	115	61,8			329	92,2				1
Former Smoker	8	4,3			4	1,1			**0,004**	4,25 (1,05-17,2)
Smokers	23	12,4			24	6,7				2,03 (1,14-3,64)
Use of alcohol every day	103				167	46,8			0,057	1,41 (0,99-2,02)
"How is your health in general?"										
Very good/Good	129	69,4			163	45,7				1
Fair/Poor/Very poor	57	30,6			141	39,5			**0,000**	0,51 (0,34-0,77)
Proxy and Missing	0	0,0			53	14,8				not assessable
Attitude towards life										
Optimistic	109	58,6			141	39,5				1
Neither optimistic nor pessimistic/Pessimistic	76	40,9			164	45,9			**0,000**	0,60 (0,41-0,87)
Proxy and Missing	1	0,5			52	14,6				0,02 (0,00-0,18)
Number of diseases										
0-2	92	49,5			97	27,2			**0,000**	1
≥ 3	94	50,5			260	72,8				0,38 (0,26-0,55)
BMI (Body Mass Index)										
≤ 21	33	17,7			67	18,8				1
22-27	95	51,1			123	34,5			0,175	1,56 (0,94-2,61)
≥ 28	40	21,5			67	18,8				1,21 (0,66-2,19)
500 metres walking ability without aids	114	61,3			63	17,6			**0,000**	7,39 (4,94-11,04)
Going up and down the stairs without help	166	89,2			147	41,2			**0,000**	11,86 (6,94-20,26)
Hand Grip (Kg) *										
First quartile	9	4,8			87	24,4				1
Second quartile	30	16,1			105	29,4				2,76 (1,17-6,50)
Third quartile	61	32,8			66	18,5			**0,000**	8,93 (3,99-20,0)
Fourth quartile	85	45,7			38	10,6				21,6 (9,17-50,9)
Could not complete	1	0,5			61	17,1				0,15 (0,02-1,31)
Ability to perform Chair Stand test	135	72,6			81	22,7			**0,000**	9,02 (5,98-13,6)
No Fall within the last year	126	67,7			212	59,4			0,057	1,43 (0,99-2,08)
No Hospitalization within the last year	159	85,5			258	72,3			**0,001**	2,26 (1,41-3,63)
No Loss of weight within the last year	156	83,9			274	76,8			0,052	1,57 (1,02-2,44)
Vital Status										
Not alive	37	19,9			149	41,7			**0,000**	1
Alive	149	80,1			208	58,3				2,88 (1,93-4,31)

Table 4.28 – Model N. 2: Univariate Analysis on f*emales* (OR are adjusted for family cluster)

	Total									
	The Best 2 (SMMSE ≥ 24 and ADL = 5) n=286				*The Others 2 (SMMSE < 24 or ADL < 5)* n=479					
	N	**%**	**mean**	**SD**	**N**	**%**	**mean**	**SD**	***p* Value**	**OR (95% CI)**
Age			92,5	2,1			94,5	3,1	**0,000**	0,75 (0,70-0,79)
Centre										
UNIBO	180	62,9			369	77,0			**0,000**	1
ISS	106	37,1			110	23,0				1,96 (1,34-2,83)
Marital status										
Never Married	37				61					1
Married	49				45				**0,007**	1,95 (1,09-3,50)
Widow/Widowerer , Divorced, Separated	203				373					0,98 (0,60-1,59)
Cohabitation										
Subjects living alone	111				57				**0,000**	1
Subjects living with others	175				422					0,21 (0,15-0,31)
Education										
Years at school			6,8	4,3			5,2	3,8	**0,000**	1,10 (1,05-1,14)
Smoking										
Never Smoker	192				380					1
Former Smoker	11				8				**0,000**	2,72 (1,00-7,36)
Smokers	83				90					1,82 (1,30-2,56)
Use of alcohol every day	175				252				**0,018**	1,43 (1,06-1,93)
"How is your health in general?"										
Very good/Good	205				230					1
Fair/Poor/Very poor	81				179				**0,000**	0,51 (0,36-7,71)
Proxy and Missing	0				70					not assessable
Attitude towards life										
Optimistic	177				196					1
Neither optimistic nor pessimistic/Pessimistic	108				213				**0,000**	0,56 (0,41-0,77)
Proxy and Missing	1				70					0,02 (0,00-0,12)
Number of diseases										
0-2	145				147				**0,000**	1
≥ 3	141				332					0,43 (0,32-0,58)
BMI (Body Mass Index)										
≤ 21	46				77					1
22-27	144				182				0,421	1,32 (0,85-2,06)
≥ 28	75				98					1,28 (0,78-20,9)
500 metres walking ability without aids	183				101				**0,000**	6,65 (4,77-9,27)
Going up and down the stairs without help	259				223				**0,000**	11,01 (6,99-17,35)
Hand Grip (Kg) *										
First quartile	17				131					1
Second quartile	51				134					2,93 (1,59-5,42)
Third quartile	91				88				**0,000**	7,97 (4,39-14,46)
Fourth quartile	126				53					18,32 (9,76-34,29)
Could not complete	1				73					0,10 (0,01-0,81)
Ability to perform Chair Stand test	212				118				**0,000**	8,76 (6,27-12,25)
No Fall within the last year	77				209				**0,001**	1,69 (1,23-2,33)
No Hospitalization within the last year	237				354				**0,004**	1,70 (1,18-2,47)
No Loss of weight within the last year	247				371				**0,002**	1,84 (1,24-2,73)
Vital Status										
Not alive	61				195				**0,000**	1
Alive	225				284					2,53 (1,82-3,52)

Table 4.29 – Model N.2: Univariate Analysis on all 90+ subjects (OR are adjusted for family cluster)

4.6.7 Model N.2: parameters associated with the health status

On the basis of the "Model N.2" we proposed, we evaluated the possible associations between a series of parameters (gender, age, education, self-reported health, attitude towards life, number of diseases, walking ability for 500 metres without aids, going up and down the stairs without anyone's help, handgrip, chair stand test, absence of fall within the last year, absence of hospitalisation within the last year and absence of weight loss within the last year) and the health status of 90+ siblings. The analysis was performed in males and females separately and the OR results were adjusted for family cluster (**Table 4.30**).

When the logistic regression model is applied to *males*, education (p = 0.006), attitude towards life (p = 0.030), hand grip (p = 0.000) and chair stand (p = 0.006) show a correlation with the health status. When the multivariate analysis model is applied to *females*, education (p = 0.003), hand grip (p = 0.000) and chair stand (p = 0,005) continue to be correlated with the health status and new variables such as age (p = 0.000), the ability of going up and down the stairs without anyone's help (p = 0,005) and the absence of hospitalisation within the last year (p = 0.043) show a correlation with the health status. When the model is applied to the *total population*, all the previous parameters that were associated with the health status in males or in females, except for the absence of hospitalisation within the last year, show a strong correlation with the health status.

In summary, **age**, **education**, **the attitude towards life, the ability of going up and down the stairs without anyone's help, hand grip** and **chair stand** show a strong correlation with the health status.

Characteristic	*Males* n=201		*Females* n=489		*Total* n=692	
	OR (95% CI)	***p* Value**	**OR (95% CI)**	***p* Value**	**OR (95% CI)**	***p* Value**
Gender					0,92 (0,6-1,4)	0,687
Age	0,87 (0,75-1)	0,058	0,82 (0,74-0,9)	**0,000**	0,84 (0,78-0,91)	**0,000**
Education	1,12 (1,03-1,22)	**0,006**	1,11 (1,04-1,18)	**0,003**	1,11 (1,05-1,17)	**0,000**
Self-reported Health (*Fair/Poor/Very poor*)	1,23 (0,48-3,17)	0,666	1,48 (0,88-2,48)	0,138	1,37 (0,88-2,15)	0,164
Attitude towards life (*Neither optimistic nor pessimistic/Pessimistic*)	0,42 (0,19-0,92)	**0,030**	0,69 (0,42-1,15)	0,155	0,65 (0,43-0,98)	**0,039**
Number of diseases ≥ 3	0,91 (0,41-2,01)	0,811	0,67 (0,4-1,14)	0,141	0,78 (0,52-1,17)	0,232
500 metres walking ability without aids	1,39 (0,59-3,29)	0,456	1,48 (0,83-2,63)	0,187	1,4 (0,87-2,26)	0,168
Going up and down the stairs without help	1,81 (0,52-6,34)	0,354	2,72 (1,35-5,49)	**0,005**	2,38 (1,3-4,34)	**0,005**
Hand Grip (Kg)						
Third quartile	5,37 (1,61-17,89)	**0,006**	4,03 (1,74-9,34)	**0,001**	4,32 (2,21-8,44)	**0,000**
Fourth quartile	9,89 (3,02-32,41)	**0,000**	8,04 (3,21-20,16)	**0,000**	8,62 (4,18-17,76)	**0,000**
Ability to perform Chair Stand test	3,32 (1,41-7,86)	**0,006**	2,26 (1,29-3,98)	**0,005**	2,58 (1,61-4,14)	**0,000**
No Fall within the last year	1,84 (0,8-4,23)	0,152	0,79 (0,47-1,34)	0,382	1,06 (0,69-1,61)	0,792
No Hospitalization within the last year	0,51 (0,2-1,26)	0,143	1,89 (1,02-3,51)	**0,043**	1,22 (0,74-2)	0,440
No Loss of weight within the last year	2,04 (0,69-6,05)	0,199	0,65 (0,36-1,2)	0,169	0,93 (0,53-1,6)	0,781

Table 4.30 – Model N.2: multivariate analysis model on the health status of 90+ siblings
(OR are adjusted for family cluster)

4.6.8 Model N.2: family history and health status of GEHA 90+ siblings at the recruitment time

This analysis aimed at finding a possible **relationship between the family history of GEHA 90+ siblings and their health status at the recruitment time**. As for Model N.1, it was performed on the 354 families with at least 2 nonagenarian siblings (in the families with more than 2 siblings, the proband was compared only with the second sibling according to the birth order) and results are reported in **Table 4.31**. Firstly, we identified the families where the proband and the second sibling shared the health status category, as defined by the "Model N.2" we proposed, and the families where they were discordant for the health category. We found that the proband and the second sibling shared the health status category "The Best 2" in 69 families (19.5%, hereafter identified as ***"Concordant Good Families"*** and representing "The Best 2" families of the study population), they shared the health status category "The Others 2" in 161 families (45.5%, hereafter identified as ***"Concordant Bad Families"***) and they did not share the health status category in 124 families (35.0%, hereafter identified as ***"Discordant Families"***). In summary, the siblings shared the health status in about 65% of the families and they were discordant in about 35% of the families. No difference in **gender composition** was found in the three groups of families, even if in "Concordant Good Families" we found an higher percentage of MM sibpairs (13%) in comparison with "Discordant families" (12.1%) and "Concordant Bad Families" (8.7%), indicating that nonagenarian males are healthier than females. On the contrary, a significant difference is present in the **age** of 90+siblings: it progressively increases passing from "Concordant Good Families" (92.7 yrs) to "Discordant Families" (93.7 yrs) and finally to "Concordant Bad Families" (94.6yrs), and concomitantly also the delta age between the proband and the second sibling increases in the three family groups, as expected. As regards the **parents age of death**, we discovered that the mean of the mother age of death is higher in "Concordant Good Families" in comparison to the other family groups, reacheing 78.5 years. Moreover, we checked if the **dimension of the total sibship** influenced the health status of the recruited 90+ siblings and, reassuringly, we found that the mean number of siblings was about 5-6 in all the three family groups, indicating that the health status of 90+ subjects, as defined by Model N.1, is not biased by the initial sibship of the family to which the belong to. **Useful definitions:**

Concordant Good Families = both siblings are in "The Best 1" category
Concordant Bad Families = only one sibling is in "The Best 1" category
} *Concordant Families*

Discordant Families = both siblings are in "The Others 1" category

	Concordant Good Families (11)				*Discordant Families (10)*				*Concordant Bad Families (00)*							
	N	**%**	**mean**	**SD**	**N**	**%**	**mean**	**SD**	**N**	**%**	**mean**	**SD**	***p* Value**	***p* Value (11 vs 00)**	***p* Value (11 vs 10)**	***p* Value (10 vs 00)**
Families with at least 2 nonagenarian siblings (n=354)	69	19.5			124	35.0			161	45.5						
Siblings Gender Composition																
MM	9	13.0			15	12.1			14	8.7						
MF	11	15.9			24	19.4			33	20.5			0.638			
FM	16	23.2			21	16.9			24	14.9						
FF	33	47.8			64	51.6			90	55.9						
Age																
Siblings Age			92.7	1.4			93.7	2.0			94.6	2.4		0.000	0.000	0.000
Siblings Delta Age			2.4	1.1			3.4	2.1			3.6	2.3		0.000	0.000	0.261
Parents Age of Death																
Father			74.1	16			74.0	15.4			73.0	16.6				
Mother			78.5	17.1			78.5	16.5			76.7	17.2				

	Concordant Good Families (11)				*Discordant Families (10)*				*Concordant Bad Families (00)*						
	N	**%**	**mean**	**SD**	**N**	**%**	**mean**	**SD**	**N**	**%**	**mean**	**SD**	***p* Value (0 vs 11)**	***p* Value (10 vs 11)**	***p* Value (0 vs 10)**
Total Sibship (included 90+ interviewed subjects)			5.5	2.5			5.7	2.6			5.8	2.5	0.207	0.310	0.363
Alive siblings/Total Sibship (at recruitment time)			0.6	0.3			0.6	0.2			0.6	0.2	0.905	0.914	0.428

Table 4.31 – Model N.2: influence of the family history on the health status of 90+ siblings as defined by the "Model N.2"

4.7 CONCORDANCE OF THE HEALTH AND THE FUNCTIONAL STATUS AMONG 90+ SIBLINGS

On the basis of the results on the level of concordance of the health status among siblings as defined by the "Model N.1", we wanted to further explore the issue of the concordance among siblings, in order to give value to the GEHA population which is composed of 90+ siblings (and not simply of nonagenarian singletons). To this aim, we analysed if the proband and the second sibling were **concordant** or **discordant** for **single variables related to the health and the functional status**. The analysis was performed on the 354 families with at least 2 nonagenarian siblings (in the families with more than 2 siblings, the oldest was compared only with the second sibling according to the birth order), by using two different approaches:

(1) the "Probandwise Concordance" test, a measure of the proportion of families where siblings are concordant for a specific item out of the families where at least one sibling is able to perform the item. This analysis allowed us to measure the percentages of families where the oldest and the second siblings obtained the same result in a specific item.

(2) the "Conditional Logistic Regression" test, a prediction of the ability of the youngest sibling to be positive to a test given the fact that the oldest sibling was or not positive to the specific test.

As regards the concordance for the **health status**, firstly we confirmed the number of Concordant Families for the health status as defined by the "Model N.1" and we found that the prediction for the second sibling to be in the same category of the oldest is significantly high. Reassuringly, also when the "Model N.2" is evaluated, even a stronger concordance was found between siblings. Then, we checked other single variables for the detection of the health status, such as the number of current diseases (as a general indicator of the health status), some past diseases, some haematological and biochemical parameters and the self-reported health. We found that haematological and biochemical parameters are the most concordant, followed by the myocardial infarction and cancer which occurred in the past and also the number of current diseases (**Table 4.32**).

As regards the concordance for the **functional status**, we checked the following items: ADL, ability to read newspaper without glasses, ability to face someone at 4 metres without glasses, ability to walk 500 metres without aids, Hand Grip, Chair Stand, SMMSE. Results indicate that the physical status (ADL, 500 metres walking), the physical performance (hand grip test, chair stand test) and the cognitive status (SMMSE) are highly concordant between the proband and the second sibling; on the contrary vision and hearing abilities does not seem to be shared by siblings (**Table 4.33**).

	Concordant		*Discordant*		*Discordant*		*Concordant*		*Probandwise Concordance*			*Conditional Logistic*	
	Sib1=yes	***Sib2=yes***	***Sib1=no***	***Sib2=yes***	***Sib1=yes***	***Sib2=no***	***Sib1=no***	***Sib2=no***	***Probability***	***Inf***	***Sup***	***OR***	***95% CI***
Classifications for health status													
Model N.1	**The Best**	**The Best**	**The Others**	**The Best**	**The Best**	**The Others**	**The Others**	**The Others**					
	26		71		33		224		0.33	0.24	0.43	2.15	1,42-3,25
Model N.2	**The Best**	**The Best**	**The Others**	**The Best**	**The Best**	**The Others**	**The Others**	**The Others**					
	69		86		38		161		0.53	0.45	0.60	2.26	1,54-3,32
Number of current diseases	**0-2**	**0-2**	**>=3**	**0-2**	**0-2**	**>=3**	**>=3**	**>=3**					
	65		70		69		150		0.48	0.41	0.56	1.01	0,73-1,41
Self-reported past diseases													
Myocardial infartion	4		18		15		316		0.19	0.03	0.36	1.20	0,6-2,38
Stroke, Cerebral thrombosis / Haemorrhage	5		41		48		259		0.10	0.02	0.18	0.85	0,56-1,29
Cancer	4		37		33		279		0.10	0.01	0.19	1.12	0,70-1,79
Hip fracture	5		40		47		261		0.10	0.02	0.19	0.85	0,56-1,30
Hematological and Biochemical Parameters													
Creatinine	**<2 mg/dl**	**<2 mg/dl**	**>=2 mg/dl**	**<2 mg/dl**	**<2 mg/dl**	**>=2 mg/dl**	**>=2 mg/dl**	**>=2 mg/dl**					
	221		12		8		0		0.96	0.94	0.98	1.50	0,61-3,67
Haemoglobin	**>=10 g/dl**	**>=10 g/dl**	**<10 g/dl**	**>=10 g/dl**	**>=10 g/dl**	**<10 g/dl**	**<10 g/dl**	**<10 g/dl**					
	237		6		3		0		0.98	0.97	0.99	2.00	0,50-8,00
PCR	**<1**	**<1**	**>=1**	**<1**	**<1**	**>=1**	**>=1**	**>=1**					
	104		27		23		9		0.8	0.75	0.86	1.17	0,67-2,05
How is your health in general?	**Very good/ Good**	**Very good/ Good**	**Fair/Poor/ Very poor**	**Very good/ Good**	**Very good/ Good**	**Fair/Poor/ Very poor**	**Fair/Poor/ Very poor**	**Fair/Poor/ Very poor**					
	126		57		61		51		0.68	0.63	0.73	0.93	0,65-1,34

Table 4.32 – Concordance for the Health Status

	Concordant		*Discordant*		*Discordant*		*Concordant*		*Probandwise Concordance*			*Conditional Logistic*	
	Sib1=yes	***Sib2=yes***	***Sib1=no***	***Sib2=yes***	***Sib1=yes***	***Sib2=no***	***Sib1=no***	***Sib2=no***	***Probability***	***Inf***	***Sup***	***OR***	***95% CI***
Five-items ADL	**ADL=5**	**ADL=5**	**ADL<5**	**ADL=5**	**ADL=5**	**ADL<5**	**ADL<5**	**ADL<5**					
	112		91		43		43		0,63	0,57	0,68	2,12	1,47-3,04
Six-items ADL	**ADL>=4**	**ADL>=4**	**ADL<4**	**ADL>=4**	**ADL>=4**	**ADL<4**	**ADL<4**	**ADL<4**					
	176		84		40		54		0,74	0,7	0,78	2,10	1,44-3,06
Reading newspaper without glasses	42		80		72		160		0,36	0,28	0,43	1,11	0,81-1,53
Face someone 4 metres away without glasses	181		71		63		30		0,73	0,69	0,77	1,13	0,80-1,58
500 metres walking	65		89		37		163		0,51	0,43	0,58	2,40	1,64-3,53
Hand Grip	**3^{rd}-4^{th} quartile**	**3^{rd}-4^{th} quartile**	**$<3^{rd}$ quartile**	**3^{rd}-4^{th} quartile**	**3^{rd}-4^{th} quartile**	**$<3^{rd}$ quartile**	**$<3^{rd}$ quartile**	**$<3^{rd}$ quartile**					
	97		94		44		119		0,58	0,52	0,65	2,14	1,49-3,06
Chair Stand test ability	83		92		44		135		0,55	0,48	0,62	2,09	1,46-2,99
SMMSE corrected (Franceschi C et al., 2000)	**>=20**	**>=20**	**<20**	**>=20**	**>=20**	**<20**	**<20**	**<20**					
	170		65		30		31		0,78	0,74	0,82	2,17	1,41-3,34
SMMSE corrected (Nybo H et al., 2003)	**>=24**	**>=24**	**<24**	**>=24**	**>=24**	**<24**	**<24**	**<24**					
	113		70		37		76		0,68	0,62	0,74	1,89	1,27-2,82

Table 4.33 – Concordance for the Functional Status

4.8 SURVIVAL ANALYSIS ON GEHA 90+ SIBLINGS AT JANUARY 1st 2009 (GEHA as a longitudinal study)

4.8.1 Basic information about the vital status of GEHA 90+ siblings

The vital status of GEHA 90+ siblings was checked at **January 1st, 2009** and the results of the survival analysis, as reported in **Table 4.34**, indicate that **256 out of 765 subjects died** (**33.5%**) during the follow-up, with a similar proportion in UNIBO and ISS. The mortality was analogous in males and in females (31.5% versus 34.3%) and it progressively increased with increasing age of the subjects at the recruitment time: only 24.7% of 90-93 yrs subjects died, while 40% of 94-98 yrs subjects died and finally 61.8% of ≥ 99 yrs subjects died (p = 0.000). p values were calculated according to Cox regression-based test for equality of survival curves.

	Status				
	Not Alive n=256 (33,5%)		*Alive* n=509 (66,5%)		
	N	%	N	%	*p* Value
RECRUITING CENTRE					
UNIBO	190	34,6	359	65,4	0,493
ISS	66	30,6	150	69,4	
GENDER					
Males	70	31,5	152	68,5	0,878
Females	186	34,3	357	65,7	
AGE (at recruitment time)					
90-93 years	100	24,7	305	75,3	
94-98 years	122	40,0	183	60,0	0,000
≥ 99 years	34	61,8	21	38,2	

Table 4.34 – Basic information about the vital status of GEHA nonagenarian siblings (at January 1st, 2009)

4.8.2 Survival and Health Status of GEHA 90+ siblings at recruitment time

In **Table 4.35** the vital status of GEHA 90+survival siblings is shown in relation to their health status according to the different classification methods of the health status that were adopted in the previous dissertation. *p* values were calculated according to Cox regression-based test for equality of survival curves.

As far as the **classification proposed by Gondo** is concerned, the "Exceptional" gross mortality is 18.4%, the "Normal" gross mortality is 21.5%, the "Frail and Fragile" gross mortality is 40.7% and the "Proxy" gross mortality is the highest and reaches 53.1%. The analysis of the survival curves does not show any difference between the "Exceptional" group and the "Normal" group, but it shows a relevant difference between the "Normal" group and the "Frail and Fragile" group and also between the "Frail and Fragile" group and the "Proxy" group (**Figure 4.3**).

As far as the **classification proposed by Franceschi** is concerned, the category "A" gross mortality is 16.9%, the category "B" gross mortality is 29.5%, the category "C" gross mortality is 38.5% and the "Proxy" gross mortality is again the highest and reaches 53.1%. The analysis of the survival curves shows a relevant difference between the "A" group and the "B" group, between the "B" group and the "C" group and also between the "C" group and the "Proxy" group (**Figure 4.4**).

As far as the **classification proposed by Evert** is concerned, the "Escapers" gross mortality is 22.9%, the "Delayers" gross mortality is 33.5%, the "Survivors" gross mortality is 37% and the "Not applicable" gross mortality is again the highest and reaches 42.4%. The analysis of the survival curves shows a relevant difference between the "Escapers" group and the "Survivors" group, as well as between the "Escapers" group and the "Delayers" group, but it does not show any difference between the "Delayers" group and the "Survivors" group (**Figure 4.5**).

As far as the **"Model N.1"** is concerned, "The Best 1" gross mortality is 16.9% and "The Others 1" gross mortality is 38.4%. The analysis of the survival curves shows a relevant difference between "The Best 1" group and the "The Others 1" group (**Figure 4.6**).

As far as the **"Model N.2"** is concerned, "The Best 2" gross mortality is 21.3% and "The Others 2" gross mortality is 40.7%. The analysis of the survival curves shows a relevant difference between "The Best 2" group and the "The Others 2" group (**Figure 4.7**).

	Status				
	Not Alive **n=256 (33,5%)**		***Alive*** **n=509 (66,5%)**		
	N	**%**	**N**	**%**	***p* Value**
Gondo et al., 2006					
Exceptional	9	18,4	40	81,6	0,000
Normal	59	21,5	215	78,5	
Frail and Fragile	154	40,7	224	59,3	
Proxy	34	53,1	30	46,9	
Franceschi et al., 2000					
A	28	16,9	138	83,1	0,000
B	59	29,5	141	70,5	
C	60	38,5	96	61,5	
Proxy	34	53,1	30	46,9	
Evert et al., 2003					
Escapers	16	22,9	54	77,1	0,070
Delayers	179	33,5	356	66,5	
Survivors	47	37,0	80	63,0	
Not Applicable	14	42,4	19	57,6	
Model N.1					
"The Best 1"	30	16,9	147	83,1	0,000
"The Others 1" *(all the others subjects)*	226	38,4	362	61,6	
Model N.2					
"The Best 2" *(SMMSE ≥ 24 and Five-items ADL = 5)*	61	21,3	225	78,7	0,000
"The Others 2" *(all the others subjects)*	195	40,7	284	59,3	

Table 4.35 – Vital status of GEHA 90+ siblings according to their health status at the recruitment time

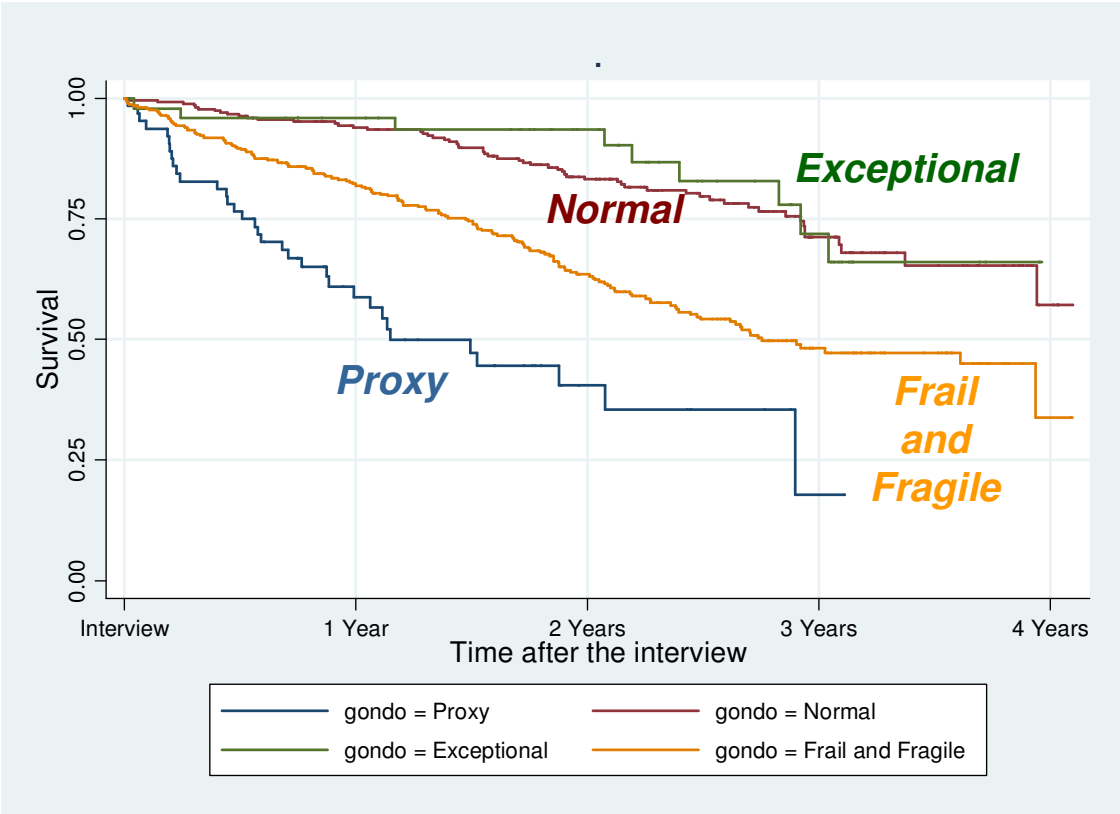


Figure 4.3 - Kaplan Meyer curve for survival on the basis of Gondo Classification

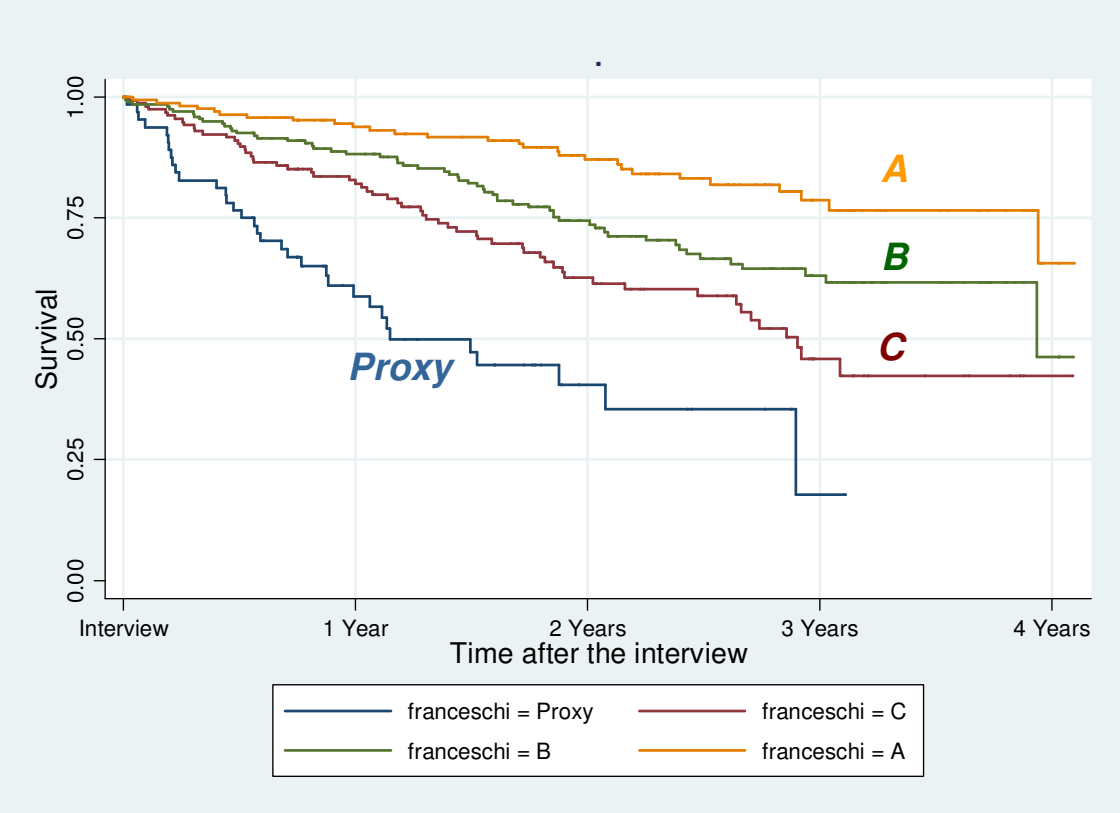


Figure 4.4 – Kaplan Meyer curve for survival on the basis of Franceschi Classification

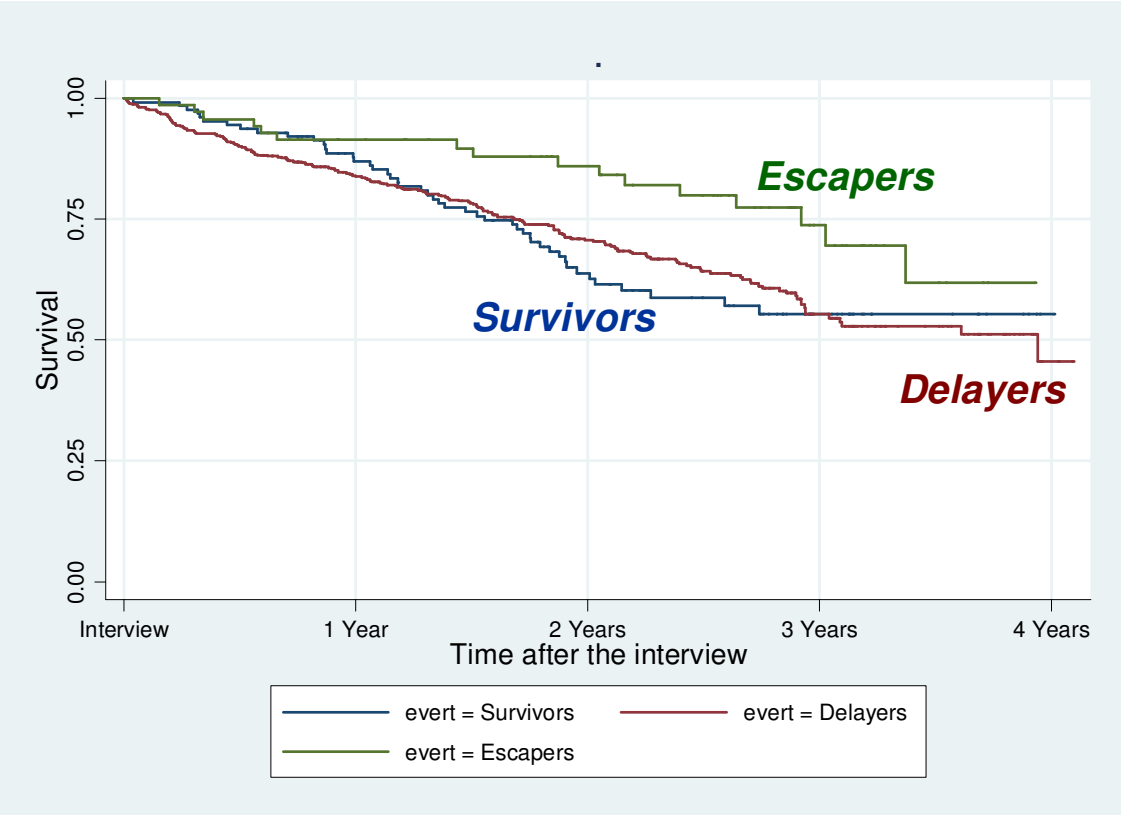


Figure 4.5 – Kaplan Meyer curve for survival on the basis of Evert Classification

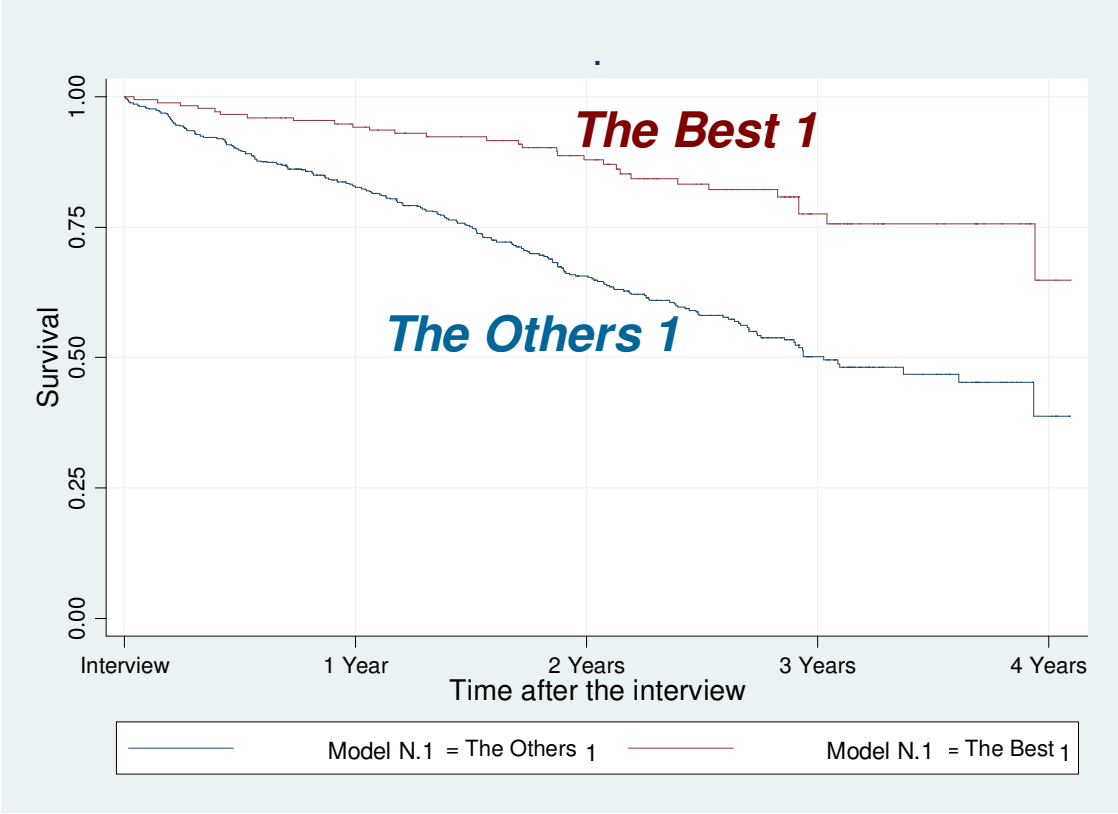


Figure 4.6 – Kaplan Meyer curve for the "Model N.1" on Health Status of 90+ siblings

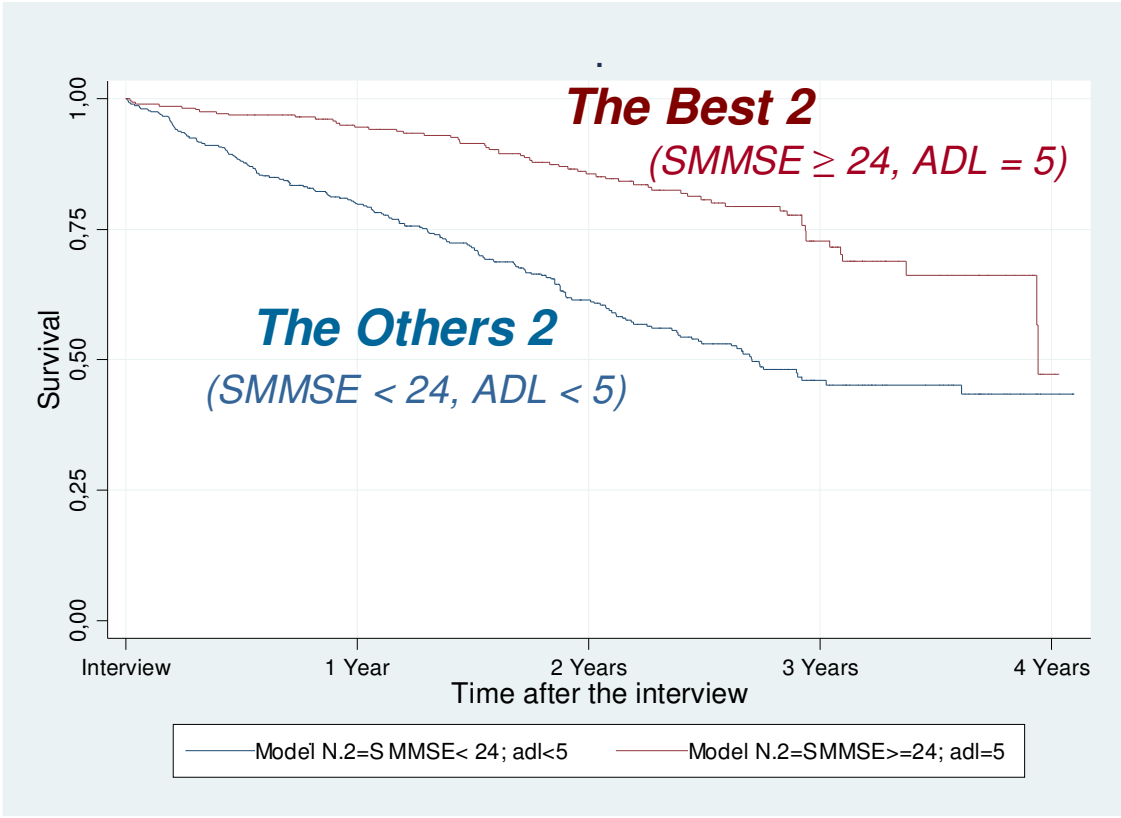


Figure 4.7 – Kaplan Meyer curve for the "Model N.2" on Health Status of 90+ siblings

Moreover, applying a multivariate Cox regression model estimating the role of health status as defined by the **"Model N.1"** on **survival**, we found that it was significantly correlated with survival, also considering gender and age at the recruitment time. Gender is not significant for survival, while as expected, the probability of death progressively increases with increasing age, as reported in **Table 4.36** and **Figure 4.8**.

Characteristic	HR (95% CI)	*p* Value
Gender		
Males	1	
Females	0,95 (0,73-1,24)	0,691
Age at recruitment time		
90-93 years	1	
94-98 years	1,62 (1,23-2,14)	0,001
≥ 99 years	3,56 (2,40-5,28)	0,000
Model N.1		
"The Others 1" *(all the others subjects)*	1	
"The Best 1"	0,42 (0,28-0,62)	0,000
Number of observations: 765; Number of family clusters: 364		

Table 4.36 – Hazard Ratio (HR) and 95% Confidence Intervals (CI) for GEHA 90+ siblings
(results were adjusted for family cluster)

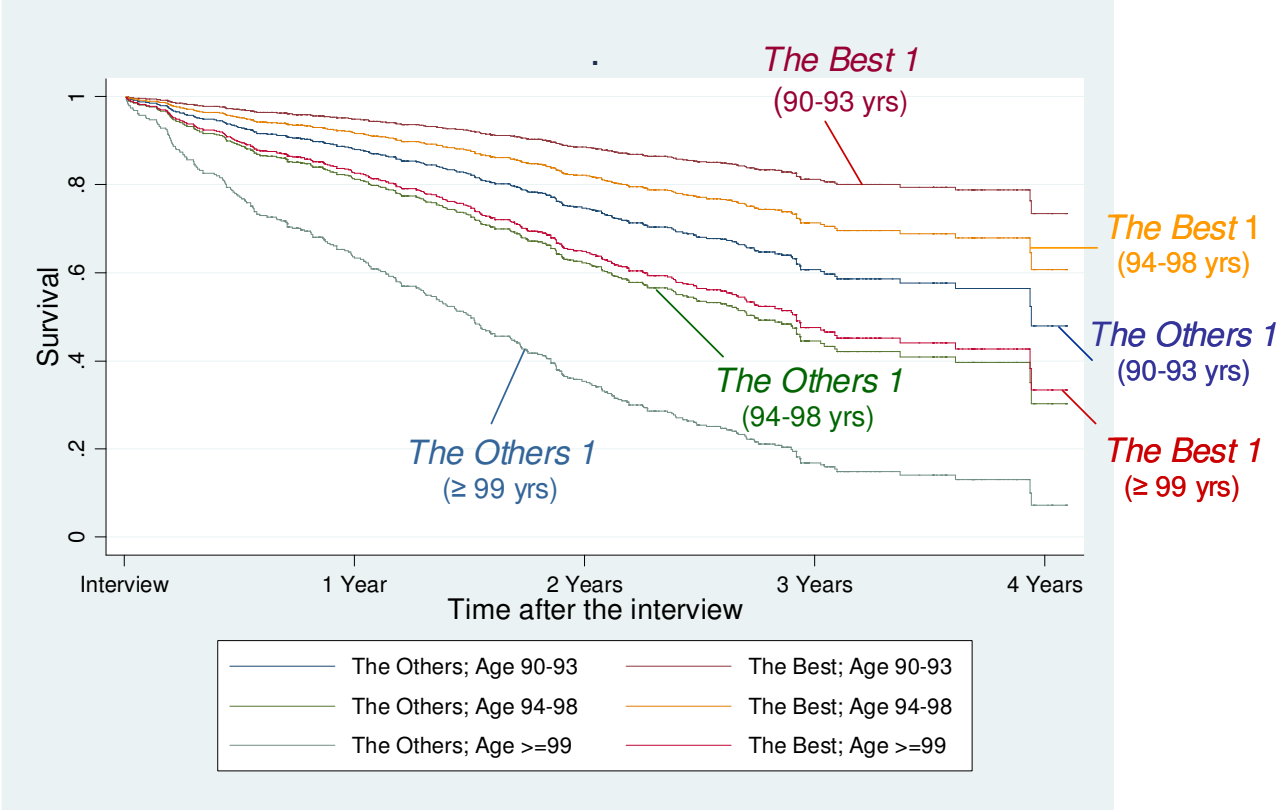


Figure 4.8 – Cox Regression for survival on the basis of the "Model N.1" (by age at recruitment time)

Moreover, applying a multivariate Cox regression model estimating the role of health status as defined by the **"Model N.2"** on **survival**, we found that it was significantly correlated with survival, also considering gender and age at the recruitment time. Gender is not significant for survival, while as expected, the probability of death progressively increases with increasing age, as reported in **Table 4.37** and **Figure 4.9**.

Characteristic	HR (95% CI)	*p* Value
Gender		
Males	1	
Females	0,91 (0,70-1,20)	0,512
Age at recruitment time		
90-93 years	1	
94-98 years	1,60 (1,21-2,11)	0,001
≥ 99 years	3,15 (2,11-4,71)	0,000
Model N.2		
"The Others 2" *(all the others subjects)*	1	
"The Best 2" *(SMMSE ≥ 24 and Five-items ADL = 5)*	0,45 (0,34-0,60)	0,000
Number of observations: 765; Number of family clusters: 364		

Table 4.37 – Hazard Ratio (HR) and 95% Confidence Intervals (CI) for GEHA 90+ siblings
(results were adjusted for family cluster)

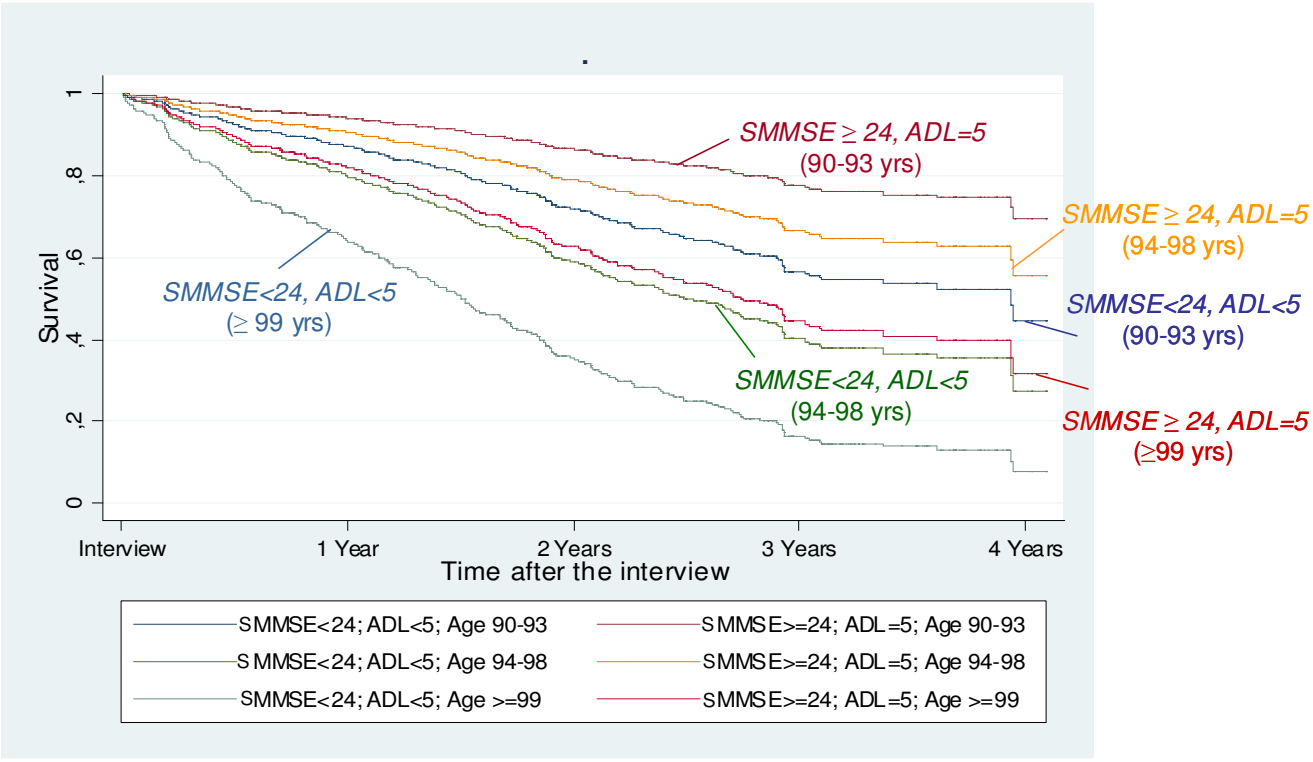


Figure 4.9 – Cox Regression for survival on the basis of the "Model N.2" (by age at recruitment time)

4.8.3 Role of Haematological and Biochemical Parameters on survival of GEHA 90+ siblings

To evaluate the influence of haematological and biochemical parameters on mortality we performed five Cox Regression models containing haematological and biochemical variables (such as haemoglobin, leukocytes, creatinine, glucose, GPT, total cholesterol, HDL-C and trygliceride) plus different parameters for the assessment of the functional and the health status. The fifth model contains also CRP among biochemical parameters, given its important role in the inflammatory status, and it is performed only on UNIBO GEHA population (**Table 4.38**).

On the whole, we confirmed that age is significantly associated with mortality, while gender was not.

In the *first model*, the "Model N.1" is strongly associated with mortality, and also creatinine and glucose levels seem to predict mortality.

In the *second model*, instead of the synthetic index given by the "Model N.1", we included the three single variables hidden inside the classification (intact cognitive function -SMMSE ≥ 20-, ability to walk 500 metres without aids and haemoglobin levels) and we noticed that they are all associated with mortality, together with creatinine level.

In the *third model*, the "Model N.2" is strongly associated with mortality together with haemoglobin and creatinina levels.

In the *fourth model*, instead of the synthetic index given by the "Model N.2", we included the two single variables hidden inside the classification (intact cognitive function -SMMSE ≥ 24- and good physical function –ADL = 5-) and we obtained that they are both associated with mortality together with haemoglobin and creatinine levels.

In the *fifth model*, we found that the health status as assessed by the "Model N.1" still remains a strong predictor of mortality, together with CRP and creatinine levels.

Cox Regression	**First Model** *(Model N.1)*			**Second Model** *(Model N.1 expanded)*			**Third Model** *(Model N.2)*			**Fourth Model** *(Model N.2 expanded)*			**Fifth Model** *(Model N.1, only UNIBO)*		
Number of observations	546			501			544			501			388		
Number of family cluster	300			290			299			290			211		
Characteristic	**HR**	**(95% CI)**	***p* Value**	**HR**	**(95% CI)**	***p* Value**	**HR**	**(95% CI)**	***p* Value**	**HR**	**(95% CI)**	***p* Value**	**HR**	**(95% CI)**	***p* Value**
Gender															
Males		1			1			1			1			1	
Females	1,12	(0,79-1,58)	0,513	0,8	(0,53-1,19)	0,27	0,89	(0,61-1,29)	0.531	0,78	(0,52-1,18)	0,248	0,92	(0,62-1,37)	0,688
Age at recruitment time															
90-93 years		1			1			1			1			1	
94-98 years	1,7	(1,19-2,41)	**0,003**	1,49	(1,00-2,24)	**0,051**	1,63	(1,14-2,34)	**0,008**	1,5	(1,01-2,21)	**0,043**	1,56	(1,0-2,42)	**0,049**
>=99 years	3,06	(1,72-5,45)	**0,000**	2,54	(1,32-4,86)	**0,005**	2,64	(1,48-4,71)	**0,001**	2,42	(1,27-4,61)	**0,007**	2,62	(1,27-5,41)	**0,009**
Model N.1															
"The Others 1"		1												1	
"The Best 1	0,46	(0,30-0,71)	**0,001**										3,57	(2,0-6,25)	**0,000**
Model N.2															
"The Others 2"								1							
"The Best 2							0,55	(0,38-0,80)	**0,002**						
SMMSE categories															
1-12					1										
13-19				0,62	(0,29-1,30)	0,204									
>=20				0,38	(0,18-0,80)	**0,011**									
SMMSE categories															
0-17											1				
18-23										0,71	(0,41-1,22)	0,212			
>=24										0,55	(0,33-0,92)	**0,022**			
Walking about 500m															
No					1										
Yes				0,65	(0,45-0,942)	**0,023**									
ADL scale categories															
0-2											1				
3-4										0,75	(0,47-1,21)	0,244			
5										0,53	(0,34-0,83)	**0,001**			
Hematologica and Biochemical Parameters															

Haemoglobin				0,80	(0,71-0,90)	**0,000**	0,81	(0,73-0,90)	**0,000**	0,79	(0,71-0,88)	**0,000**			
Leukocytes	1,03	(0,99-1,08)	0,159	1,02	(0,97-1,07)	0,359	1,03	(0,99-1,08)	0,279	1,02	(0,97-1,07)	0,408	1,01	(0,96-1,07)	0,618
Creatinine	1,7	(1,21-2,40)	**0,002**	1,66	(1,19-2,32)	**0,003**	1,47	(1,05-2,06)	**0,026**	1,59	(1,13-2,22)	**0,007**	1,69	(1,01-2,82)	**0,044**
Glucose	1	(1,0-1,01)	**0,050**	1	(1,00-1,01)	0,182	1,00	(0,99-1,02)	0,609	1	(1,0-1,0)	0,133	1	(1,0-1,0)	0,594
ALT (GPT)	0,99	(0,96-1,02)	0,414	0,99	(0,95-1,03)	0,623	1,00	(0,99-1,00)	0,116	0,99	(0,96-1,03)	0,682	1	(0,92-1,02)	0,208
Total Cholesterol	1	(0,98-1,01)	0,72	1,01	(0,99-1,02)	0,256	1,00	(0,97-1,02)	0,792	1,01	(1,0-1,03)	0,126	1	(0,99-1,03)	0,285
HDL-C	1	(0,99-1,0)	0,473	1	(0,99-1,00)	0,553	1,00	(0,99-1,00)	0,412	1	(1,0-1,0)	0,321	1	(0,99-1,0)	0,329
Tryglicerides	1	(0,99-1,0)	0,546	1	(0,99-1,00)	0,742	1,00	(0,99-1,00)	0,613	1	(1,0-1,0)	0,406	1	(1,0-1,0)	0,776
CRP													1,16	(1,03-1,31)	**0,017**

Table 4.38 - Hazard Ratio (HR) and 95% Confidence Intervals (CI) for GEHA 90+ siblings (results were adjusted for family cluster)

5. DISCUSSION

5.1 RECRUITMENT OF GEHA 90+ SIBLINGS

One of the aims of the present study was to outline the recruitment procedure that was standardized at the beginning of the GEHA project to be followed by all the European recruiting units, including UNIBO and ISS. The **set up of a standardized protocol to assess cognitive status, physical performances and health status of European nonagenarian subjects** represents a great success of the project, for many reasons: 1. it gives power and reliability to the whole project and to future results on genetics; 2. it allows the performance of comparisons between 90+ siblings recruited in different European regions (taking into account both population background, such as differences in genetic variation, birth weight, and childhood growth, and sociocultural factors, such as differences in lifestyle and public health care for old people); 3. it could be also considered as a reference method for further study on longevity; 4. it helps the European gerontological research to establish common criteria and methodologies which can impact on important areas (public health and policy makers).

The strength of this study is that the same design and the same recruitment procedure were used in each region. Furthermore, the examiners in the various regions went through a common training course.

In this contest it was possible to compare **Italian 90+ siblings recruited in Northern Italy** (by UNIBO recruiting unit) **and in Central-Southern Italy** (by ISS). Firstly we noticed that the percentage of families that gave a positive response to participate in the GEHA study is 25.5%, with a higher proportion among UNIBO subjects; this difference could be related to the inclusion of volunteers sibpairs who spontaneously offered to enter the study and were recruited by UNIBO, but not by ISS staff. It is worth pointing out that this difference did not represent a bias for the analysis, even if it could be predicted that volunteer families are selected among the whole 90+ sibpairs and are healthy. Indeed, the health and the functional status of 90+ siblings was not different between centres with all the methods that were adopted; unexpectedly, ISS subjects were actually in better shape.

Moreover, we noticed that the families did not enter the study was 69.2%. Among families did not enter the study, an higher percentage of death was present in UNIBO respect to ISS. That was because the data on 90+ sibpairs obtained from the Registry Office at the far beginning of the project were not updated periodically with the mortality status; therefore, with the passing of time, some 90+ subjects died and recruiters became aware of this only after having contacted them.

It is worth pointing out that the **25.5% of positive response** to participate in the study and the **69.2% of impossibility to recruit the sibpair** differ from the initial theoretical assumption of GEHA consortium who estimated that overall there would be about 50% of positive and 50% of negative responses. This result is noteworthy also because the percentage of positive response would diminish further to 22% if volunteers sibpairs recruited by UNIBO were excluded by the scoring. However, even if different from expectation, these results are precious and useful during the planning phase of future studies on long-living subjects.

5.2 PHENOTYPIC CHARACTERISTICS OF GEHA 90+ SIBLINGS RECRUITED BY UNIBO AND ISS RECRUITING UNITS

One of the main objectives of the GEHA projects is to find genes associated with longevity and healthy aging. It was assumed that this study could be conducted on 90+ sibpairs because they are selected and represent a peculiar and extreme phenotype which is an appropriate model to study longevity. In the present analysis we explored whether the recruitment strategy has resulted in a population enriched for hereditable component for exceptional longevity. We found that the characteristics of GEHA families recruited by UNIBO and ISS are comparable to those obtained by Schoenmaker M *et al.* on the families enrolled within the Leiden Longevity Study (Schoenmaker *et al.*, 2006). Moreover, we demonstrated that 90+ siblings enrolled in the **GEHA study** actually belonged to **families enriched in long-living members**, as predicted at the beginning of the project. To test longevity throughout families we studied the parents of 90+ siblings. They were born on average in 1880-1890 and they died beyond the life expectancy of their birth cohort; in addition, it should noted that they lived in an historical period where the environmental conditions were particularly unfavourable (they survived to the First World War, the Second World War and they also escaped to the infective Spanish influenza). This finding represents another strength that gave value and power to the project, it supports the results obtained by the possible phenotypic and genotypic analysis performed on the recruited population and it allows to draw conclusions in the field of longevity.

In our analysis we described and compared the phenotypic characteristics of 90+ siblings recruited by UNIBO and ISS. As regards the **cognitive status**, the main findings indicate that 56.2 % of the population is cognitively intact (SMMSE $\geq$ 24) according to the cut-off points used by Nybo *et al.* in a study on Danish nonagenarians (Nybo *et al.*, 2003); this percentage reaches 74.2% when the cut-off points indicated by Franceschi in a study on Italian centenarians were used (Franceschi *et al.*, 2000a). An higher proportion of cognitively unimpaired subjects is present in ISS subjects in respect to UNIBO (probably related to their higher education level) and males, even if fewer in number, obtained higher score in SMMSE than females.

As regards the **physical status**, the main findings indicate that 50.8% of the population is not disabled for all the basic ADL (ADL = 5), and this result is similar to that found in a study on Danish nonagenarians (Nybo *et al.*, 2001a); this percentage reaches 67.7% when the cut-off points indicated by Franceschi in a study on Italian centenarians were used (Franceschi *et al.*, 2000a). The highest disability was related to the "bathing" ability. Since ISS subjects present higher scores at ADL than UNIBO subjects, the difference was not significant indicating the

populations are homogeneous as regards the self-reported level of independency, measured by ADL. Also results about hand grip strength and chair stand test were not different in UNIBO and ISS populations. Interestingly, it should be noted that the mean hand grip strength values (23.7 Kg for males and 14.4 Kg for females) were much more similar to those measured in nonagenarians from Southern Denmark, than to Calabria region (Jeune *et al.*, 2006).

As regards **lifestyle**, we noticed that healthy behaviours shaped the life of 90+ siblings: most of them (74.8%) never smoked and when it occurred it is correlated with bad health conditions and non-autosufficiency, indicating that it compromises health status and the quality of life even in long living subjects, as reported in a study on Italian centenarians (Nicita-Mauro *et al.*, 2007). The highest proportion of smokers was found among ISS subjects, probably because they all live in the city where it was easier to access to cigarettes and where the habit to smoke was culturally more elevated than in the countryside.

As regards the **health status**, we found that most of 90+ siblings delayed the on-set of the major age-related diseases after 80 years of age (69.9%), in accordance with the "compression of morbidity" hypothesis (Evert *et al.*, 2003). However, only a low percentage of 90+ siblings do not report any disease at the recruiting time (4.3%) and do not take drugs (8.9%), even if half of the population can be considered as cognitively intact and physical independent. As suggested by Jeune *et al.* in a study on Danish centenarians (Andersen-Ranberg *et al.*, 2001), this apparent paradox could be explained by the high prevalence among long-living subjects of several common diseases (such as hypertension and CVD), which do not prevent nonagenarians from being cognitively intact and physically not disabled. This evidence suggests that in long living subjects "healthy aging" could be defined as the condition where good physical and cognitive abilities and autonomy in the daily life are maintained.

In the worldwide scenario, it is emerging that also psychological measures, together with cognitive and functional factors, revealed to be the most effective measures to define the health status because they contact most of the fields responsible of the age-related decline (Passarino *et al.*, 2007). To this regard, we found that about 62% of 90+ siblings considered their health as "Very Good" or "Good" and half of them declared to be "Optimistic", indicating that a positive attitude towards life contributes to attain longevity. In fact, as reported by Selim *et al.* in the *1999 Large Health survey of Veteran Enrollees*, centenarians were psychologically resilient despite of their poor physical health, they reported feeling peaceful and calm most of time and they do no report themselves as experiencing progressive decline (Selim *et al.*, 2005). Moreover, a study on centenarians living in central Italy indicates that centenarians have a peculiar personality, characterised by: low exploratory activity, good resistance to frustrations and physical stress, low

pessimistic and anticipatory anxiety, *a priori* persistence, autonomy, self-trascendence (feeling themselves as part of society or humanity or universe) (Sorbi *et al.*, unpublished data).

Results about **haematological and biochemical parameters** indicates that most of the parameters fell within the standard ranges valid for the adult population and are very similar to those reported in a study on Italian centenarians (The Italian Multicentric Study on Centenarians (IMSC), 1998). The evaluation of which haematological and biochemical risk factors could be related to mortality will be discussed later in the dissertation.

5.3 ASSESSMENT OF THE HEALTH AND THE FUNCTIONAL STATUS OF GEHA 90+ SIBLINGS

A major aim of GEHA is to identify gene(s) and gene variant(s) related to successful/healthy and unsuccessful aging. To this purpose the recruited sibpairs must be carefully assessed as far as their health status is concerned, in order to correctly classify all of them. In the present analysis we were particularly interested in **discovering the group of "best" subjects** in order to drive or to compare phenotypic with genetics results. A validated, universal and comprehensive model to define healthy aging is not available for long-living subjects. Therefore, based on the previous experience of some research groups, one also participating in this IP, it seemed reasonable to adopt as a starting point the classifications proposed in three studies on centenarians:

1. the **Tokyo Centenarian Study** (Gondo *et al.*, 2006) categorized people on the bases of their functional characteristics into four phenotypes: "Exceptional", who had intact visual and hearing function, were fully independent and had excellent cognitive functions; "Normal", who were somewhat independent and had good cognitive functions; "Frail", who were impaired for the functional status or the cognitive status; "Fragile", who were totally dependent and had severely impaired cognitive functions.

2. the **Italian Study** (Franceschi *et al.*, 2000a) categorized people into three different phenotypes: "A", who had good functional status without specific morbidity history; "B", who were in intermediate condition; and "C" who had poor functional status with a history of morbidity. In addition they subdivided group "C" into "C1", where cognitive impairment was evident; "C2", where both physical and cognitive impairment were observed; and "C3", where physical impairment was evident.

3. the **New England study** (Evert *et al.*, 2003) used retrospective morbidity profiles and categorized people into three phenotypes: the "Escapers" who could accomplish disease-free aging until they reached 100 years, the "Delayers", who developed disease only very late in life, and the "Survivors", who survived with disease.

These three classification systems have advantages and disadvantages, since the Gondo is almost a classification of functional status, the Franceschi is a mix of function and morbidity and the Evert is only based on morbidity history.

The first part of this study consisted in a **methodological work** where the three classification methods were applied to the population sample to assess the health status. Then, to evaluate the

validity of the classifications, we assessed the **mortality of 90+ siblings at January 1st, 2009** as the main external outcome and we compared the survival curves among the groups.
At the beginning of the study, **it was anticipated that about half of the recruited sibpairs would have been in good health** and thus **about a quarter or more would have presented both members of the sibship in good health**. As a matter of fact, this prediction has revealed to be too generous, since **from 6.4% to 37% of 90+ siblings were considered as "healthy", depending on the different classification methods** we adopted.

Firstly, according to the functional classification proposed by **Gondo**, only 6.4% of 90+siblings were categorized as "Exceptional" and 35.8% as "Normal" (also considered as being healthy). Indeed, as expected, no difference in mortality was found between "Exceptional" and "Normal" subjects. This result probably indicates that visual and hearing functions are so much peculiar and elaborate that physiologically decade with increasing age, but they are not representative of a successful aging in terms of mortality. However, it could be envisaged that vision and hearing abilities play a role in the quality of life of nonagenarians and centenarians, allowing them to maintain a social and active life also at very old age. In summary, in Gondo classification the number of subjects classified as "Exceptional" is small and very often no differences are present between "Exceptional" and "Normal", for example for mortality, so it could be use to appropriately discriminate two large groups of centenarians according to their functional status because it uses a multiple-domain approach for the assessment of the functional and the cognitive status (for example MMSE and Barthel Index), which is more reliable than the single domain approach.

Secondly, according to **Franceschi** classification, 21.7% of 90+ siblings were categorised as "A" (good physical and cognitive status). Franceschi classification is based on a mix of functional, morbidity and haematological-biochemical parameters, because it is not only based on a geriatric-functional concept of aging, but it treats aging as a complex phenomenon operating at many different levels, that should be assessed with different parameters together. On the one hand this approach could be debatable because it assessed the phenotype (healthy/intermediate/unhealthy) with a mixture of causative factors (medical, biological status, environment, stochasticity) and effects (cognitive or physical function). Furthermore, when it is applied to our population, it was found a very high proportion of "not applicable" subjects (23.4%), which invalid some of the comparisons between UNIBO and ISS (the proportion is higher in ISS than in UNIBO); this is due to the presence of haematological-biochemical

parameters inside classification, missing for some of the recruited subjects and that make difficult to compare results with other studies (the proportion of blood samples always varies very much between studies). On the other hand, this has an high discriminatory capacity in terms of mortality because significant differences were found when the survival curves of group "A", "B" and "C" were compared. It could be considered a good predictor of mortality.

Thirdly, according to **Evert** classification, 9.2% of subjects were categorized as "Escapers" and most of the subjects (69,9%) were categorised as "Delayers", indicating that the discriminatory capacity is low, as confirmed also by the survival analysis. This classification in fact is based on self-reported data on morbidity, whose reliability is uncertain, and it does not describe the real phenotype because it is not able to distinguish between a subject categorised as "Survivors" with high functional status or with frailty. It emphasizes participants' medical history and it allows exploration of the effect of disease-associated factors on longevity, under the "compression of morbidity hypothesis", which suggests that the onset of illnesses is delayed among centenarians. However, it has the disadvantage that it is difficult to identify those factors that either protect or delay the aging process.

Finally, within the scenario of high heterogeneity of nonagenarians and centenarians, it was envisaged to find a **simplified set of criteria** to classify very old people, in order to have an **operational tool for distinguishing healthy from non healthy subjects**.
To this aim, we compared classification by Gondo and Franceschi and we were driven towards a Model N. 1 for the identification of "The Best 1" group of subjects on the basis of SMMSE $\geq$ 20, 500 metres walking ability without aids and haemoglobin > 10 g/dl.
Additionally, we constructed a Model N. 2 for the identification of "The Best 2" group of subjects on the basis of the most valid functional items that most aging researcher collect, i.e. those who are not disabled on the basis of five-items ADL (can carry out all five basic items) and not cognitively impaired (SMMSE score $\geq$ 24).

Advantages and disadvantages of Model N.1 ("Franceschi = A or Gondo = Exceptional")
The model N.1 we proposed for the identification of "The Best 1" group of subjects was based on three parameters: one about cognitive status, one about physical ability and one haematological parameters (haemoglobin). According to model N.1, "The Best 1" group of subjects is composed of 23% of 90+ siblings. On the one hand this model has the advantage to come out from an empirical analysis on phenotypic data and not from *a priori* assumption and,

interestingly, it suggests that the most effective measures to define the health status in nonagenarians are a cognitive measure (represented by SMMSE in this case), a functional measure (500 metres walking ability in this case) and the haemoglobin (a predictor of mortality in many studies on centenarians), because they contact most of the fields responsible of the age-related decline. The "500 metres walking ability" confirmed that the functional parameters have a major role in categorizing for the health status of nonagenarians and it was already found to be associated with mortality in elderly subjects (McDermott *et al*., 2008). Moreover, this classification is also able to select the best group of subject from the whole population and its discriminatory capacity was validated with the survival analysis, also corrected for age.

On the other hand, it should be noted that only Italian recruiting units collected haematological and biochemical parameters on GEHA 90+ siblings, because the clinical check-up was not a compulsory activity of the GEHA project. Therefore, it would have not been possible to apply the model N.1 we proposed to the other GEHA dataset collected by European units and it would have been difficult to compare our results with other studies because the proportion of blood samples varies very much between studies. Indeed, even in our population the laboratory parameters were available only for 79% of subjects, indicating that it was not possible to classify all of them according to this model. Additionally, it would not be totally appropriate to compare results of haematological and biochemical parameters when they are performed in different laboratories; in this sense, a good study design would imply the centralization of clinical tests in a single laboratory (not always feasible in study on European scale).

However, with all these limitations in mind, Model N.1 could be considered as a good predictor of mortality because significant differences were found when the survival curves of "The Best 1" and "The Others 1" group were compared, also when 90+ siblings are divided for age at recruitment time.

Finally, it should be noted that according to model N. 1, the proportion of families where both siblings are in "The Best 1" group is 7%, a bit less than the initial prevision.

Advantages and disadvantages of Model N.2 ("Functional Classification")

To overcame some of the limits of Model N.1, we suggested a model N. 2 for the identification of "The Best 2" group of subjects, based on **five-item ADL scale and SMMSE** which can be used in the comparisons with results from a lot of studies and represent the most valid functional items that most aging research collect (and thereby avoiding morbidity items which differ very much between regions and studies). "The Best 2" category is defined as **"non-disabled and cognitively intact", i.e. "independent" (SMMSE ≥ 24 and ADL = 5)**. According to model

N.2, “The Best 2” group of subjects is composed of 37% of 90+ siblings. As well as model N.1, also Model N.2 is a good predictor of mortality because significant differences were found when the survival curves of “The Best 2” and “The Others 2” group were compared, also when 90+ siblings are divided for age at recruitment time. When the health status is defined by model N. 2, the proportion of families where both siblings are in “The Best 2” group is 19%, higher in respect to model N.1 and closer to the initial assumption.

On the whole, this analysis suggests that the parameters related to functional abilities should be included in the assessment of health status in the elderly (functional parameters have a major role in categorizing for the health status). Moreover, this explorative analysis through the application of the available classification models and the **new criteria** that were proposed are useful for the future genetic analysis since they were **validated as predictors of mortality by using mortality data**.

As emerged in previous studies on centenarians (Franceschi *et al.*,2000), we found that **men and women** follow different trajectories to reach longevity. Indeed, in this study we confirmed that the determinants that allow males and females to attain extreme longevity in good health are different: male nonagenarians show a more homogeneous phenotype than females, and, though far fewer in number, tend to be healthier than females. When the health status is defined by model N.1, the parameters influencing males health status are few and are only functional (going up and down the stairs, hand grip and chair test); on the contrary, females are more complex and the health status is explained both by the functional status with a much higher proportion than males, and by comorbidity. When the health status is defined by model N.2, education plays a role on the health status both in males and females and also the attitude towards life is associated to the health status in males. In addition, it should be noted that the health status is less associated with mortality in males than in females because males are healthier, but their life-expectancy is shorter than females and they die suddenly.

Interestingly, it should be noted that **UNIBO** and **ISS** recruiting units followed some **methodological differences in the recruitment**, such as UNIBO recruitment of families that spontaneously offered to participate in the study after some press release on local newspaper or some local magazine or some TV program where Prof. Franceschi explained the main aim of the GEHA project and asked to all 90+ sibpairs living in Northern Italy to contact the recruiting unit to take part in the project. Since ISS did not adopt this strategy but only contacted 90+ sibpairs

on the basis of anagraphe lists, it could represent a recruitment bias of selection (the volunteers are supposed to be in a better health status). Moreover, the descriptive part showed that some cultural and social differences were present between UNIBO and ISS subjects, such as level of education, type of occupation, type of residence, SMMSE, etc…Nevertheless, the results related to the **health status** and **mortality** are **very similar in UNIBO and ISS**. Actually, when the health status is defined by model N.1, ISS 90+ subjects (both males and females) have a higher probability than UNIBO subjects to be classified as "The Best 1". This result is reproduced also when the health status is defined by model N.2, but only on the total population. This result was very reassuring because it **justifies the choice of unify the two population in the same data analysis**.

In conclusion, we could state that this analysis contributed to the **definition of "successful" and "unsuccessful" aging** and categorising a very large cohort of our most elderly subjects into "successful" and "unsuccessful" groups provided an unrivalled opportunity to detect some of the basic genetic/molecular mechanisms which underpin good health as opposed to chronic disability.

5.4 CONCORDANCE OF THE HEALTH AND THE FUNCTIONAL STATUS AMONG GEHA 90+ SIBLINGS

The peculiarity of GEHA population resides in the presence of 90+ siblings and not simply of nonagenarians singletons. Therefore, it constitutes the election model for the identification of the parameters which are concordant among long-lived siblings and on the contrary of those parameters which are discordant among siblings. It would be of great interest to find out the concordant or discordant factors because siblings share half of the genome, they share mtDNA inherited by their mother and they have also shared the early events in life. Therefore, it is supposed that:

- ***CONCORDANT*** variables have an important ***FAMILIAR*** component, which could be determined by genetics or by environment or by stochasticity (and it has to be defined);
- ***DISCORDANT*** variables are *NOT FAMILIAR* and could be determined by the environment or stochasticity.

We are aware that the issue of concordance among 90+siblings is at the same time complicated but very intriguing and informative because it could be propaedeutic for geneticians and it could lead the future genetics analysis. Actually, the evaluation of Concordant and Discordant Families contributes to identify the best families where both siblings have the same good functional status and the same good health status (it is rare to became nonagenarian, more to became nonagenarian in good health, even more to have a 90+ sibling and even more that both of them are healthy).

The percentage of **"Concordant Good Families"** (where both siblings are in good health) is **7.3%** according to Model N.1, and it reaches **19.5%** according to Model N.2. These results are a bit lower than the expected 25% assumed at the beginning of the project. Moreover, it is note worthy that the proportion of "Concordant Families", where both siblings share the same health category is higher than the percentage of "Discordant Families" both when the health status is defined by the Model N.1 (70% of "Concordant Families") and by Model N.2 (65% of "Concordant Families"). So, we can wonder why they are discordant, because probably the discordant families are much more informative than the concordant families.

Interestingly, trying to find out those items that were concordant among siblings, we demonstrated that **parameters related to cognitive status and physical abilities are those with the highest concordance level between the proband and the second sibling**.

What are the future perspectives in the field of concordance among siblings?

These findings on the concordance of the functional and the health status among siblings could be adopted as the starting point for the determination of a sort of synthetic index of “global concordance”, containing only a restricted core of concordant variables to be assessed in order to lead genetics analysis.

5.5 SURVIVAL ANALYSIS ON GEHA 90+ SIBLINGS

On the basis of the demographic mortality curve it was predicted that, on average, for 90+ old males in the countries GEHA studied, somewhat more than half will die in 3 years, whereas for 90+ females, this figure will be somewhat less than half, assuming that they are random people from the EU. However, the sibs who were recruited were (by definition) in sibpairs and hence they are likely to be exceptionally healthy. On the other hand, many of the recruited sibs were not 90 but more than 90, and mortality increases rapidly with age. Furthermore, some of the sibs who were interviewed early in Year 1 of GEHA study, were followed for about 5 years until January 1st 2009. Indeed, since the recruitment finished 4 years from the beginning of GEHA, it was possible to follow most of the sibs for about 2-3 years or more. On the whole, the **demographic prediction** indicates that **around a half of the sibs will die during the study period** and that it will be possible to discriminate between people who die within 3 to 4 years and people who survive longer. Having a large number of people in both categories, this fact added power to the analysis.

During the follow-up **33.5%** of 90+ siblings died, with a similar proportion in UNIBO and ISS. The **mortality was analogous in males and females**, but it progressively **increased with increasing age**.

These data about the vital status of GEHA 90+ siblings are to be considered a powerful and extraordinary source because they allow to confirm and validate all the models and analysis on the definition of "healthy aging". Survival data are indeed a robust outcome for the validation of methods aimed at defining the health status of nonagenarians and centenarians, and also for the validation of the genetic analysis included as integrant part of the GEHA project.

In particular, they let us demonstrate that **Franceschi classification** has a good predictive capacity, even if it is based on morbidity and functional parameters together. Similarly, also **Model N.1** and **Model N.2** have an extraordinary good **discriminatory capacity in terms of survival**, also when the age at recruitment time is considered. Even if one is based on functional and haematological parameters and the other has only a functional base, they predict mortality at the same level. On the contrary, the mortality curves of the groups defined by Gondo classification do not differ between subjects categorized as "Exceptional" or "Normal", indicating that survival is not influenced nor by vision neither by hearing function. Similarly, also the mortality curves defined by Evert classification do not discriminate between subjects categorized as "Delayers" and "Survivors".

Finally, we checked what factors are related to mortality of GEHA 90+ siblings because it was emerging that the predictors of morbidity and mortality (for example among haematological and biochemical parameters) change with increasing age.

Indeed, common risk factors for the adult and the elderly population, such high levels of total cholesterol, LDL and tryglicerides or low levels of HDL, lose their importance in long-living subjects, such as nonagenarians and centenarians. For example, it was demonstrated that increased amounts of total cholesterol may provide a protective effect for elderly individuals (Melton *et al.*, 2006).

Do **haematological and biochemical parameters** play a role on **mortality**? Are they predictive?

A factor analysis on subjects from 40 to 108 years on 7 haematological and biochemical parameters (total cholesterol, tryglicerides, glucose plasma levels, C reactive protein, fibrinogen, white blood cell count and haemoglobin) revealed consistent clusters of variables that were different in subjects of different age. The group of very old subjects presented a decrease of complexity respect to younger and elderly groups (from three clusters which explained the seven parameters to only two clusters) and a concomitant increase of variability. With increasing age the glucidic factor and the lipidic factor reduced to one cluster (as if the regulation of glucidic and lipidic metabolisms became more and more integrated in longevity), while the inflammatory factor remain separate. Moreover, the percentage of variability explained by the inflammatory factor increases with age, supporting the hypothesis of "inflammaging" (Franceschi *et al.*, 2000b). These data could be considered as the result of the combined effects of selective and remodelling forces that act together to achieve human longevity (data still unpublished). Another evidence indicating that with increasing age the phenotype becomes less complex come from a study by Passarino *et al.* (Passarino *et al.*, 2007), who demonstrated that the use of parameters reflecting cognitive, psychological and physical function to study the aging phenotype is useful among old subjects (65-85 years) because it is a discrete measure of frailty, but among nonagenarian subjects it loses its discriminatory function, indicating that nonagenarians lose variability in terms of frailty. In this scenario, it could be assumed that **after 90 years of age the predictive capacity of haematological parameters in relation to mortality decreased**.

Nevertheless, in our analysis we demonstrated that **haemoglobin, creatinine and also CRP** are those parameters which play the **most important role in terms of survival probability**. Actually, haemoglobin and creatinine are associated with survival also when the health status is defined only by functional parameters. This evidence suggests that haematological and biochemical parameters continues to be associated with survival also after 90 years of age. To

clarify better this issue it would be now important to deepen those pathways that are hidden behind haemoglobin and creatinine and that are probably the key ones. Moreover, in nonagenarians the **functional status** (cognitive function and autosufficiency for the basic ADL) gains importance both as a determinant of the health status and also as a predictor of mortality. To this regard, it is worth noting that, at an even more exceptional old age (after age 100) survival is mainly dependent on physiological reserve, physical and cognitive function, and that in very old stochastic determinants may dominate over programmed factors, such as family longevity, in determining survival, as found in a study on Swedish centenarians (Hagberg and Samuelsson, 2008). Interestingly, the association between the **health status** and the **vital status** is **protective only for females**, suggesting that for males the fact of being classified in "The Best" category is less protective than for females, in accordance with the **male-female health-survival paradox** (Oksuzyan *et al.*, 2008).

Thus, these findings open a huge, but fundamental question: which is the core of parameters that are sufficient to determine the health status in males and females? We can assume that they hide a thick network of metabolisms and regulatory systems, thus representing the summary of a very complex system. The definition of this essential core will be fundamental for improving quality of life of elderly and for defining *ad hoc* assistance programmes.

5.6 POTENTIAL IMPACT OF THE STUDY AND THE GEHA PROJECT

This study, in accordance with the main objectives of the whole GEHA project, represents one of the first attempt to identify the biological and non biological determinants of successful/unsuccessful aging and longevity. Here, the analysis was performed on 90+ siblings recruited in Northern and Central Italy and it could be used as a **reference for others studies in this field on Italian population**. Moreover, it would be welcome if it could be replicated in other European regions in order to evaluate if the results are reproduced or not, for example owing to historical and genetic differences of the populations. Therefore, it could **give rise to a series of phenotypic analysis on the global GEHA dataset** (collected by all the recruiting units), that will complete the genetic analysis, according to the multidisciplinary perspective which is at the bases of the GEHA project. Indeed, particular genes and genes variants associated with successful/unsuccessful aging and longevity could be used as new and innovative targets for diagnostic and therapeutic strategies of age-related pathologies and disabilities. These findings also represent a starting point for new activities to be developed and exploited by the European biotech companies which are part of GEHA consortium.

In particular the development of the following outcomes can be predicted:

- **development of *ad hoc* protocols**, standardized at the European scale, for the assessment of the health status of the oldest old;
- **development of new *ad hoc* algorithms** capable of combining clinical, social and genetic data in order to identify subgroups of old people at higher risk for the development of age-related diseases/disabilities;
- **development of *ad hoc* microarrays** for the assessment of successful (healthy)/unsuccessful aging;
- **development of molecular biology methods** capable of exploiting the knowledge related to the genes associated with healthy aging and longevity to counteract the activity of genes related to major age-related diseases and disabilities;

5.7 CONTRIBUTION TO POLICY DEVELOPMENTS

Increasing the proportion of Europeans who benefit from **healthy aging** would permit an increasing percentage of the older members of the European Community to continue a socially and economically productive life. The topic of the biological determinants of healthy aging will allow to identify new markers to be utilized for the identification of subgroups of old European citizens having a higher risk to develop age-related diseases and disabilities.

The GEHA project **has a real possibility of directing major preventive medicine strategies** for the new epidemic of chronic disease in the 21st century as well as having a positive economic impact on the European Community.

6. CONCLUSIONS

With the present work we aimed at characterizing GEHA 90+ siblings phenotypes and at identifying biological and non biological determinants of successful/unsuccessful aging and longevity. Specifically, the *major objectives* were the following:

1. to outline the **recruitment procedure of 90+ siblings from 11 European regions**;
2. to assess **90+ Italian siblings** as far as their **health/functional status** is concerned on the basis of the classification methods proposed in previous studies on centenarians, and to validate the results by using **mortality** data;
3. to investigate the **concordance of health and functional status among 90+ siblings**.

This study gives interesting insights in this direction and the *key messages* to be remember could be summarised as follow:

- a **standardized protocol to assess cognitive status, physical performances and health status of European nonagenarian subjects** was set up, in respect to ethical requirements, and it is available as a reference for other studies in this field;
- the proportion of **positive response to participate in the study** reached **25.5%** (instead of the initial theoretical assumption of 50% of positive response);
- GEHA families are **enriched in long-living members and extreme survival**, and represent an appropriate model for the identification of genes involved in healthy aging and longevity;
- two simplified sets of criteria to classify 90+ sibling according to their health status were proposed, as **operational tools for distinguishing healthy from non healthy subjects**;
- the proportion of **90+ siblings in good health** was **23%** (according to Model N.1) or **37%** (according to Model N.2) (instead of the expected 50%) and the proportion of families were both siblings were in good health was 7.3% (according to Model N.1) or 19.5% (according to Model N.2);
- **cognitive** and **functional parameters** have a major role in categorizing 90+ siblings for the **health status**;
- parameters such as **education** and **good physical abilities** (500 metres walking ability, going up and down the stairs ability, high scores at hand grip and chair stand tests) are associated with a **good health status** (defined as "cognitive unimpairment and absence of disability");
- male nonagenarians show a more **homogeneous phenotype** than females, and, though far fewer in number, tend to be **healthier than females**;
- the **33.5% of the recruited 90+ siblings died during the study period** (slightly less than the expected 50% of death);
- in males the good health status is not protective for survival, confirming the **male-female health survival paradox**;

- **survival after age 90 was dependent mainly on intact cognitive status** and **absence of functional disabilities**;
- **haemoglobin** and **creatinine** levels are both associated with **longevity**;
- the most **concordant** items **among 90+ siblings** are related to the **functional status (cognitive status and physical abilities)**, indicating that they contain a **familiar** component. It is still to be investigated at what level this familiar component is determined by genetics or by environment or by the interaction between genetics, environment and chance (and at what level).

7. REFERENCES

Abbott, A., 2004. Ageing: growing old gracefully. Nature. 428: 116–118.

Andersen-Ranberg, K., Schroll, M., Jeune, B., 2001. Healthy centenarians do not exist, but autonomous centenarians do: a population-based study of morbidity among Danish centenarians. JAGS. 49: 900-908.

Andres, R., Tobin, J.D., 1977. Endocrine Systems. From Handbook of Biology of Aging. Van Nostrand Reinhold, New York.

Atzmon, G., Schechter, C., Greiner, W., *et al.*, 2004. Clinical phenotype of families with longevity. J. Am. Geriatr. Soc. 52: 274–277.

Bafitis, H., Sargent, F., 1977. Human physiological adaptability through the life sequence. J. Gerontol. 32, 402–410.

Barbieri, M., Bonafé, M., Franceschi, C., *et al.*, 2003. Insulin/IGF-I-signaling pathway: an evolutionarily conserved mechanism of longevity from yeast to humans. Am. J. Physiol. Endocrinol. Metab. 285: E1064–E1071.

Barbieri, M., Bonafé, M., Rizzo, M.R., *et al.*, 2004. Gender specific association of genetic variation in peroxisome proliferator-activated receptor (PPAR)gamma-2 with longevity. Exp. Gerontol. 39: 1095–1100).

Barzilai, N., Atzmon, G., Schechter, C., *et al.*, 2003. Unique lipoprotein phenotype and genotype associated with exceptional longevity. JAMA 290: 2030–2040.

Bathum, L., Christiansen, L., Tan, Q., *et al.*, 2005. No evidence for an association between extreme longevity and microsomal transfer protein polymorphisms in a longitudinal study of 1651 nonagenarians. Eur. J. Hum. Genet. 13: 1154–1158.

Beekman, M., Blauw, G.J., Houwing-Duistermaat, J.J., *et al.*, 2006. Chromosome 4q25, microsomal transfer protein gene, and human longevity: novel data and a meta-analysis of association studies. J. Gerontol. A Biol. Sci. Med. Sci. 61: 355–356.

Bellizzi, D., Rose, G., Cavalcante, P., *et al.*, 2005. A novel VNTR enhancer within the SIRT3 gene, a human homologue of SIR2, is associated with survival at oldest ages. Genomics 85: 258–263.

Bellizzi, D., Cavalcante, P., Taverna, D., *et al.*, 2006. Gene expression of cytokines and cytokine receptors is modulated by the common variability of the mitochondrial DNA in c-hybrid cell lines. Genes Cells 11: 883–891.

Bellizzi, D., Dato, S., Cavalcante, P., *et al.*, 2007. Characterization of a bidirectional promoter shared between two human genes related to aging: SIRT3 and PSMD13. Genomics 89: 143–150.

Bonafé, M., Olivieri, F., Cavallone, L., *et al.*, 2001. A gender–dependent genetic predisposition to produce high levels of IL-6 is detrimental for longevity. Eur. J. Immunol. 31: 2357–2361.

Bonafé M., Barbieri, M., Marchegiani, F., *et al.*, 2003. Polymorphic variants of insulin-like growth factor I (IGF-I) receptor and phosphoinositide 3-kinase genes affect IGF-I plasma levels and human longevity: cues for an evolutionarily conserved mechanism of life span control. J. Clin. Endocrinol. Metab. 88: 3299–3304.

Bonafé, M., Barbi, C., Olivieri, F., *et al.*, 2002. An allele of HRAS1 3'variable number of tandem repeats is a frailty allele: implication for an evolutionarily conserved pathway involved in longevity. Gene 286: 121–126.

Candore, G., Balistreri, C. R., Listì, F., *et al.*, 2006. Immunogenetics, gender, and longevity. Ann. NY Acad. Sci.1089, 516–537.

Capri, M., Salvioli, S., Monti, D., *et al.*, 2008. Human longevity within an evolutionary perspective: The peculiar paradigm of a post-reproductive genetics. Experimental Gerontology. 43: 53–60.

Carrieri, G., Bonafé, M., De Luca, M., *et al.*, 2001. Mitochondrial DNA haplogroups and APOE4 allele are non-independent variables in sporadic Alzheimer's disease. Hum. Genet. 108: 194–198.

Carrieri, G., Marzi, E., Olivieri, F., *et al.*, 2004. The G/C915 polymorphism of transforming growth factor beta1 is associated with human longevity: a study in Italian centenarians. Aging Cell 3: 443–448.

Caruso, C., Candore, G., Colonna-Romano, G., *et al.*, 2005. Inflammation and life-span. Science 14 (307), 208–209.

Caselli, G., Pozzi, L., Vaupel, J.W., *et al.*, 2006. Family clustering in Sardinian longevity: a genealogical approach. Exp. Gerontol. 41: 727–736.

Cevenini, E., Invidia, L., Lescai, F., *et al.*, 2008. Human models of aging and longevity. Expert Opin Biol Ther. 8(9): 1393-405.

Christiansen, L., Bathum, L., Andersen-Ranberg, K., *et al.*, 2004. Modest implication of interleukin-6 promoter polymorphisms in longevity. Mech. Ageing Dev. 125: 391–395.

Christensen, K., Johnson, T.E., and Vaupel., J.W., 2006. The quest for genetic determinants of human longevity: challenges and insights. Nat. Rev. Genet. 7:436–448.

Coskun, P.E., Ruiz-Pesini, E., and Wallace, D.C., 2003. Control region mtDNA variants: longevity, climatic adaptation, and a forensic conundrum. Proc. Natl. Acad. Sci. USA 100: 2174–2176.

Dato, S., Passarino, G., Rose, G., *et al.*, 2004. Association of the mitochondrial DNA haplogroup J with longevity is population specific. Eur. J. Hum Genet. 12: 1080–1082.

De Benedictis, G., Carotenuto, L., Carrieri, G., *et al.*, 1998. Gene/longevity association studies at four autosomal loci (REN, THO, PARP, SOD2). Eur. J. Hum. Genet. 6: 534–541.

De Benedictis, G., Rose, G., Carrieri, G., *et al.*, 1999. Mitochondrial DNA inherited variants are associated with successful aging and longevity in humans. FASEB J. 13: 1532–1536.

De Benedictis, G., Tan, Q., and Jeune, B., 2001. Recent advances in human gene longevity association studies. Mech. Ageing Dev. 122: 909–920.

De Benedictis, G., Franceschi, C., 2006. The unusual genetics of human longevity. Sci. Aging Knowledge Environ. 2006, pe20.

De Luca, M., Rose, G., Bonafé, M., *et al.*, 2001. Sex-specific longevity associations defined by Tyrosine Hydroxylase-Insulin-Insulin Growth Factor 2 haplotypes on the 11p15.5 chromosomal region. Exp. Gerontol. 36: 1663–1671. Erratum in Exp. Gerontol. 2002 37: 607–608.

De Martinis, M., Franceschi, C., Monti, D., *et al.*, 2005. Inflamm-ageing and lifelong antigenic load as major determinants of ageing rate and longevity. FEBS Lett. 579: 2035–2039.

Evert, J., Lawler, E., Bogan, H., Perls, T., 2003. Morbidity profiles of centenarians: survivors, delayers, and escapers. J Gerontol A Biol Sci Med Sci. 58(3): 232-237.

Fagnoni, F.F., Vescovini, R., Passeri, G., *et al.*, 2000. Shortage of circulating naive CD8(+) T cells provides new insights on immunode.ciency in aging. Blood 95, 2860–2868.

Fillenbaum, G.G., 1996. Functional ability. In: Ebrahim S, Kalache A, eds. Epidemiology in Old Age, 1st Ed. London: BMJ Publishing Group, 228-235.

Fraga, M.F., Ballestar, E., Paz, M.F., *et al.*, 2005. Epigenetic differences arise during the lifetime of monozygotic twins. Proc Natl Acad Sci U S A. 102(30): 10604-10609.

Franceschi, C., Monti, D., Sansoni, P., Cossarizza, A., 1995. The immunology of exceptional individuals: the lesson of centenarians. Immunol. Today. 16, 12–16.

Franceschi, C., Cossarizza, A., 1995. Introduction: the reshaping of the immune system with age. Int. Rev. Immunol. 12, 1–4.

Franceschi, C., Motta, L., Valensin, S., *et al.*, 2000a. Do men and women follow different trajectories to reach extreme longevity? Italian Multicenter Study on Centenarians (IMUSCE). Aging (Milano). 12(2): 77-84. Review.

Franceschi, C., Valensin, S., Bonafé, M., *et al.*, 2000b. The network and the remodelling theories of aging: historical background and new perspectives. Exp Gerontol. 35: 879-896.

Franceschi, C., Bonafé, M., Valensin, S., 2000c. Human immunosenescence: the prevailing of innate immunity, the failing of clonotypic immunity, and the .lling of immunological space. Vaccine 18, 1717–1720.

Franceschi, C., Olivieri, F., Marchegiani, F., *et al.*, 2005. Genes involved in immune response/inflammation, IGF1/insulin pathway and response to oxidative stress play a major role in the genetics of human longevity: the lesson of centenarians. Mech. Ageing Dev. 126: 351–361.

Franceschi, C., Bezrukov, V., Blanché, H., *et al.*., 2007. Genetics of healthy aging in Europe: the EU-integrated project GEHA (GEnetics of Healthy Aging). Ann N Y Acad Sci. 1100:21-45.

Franceschi, C., Capri, M., Monti, D., *et al.*, 2007. Inflammaging and antiinflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech. Ageing Dev. 128: 92–105).

Franceschi, C., Motta, L., Motta, M., *et al.*, 2008. IMUSCE. The extreme longevity: the state of the art in Italy. Exp Gerontol. 43(2): 45-52.

Geesaman, B.J., Benson, E., Brewster, S.J., *et al.*, 2003. Haplotype-based identification of a microsomal transfer protein marker associated with the human lifespan. Proc. Natl. Acad. Sci. USA 100: 14115–14120.

Gondo, Y., Hirose, N., Arai, Y., *et al.*, 2006. Functional status of centenarians in Tokyo, Japan: developing better phenotypes of exceptional longevity. J Gerontol A Biol Sci Med Sci.. 61(3):305-10.

Hagberg, B. and Samuelsson, G., 2008. Survival after 100 years of age: a multivariate model of exceptional survival in Swedish centenarians. Journal of Gerontology. 63A (11): 1219-1226.

Hjelmborg, J.V., Iachine, I., Skytthe, A., *et al.*, 2006. Genetic influence on human lifespan and longevity. Hum. Genet. 119: 312–321.

Ikeda, A., Iso, H., Toyoshima, H., *et al.*, 2006. JACC Study Group. Parental longevity and mortality amongst Japanese men and women: the JACC Study. J. Intern. Med. 259: 285–295.

Jeune, B., Skytthe, A., Cournil, A., *et al*., 2006. Handgrip strength among nonagenarians and centenarians in three European regions. J Gerontol A Biol Sci Med Sci. 61(7): 707-712.

Karasik, D., Hannan, M.T., Cupples, L.A., *et al.*, 2004. Genetic contribution to biological aging: the Framingham Study. J. Gerontol. A Biol. Sci. Med. Sci. 59: 218–226.

Katz, S., Ford, A. B., Moskovitz, R.W., *et al.*, 1963. Studies of Illness in the aged. The Index of ADL: a standardized measure of biological and psychosocial function. *JAMA*. 185: 914-919.

Katz, S., Downs, T.D., Cash H.R., *et al.*, 1970. Progress in development of the index of ADL. Gerontologist. 10: 20–30.

Kirkwood, T.B., and Franceschi, C, 1992. Is aging as complex as it would appear? New perspective in aging research. Ann N Y Acad Sci. 663: 412-417.

Kirkwood, T.B., Feder, M., Finch, C.E., *et al.*, 2005. What accounts for the wide variation in life span of genetically identical organisms reared in a constant environment? Mech Ageing Dev 126: 439-443.

Licastro, F., Candore, G., Lio, D., *et al.*, 2005. Innate immunity and inflammation in ageing: a key for understanding age-related diseases. Immune Ageing 18 (2), 8.

Lio, D., Candore, G., and Crivello, A., 2004. Opposite effects of interleukin 10 common gene polymorphisms in cardiovascular diseases and in successful ageing: genetic background of male centenarians is protective against coronary heart disease. J. Med. Genet. 41: 790–794.

Logan, J., 1744. Cato major; His discourse of Old Age. Philadelphia: Benjamin Franklin.

Magni, E., Binetti, G., Bianchetti, A., *et al.*, 1996. Mini Mental State Examination: a normative study in Italian elderly population, Eur. J. Neurol. 3: 1-5.

Marchegiani, F., Marra, M., Spazzafumo, L., *et al.*, 2006. Paraoxonase activity and genotype predispose to successful aging. J. Gerontol. A Biol. Sci. Med. Sci. 61: 541–546.

Matthews, E., Haimes, E., Duguet, A.M., *et al.* 2005. Informed consent of very old patients and modern genomics. Biogerontology 6: 81–84.

Maynard Smith, J., 1966. Theories of Aging From Topics in the Biology of aging. P.L. Interscience, Newyork, pp. 13–24.

McDermott, M.M., Tian, L., Liu, K., *et al.*, 2008. Prognostic value of functional performance for mortality in patients with peripheral artery disease. J Am Coll Cardiol. 51(15): 1482-1489.

Melton, P.E., Zlojutro, M., Kimminau, K., Crawford, M.H., 2006. Biological Aging and Cox Hazard Analysis of Mortality Trends in a Mennonite Community From South-Central Kansas. American Journal of Human Biology. 18: 387-401.

Molloy, D.W., Alemayehu, E., Roberts, R., 1991. Reliability of a Standardized Mini-Mental State Examination compared with the traditional Mini-Mental State Examination. Am J Psychiatry. 148: 102-105.

Nagi, S.Z., 1976. An epidemiology of disability among adults in the United States. Milbank Mem Fund Q; 54: 439-467.

Nebel, A., Croucher, P.J., Stiegeler, R., *et al.*, 2005. No association between microsomal triglyceride transfer protein (MTP) haplotype and longevity in humans. Proc. Natl. Acad. Sci. USA 102: 7906–7909.

Nicita-Mauro, V., Lo Balbo, C., Mento, A., *et al*., 2008. Smoking, aging and the centenarians. Exp Gerontol. 43(2): 95-101.

Niemi, A.K., Hervonen, A., Hurme, M., *et al.*, 2003. Karhunen PJ, Jylha M, Majamaa K. Mitochondrial DNA polymorphisms associated with longevity in a Finnish population. Hum. Genet. 112: 29–33.

Niemi, A.K., Moilanen, J.S., Tanaka, M., *et al.*, 2005. A combination of three common inherited mitochondrial DNA polymorphisms promotes longevity in Finnish and Japanese subjects. Eur. J. Hum. Genet. 13: 166–170.

Nybo, H., Gaist, D., Jeune, B., *et al.*, 2001a. Functional status and self-rated health in 2,262 nonagenarians: the Danish 1905 Cohort Survey. J Am Geriatr Soc. 49(5): 601-9.

Nybo, H., Gaist, D., Jeune, B. *et al.*, 2001b. The Danish 1905 cohort: A genetic epidemiological nationwide survey. J Aging Health. 13: 32–46.

Nybo, H., Petersen, H.C., Gaist, D., *et al.*, 2003. Predictors of mortality in 2,249 nonagenarians - The Danish 1905-Cohort Survey. J Am Geriatr Soc. 51: 1365-1373.

Oksuzyan, A., Juel, K., Vaupel, J.W., Christensen, K., 2008. Men: good health and high mortality. Sex differences in health and agin. Aging Clin Exp Res. 20 (2): 91-102.
Partridge, L., and Gems, D., 2002. Mechanisms of ageing: public or private? Nat Rev Genet. 3: 165-175.

Passarino, G., Montesanto, A., De Rango, F., *et al.*, 2007. A cluster analysis to define human aging phenotypes. Biogerontology. 8(3): 283-90.

Passeri, G., Pini, G., Troiano, L., *et al.*, 2003. Low vitamin D status, high bone turnover, and bone fractures in centenarians. J. Clin. Endocrinol. Metab. 88:5109–5115).

Perls, T.T., Wilmoth, J., Levenson, R., *et al.*, 2002. Life-long sustained mortality advantage of siblings of centenarians. Proc. Natl. Acad. Sci.USA99: 8442–8447.

Puca, A.A., Daly, M.J., Brewster, S.J., *et al.*, 2001. A genome-wide scan for linkage to human exceptional longevity identifies a locus on chromosome 4. Proc. Natl. Acad. Sci. USA 98: 10505–10508.

Rose, G., Passarino,G., Carrieri, G., *et al.*, 2001. Paradoxes in longevity: sequence analysis ofmtDNAhaplogroup J in centenarians. Eur. J. Hum. Genet. 9: 701–707.

Rose, G., Dato, S., Altomare, K., *et al.*, 2003.Variability of the SIRT3 gene, human silent information regulator Sir2 homologue, and survivorship in the elderly. Exp. Gerontol. 38: 1065–1070.

Ross, O.A., Mccormack, R., Curran, M.D., *et al.*, 2001. Mitochondrial DNA polymorphism: its role in longevity of the Irish population. Exp. Gerontol. 36: 1161–1178.

Salvioli, S., Olivieri, F., Marchegiani, F., *et al.*, 2006. Genes, ageing and longevity in humans: problems, advantages and perspectives. Free Radic. Res. 40, 1303–1323.

Santoro, A., Salvioli, S., Raule, N., *et al.*, 2006. Mitochondrial DNA involvement in human longevity. Biochim. Biophys. Acta 757: 1388–1399.

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

Selim, A.J., Fincke, G., Berlowitz, D.R., *et al.*, 2005. Comprehensive health status assessment of centenarians: results from the 1999 Large Health Survey of Veteran Enrollees. Journal of Gerontology. 64A (4): 515-519.

Svanborg, A., Bergstrom, G., Mellstrom, D., 1982. Epidemiological Studies on Social and Medical Conditions of the Elderly. World Health Organization, Copenaghen.

Tan, Q., Yashin, A.I., Bladbjerg, E.M., *et al.*, 2001. Variations of cardiovascular disease associated genes exhibit sex-dependent influence on human longevity. Exp. Gerontol. 36: 1303–1315.

Tan, Q., Bellizzi, D., Rose, G., *et al.*, 2002. The influences on human longevity by HUMTHO1.STR polymorphism (Tyrosine Hydroxylase gene). A relative risk approach. Mech. Ageing Dev. 123: 1403–1410.

Tanaka, M., Gong, J.S., Zhang, J., *et al.*, 1998. Mitochondrial genotype associated with longevity. Lancet 351: 185–186.

Terry, D.F., Wilcox, M., McCormick, M.A., et al., 2003. Cardiovascular advantages among the offspring of centenarians. J Gerontol A Biol Sci Med Sci. 58(5): M425-431.

Terry, D.F., Wilcox, M.A., Mccormick, M.A., *et al.*, 2004a. Lower all-cause, cardiovascular, and cancer mortality in centenarians'offspring. J. Am. Geriatr. Soc. 52: 2074–2076.

Terry, D.F., Wilcox, M.A., Mccormick, M.A., *et al.*, 2004b. Cardiovascular disease delay in centenarian offspring. J. Gerontol. A Biol. Sci. Med. Sci. 59: 385–389.

Terry, D.F., Sebastiani, P., Andersen, S.L., Perls, T.T., 2008. Disentangling the roles of disability and morbidity in survival to exceptional old age. Arch Intern Med. 168(3): 277-83.

The Italian Multicentric Study on Centenarians (IMSC), 1998. Laboratory parameters of Italian centenarians. Archives of Gerontology and Geriatrics. 27: 67-74.

Torroni, A., Achilli, A., Macaulay, V., *et al.*, 2006. Harvesting the fruit of the human mtDNA tree. Trends Genet. 22: 339–345.

Wack, A., Cossarizza, A., Heltai, S., *et al.*, 1998. Age-related modifications of the human α-βT cell repertoire due to di.erent clonal expansions in the CD4+ and CD8+ subsets. Int. Immunol. 10, 1281–1288.

Weinert, B.T., and Timiras, P. S., 2003. Invited review: Theories of aging. J Appl Physiol. 95: 1706-1716.

Williams, G.C., and Nesse, R.M., 1991. The dawn of Darwinian Medicine. Q. Rev. Biol. 66: 1-22.

Willcox, B.J., Willcox, D.C., He, Q., *et al.*, 2006. Siblings of Okinawan centenarians share lifelong mortality advantages. J. Gerontol. A Biol. Sci. Med. Sci. 61: 345–354.

Zhang, J., Asin-Cayuela, J., Fish, J., *et al.*, 2003. Strikingly higher frequency in centenarians and twins of mtDNA mutation causing remodeling of replication origin in leukocytes. Proc. Natl. Acad. Sci. USA 100: 1116–1121.

8. ACKNOWLEDGMENTS

First of all I'd like to thank **Prof. Claudio Franceschi**, for his mentoring during the last 7 years and for having involved me in this challenging GEHA project.

I'd like to thank **Prof.ssa Antonia Stazi** for having hosted me in her institute giving me the opportunity to engage on new statistical and epidemiological tools. I am really grateful to her humanity and the energy she was able to transfer me!

I'd like to thank **Prof. Bernard Jeune** for his tutoring during all the GEHA project and especially in the writing out of this thesis. I am really grateful to his great experience, the stimulating discussion about the best way to analyse GEHA data (they became my source of inspiration!), his kindness in teaching me something new in this field and his always clear and prompt answers.

A special thank to **Rodolfo** for supporting me on the analysis of the results (also in extreme condition), for coaching me on statistical issues, and for his indispensable effort in order to finish the thesis in time!!! And also for the relaxing moments in Rome after the intense work.

I'd like to thank **Liana Spazzafumo** for her support and extraordinary friendship during the last years, **Jutta Gampe** for her kindness during the GEHA training course in Rostock and **Maeve Rea**, so nice as professional, for her kind suggestions, together with all the others members of the GEHA project, for creating the basis of a scientific agora where discussing and planning the future directions in the genetics of aging.

I'd like to thank sincerely all the **GEHA 90+ siblings** to which I dedicated this thesis…without their participation the GEHA Project would not have been realized! During the recruitment I found friends, people that remained in my heart with their talks, smiles and expressions, such as Adelma, Virginia and their family!

I'd like to thank **all the people in Bologna lab**, in particular Silvana for her wide vision on the project (and also for the terrible monthly recruitment monitoring forms, so necessary as complicated!) and the other GEHA girls:

the “blonde Laura”, my “angel”, who shared with me all the numerous tasks in the lab and supported me practically and psychologically in the last months of the writing out of the thesis…Lau, we should keep on going with courage and enthusiasm…
Fede, the first lady of GEHA recruitment, for the stimulating brainstorming preceding our daily processes of decision making…
Maria, Mariota and Elisa, my fellow-travellers during the transfers to reach 90 siblings houses (how many very early rising to do blood drawings, and what unexpected places to reach!)…
Giustina, for the precious collaboration in the mortality follow-up of GEHA subjects (how many phone calls we made during Christmas holiday to Register Offices all over Italy!) and for the capacity to offer an unconventionally wise point of view in all situations…
And also Laurina and Annalaura, the youngest mates of the lab, for the stylistic suggestions about my thesis and for their sweetness…
I can’t forget Prof. Monti, my guide from my first University Degree onwards…Miriam and Stefano, for the indispensable help in problems solving in the lab…Rita, Laura B., Michy, Laura I., Catia, Serena, Stella, Elena and Aurelia for our relaxing week-ends and funny nights together…and also the guys of the lab Nico, Fra and Paolo…

Moreover, I’d like to thank others **friends** who efficiently contributed to this success: Andrea, who started this adventure with me…Fabio, Denny, Max, Pol, Jack, Manu, Chiara, Marco, Lucio and Pier for the wonderful holidays *“in contact with nature”*…Vale, Sere and Martina (and also the new born babies) for the interesting interaction and the relaxing time spent in a familiar atmosphere… Elia, Paolo T., Robby and Leo for the stimulating discussion…Cristian for the joy he was able to gave me with his enthusiasm and for “being with me” when I was in Rome… Fred for having accompanied me with his precious advices even if from far away… Ste for being an example that disease is not a limit but an opportunity (you are great!)… and Robi for the loving help in the last period of intense work…

And finally, I can’t help thanking my **family** with all my energy: my father and my mother, who made this higher step possible, by supporting me in any choice and always believing in my possibilities…incredibly, day by day they managed to understand my needs without any request, and, like a good fairy, they helped me with a delicate, silent and constant sustain… I’d like also to thank my grandparents, the mainstay of the family, for having inspired my interest for old subjects.

I am delighted to thank you all and…*without collaboration we cannot do anything good (as also the GEHA project has revealed)*!!!

Now, at the end of this scientific adventure I'd like to do some reflections and considerations…
This path was somewhat unexpected for me, but I realized now that this thesis is exactly what I dreamt during the sessions about "recruitment and set-up of GEHA questionnaires" on Bertinoro kick-off meeting…and this is a very important personal success!!!
What did it leave me?
Many memories (images, glances, life talks, emotions) and teachings from 90+ siblings, mainly from their countryside-popular culture. In particular, I remember the one on the importance of child education: *"as the blade of grass should grow erect from the very beginning in order to be health, at the same time child should receive the right tutoring from their birth, not later!"*.
While visiting so many families, I entered in close contact with many realities: rich, poor, alone or surrounded by many relatives and I became aware that one the most relevant ingredients to age in good condition is the mood and the attitude towards life…
Elderly is a complex phase of our life which could be considered as the highest exhibition of the sorrow that characterizes all our life, but at the same time it is accompanied by an increase of wisdom and an higher awareness of the events of life…old subjects represent where we came from and can efficiently help our choices with their precious teachings…

To conclude, I'd like to report the poems written by a long-living subject on the occasion of his 99° and 100° birthday:
All'ultimo degli "anta" ormai arrivato,
ma da buttare ancora non mi sento,
sarei forse un tantino esagerato
se io spostassi il mio traguardo ai 100?

La strada è lunga,
ma il di più l'ho fatto,
vado avanti senza darmi pena
se lungo o breve sarà l'ultimo tratto…
…ho il cuore in pace e l'anima serena!!!

APPENDIX A (Informed Consent Form)

GEHA
(GEnetics of Healthy Aging)

A Family Longevity Study

Informed Consent Form

(*for 90+ siblings)*

Dear [Potential Participant's Name here],

You are being asked to take part in our research study because you and your sister or brother are among the very oldest siblings in our country. We have learned your age through information we have retrieved from (name of the register or other sources).

Your participation in this study is your own decision. Please read this information letter and consent form carefully and take your time making your decision. We encourage you to talk with your family, friends and/or nursing staff, if you live in a nursing home, before you decide to take part in this research study.

Who we are:
We are a group of researchers at the (name of the research institution) who, for many years, have performed research on aging. This study is part of a large 5 year European study (The GEHA Project) which includes interviews of about 3,000 long-living pairs of brothers and/or sisters from 10 European countries. The study is sponsored by grants from the European Union. Any revenue generated from this research will be re-invested in non-profit scientific research on aging and longevity.

Purpose of the Study:
We want to investigate why some families live much longer than others. It is rare that two siblings live to very old age. This could mean that special circumstances apply to your family. Is the reason for your long life hidden in the genes of your family and, if so, in which genes? Or is it because your family has better health habits, such as eating healthy food, not smoking, and getting exercise? To investigate these questions further, it would be helpful for us if you would allow us to interview you and take a blood sample from you.

If you agree to take part in this study, there will be no direct medical benefit to you. However, we hope the information learned from this study will enable future generations to live to a healthy old age.

Study Procedures:
We will telephone you or visit you within 14 days after you have received this letter. At that time you will have the chance to ask any questions you may have. You or your relatives can also call us before that time at the following phone number (the phone number).

If you agree to participate, a time would be agreed with you at which the interview and other assessments could take place in your home. They would be performed by a nurse (or a medical doctor) from our institute. The nurse (or the medical doctor) will ask you some questions about the composition of your original family and about your health; she/he will ask you to do some physical exercises and she/he will then take a sample of blood. The questions will also include: life style, living conditions, how you manage everyday life, and your ability to remember. As part of the interview, you will be asked about your current medication (it would be helpful if you could have any medication that you take available for the nurse/the medical doctor to see).
The physical exercises will be in two points: In the first the nurse (or the medical doctor) will ask you to stand up from a chair without using your hands; in the second she/he will ask you to squeeze a hand grip. She/He will also take a sample of about 20 ml of blood from your arm.

(Eventually: she/he will ask you about the permission to make inquiries about your health status at your practitioner (medicine intake, diseases, hospitalizations).

We expect that the entire visit will last between 1 and 1½ hours.

Possible Inconveniences:
We hope that participating in this study will not inconvenience you. The interview might, perhaps, be a little tiring and there could be some bruising where the blood is taken from.

Confidentiality:
The information that you give us will be used purely for research purposes. All information that you provide will be treated confidentially. No information will be passed on to official authorities, and no people who participate in this study will be recognizable in any report or publication of the results. The study has been approved by the local Research Ethics Committee.

Concerning your Blood Sample:
Your blood sample will be used for studies of genes that might influence human health and life span. This is not a genetic study to test a risk of disease and so, we will not contact you directly regarding the genetic results of your blood sample.

We will cooperate with researchers from other research institutions who are participating in this European study. They may gain access to your sample purely for research purposes. In that case the researchers will not have any identifying information that could link your sample to you. Your sample will be kept separate from your identification and will be given a special code number that only we can identify as yours. The codes will be kept separate from your identifying information and each will be securely locked.

In principle the sample can be stored and used indefinitely in order to advance our genetic studies on longevity. **At any time you have the option to request a withdrawal of the sample and it will be destroyed.**

It is Entirely Voluntary to Participate
Please note that you may also interrupt your participation at any time, even during the home visit. A relative or someone from the nursing staff of your nursing home is welcome to be present at the visit, if you wish. If you or your family/relatives have any questions about the study, you are very welcome to contact us by telephone. Your decision will not affect your normal medical care.

We will contact you within the next 14 days and will inform you further about the study. On this occasion we will also answer your questions about the study. If, after this information and discussion, you feel able to consent to participate in the study, we shall ask you to sign a consent form. If, on the other hand, you wish to think about things a little longer, or talk to a relative or a friend, we would arrange to see you again at a convenient time for you. Before you were finally to sign the consent form, the researcher would wish to check that you fully understood the information you had been given. If you wish to participate but are not able to sign the

confirmation yourself, a family member or one of the staff members in the nursing home who know you very well may sign on your behalf.

Yours sincerely,

"I hereby confirm that having received the above information orally and in writing, I consent to participate in the GEHA Research Project.

I understand that this research is not connected to my normal medical care and my participation or withdrawal will not affect my normal medical care in any way.

I have been informed that my participation is voluntary, and that I can withdraw my consent to participate at any time without giving a reason."

_____
Name and Surname of the Participant

_____ _____
Signature of Participant Date

_____
Name and Surname of the Person signing on behalf of Participant

_____
Relationship with the Participant

_____ _____
Signature of the Person signing on behalf of Participant Date

_____
Name and Surname of the Interviewer

_____ _____
Signature of Interviewer Date

APPENDIX B (GEHA Family Questionnaire)

Family number | | | | | | | |

GEHA project

FAMILY QUESTIONNAIRE

Interviewer: _____

Date of interview: _____

Parents of eligible siblings

Father:		Source?
Name:		
Date of birth:	Place of birth:	
Date of death:	Age:	
Place of death:		

Mother:		Source?
Name:	Maiden name:	
Date of birth:	Place of birth:	
Date of death:	Age:	
Place of death:		

Marriage:

		Source?
Date of marriage:	Place of marriage:	

Origin of parents and grandparents:

From where is the....	**Europe**	**Africa**	**Asia**	**Other**	**Do not know**	**Exact Country/Region of birth**
Father	☐1	☐2	☐3	☐4	☐8	_____
Mother	☐1	☐2	☐3	☐4	☐8	_____
Father's father	☐1	☐2	☐3	☐4	☐8	_____
Father's mother	☐1	☐2	☐3	☐4	☐8	_____
Mother's father	☐1	☐2	☐3	☐4	☐8	_____
Mother's mother	☐1	☐2	☐3	☐4	☐8	_____

Children of the above parents – siblings to GEHA study subjects

Child no.	*Sex*	Name	*Date of birth*	Place of birth	Source - birth	Alive?	Date of death	Age at death	Source - death	GEHA – id-nr
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										

Eligible siblings

Please, for each sibling born before 31 October 1916 (88+), fill the following page.

Sibling No. _____ (from list of children) GEHA id-number : | | | | | | | |

<table>
<tr><td>Sex:</td><td colspan="2">Surname:
Given Name:</td><td>Married name:</td></tr>
<tr><td>Date of birth:</td><td colspan="3" rowspan="3">Address:</td></tr>
<tr><td>Vital status:</td></tr>
<tr><td>Date of death:</td></tr>
<tr><td>Eligible at date:</td><td>Telephone:</td><td colspan="2">Contact person:</td></tr>
<tr><td></td><td colspan="3">Notes:</td></tr>
</table>

(Copy this page as many times as necessary !!)

APPENDIX C (GEHA 90+ Siblings Questionnaire)

ID number | | | | | | | | | |

GEnetics of Healthy Aging – GEHA

A study of long-lived sibpairs in 10 European countries

Interview questionnaire

2004 – 2008

Interviewer: _____

Date of interview: _____

Text 1: Feasibility of the interview and obtaining informed consent

For the interviewer:

This page has to be filled in by the interviewer before the actual interview but after giving information about the project.

1. Is the participant able to …	**Yes, without any difficulty**	**Yes, with little difficulty**	**Yes, with great difficulty**	**No**
a. … see?	☐1	☐2	☐3	☐4
b. … hear?	☐1	☐2	☐3	☐4
c. … understand?	☐1	☐2	☐3	☐4
d. … speak?	☐1	☐2	☐3	☐4

---

2. Is the participant confined to his/her bed?

Yes, does not get out of bed at all..... ☐1

Yes, only out of bed when going to the toilet and taking a bath..... ☐2

No ..... ☐3

---

3. a. The participant consents to participate in the study?

Yes ..... ☐1

No ..... ☐2

b. The participant appears to assent and proxy consent obtained?

Yes ..... ☐1

No ..... ☐2

To be filled in by interviewer:

Proxy interview?

Yes ..... ☐*1*

No ..... ☐*2*

Sex of the participant:

Male ..... ☐*1*

Female ..... ☐*2*

Starting time of the interview: __ __:__ __

Text 2: SOCIO-DEMOGRAPHIC INFORMATION

I will start by asking you some questions about yourself and your family.

4. What is your date of birth? Day: ____ Month: _____ Year: _____

---

5. What is your place of birth? County/area code: _____

(parish, municipality, town …) _____

---

6. a. How many brothers and sisters did or do you have (excluding yourself, half-brothers and half-sisters)?

Number of brothers: _____

Number of sisters: _____

b. What is your birth order: _____

c. How many half-brothers and half-sisters do you have? Number: _____

---

7. a. How many children (biological) did you yourself have? Number: _____

b. How many are still living? Number: _____

---

8. What is your marital status at present?

Never married ..... ☐1

Married (indicate the age of your spouse) ..... ☐2 Age: _____

Divorced, separated ..... ☐3

Widow/widower (indicate the age of your spouse at his/her death)..... ☐4 Age: _____

If "Widowed", when did your wife/husband die? Year: _____

Educational level

9. a. For how many years did you go to school? Years _____

b. Did you receive any further education?

Yes ..... ☐1

No ..... ☐2

If "Yes", which? _____

---

10. ***To be filled in by interviewer:***

Never went to school ..... ☐1

Did not finish primary school ..... ☐2

Finished primary school ..... ☐3

First stage of secondary level education ..... ☐4

Second stage of secondary level education ..... ☐5

Recognised third level education: a third level education other than university degree..... ☐6

Recognised third level education: an initial university degree or recognized equivalent ..... ☐7

Recognised third level education: a higher university degree or post graduate ..... ☐8

Do not know ..... ☐88

---

11. Occupation

a. Have you ever had any occupation?

Yes ..... ☐1

No ..... ☐2

b. If "Yes", what was your main occupation for the greater part of your life?

Indicate exact occupation: _____

c. Has your spouse ever had any occupation?

Yes ..... ☐1

No ..... ☐2

Never had a spouse ..... ☐3

d. If "Yes", what was the main occupation of your spouse?

Indicate exact occupation: _____

---

12. ***To be filled in by interviewer (tick one in each column):***

	IP	**Spouse**
Legislators, senior officials and managers	☐1	☐1
Professionals	☐2	☐2
Technicians and associate professionals	☐3	☐3
Clerks	☐4	☐4
Service workers and shop and market sales workers	☐5	☐5
Skilled agricultural and fishery workers	☐6	☐6
Craft and related trades workers	☐7	☐7
Plant and machine operators and assemblers	☐8	☐8
Elementary occupations	☐9	☐9
Military	☐10	☐10
Not applicable	☐11	☐11
Never had a spouse		☐12

13. What type of housing do you live in?

House (incl. town house), farm..... ☐1

Apartment ..... ☐2

Special dwelling for elderly people ..... ☐3

Nursing home or residential care ..... ☐4

Other type: ..... ☐5

If the participant lives in a nursing home or residential care:

For how many years? _____ Go to Q.16

---

14. How many persons live in your household apart from yourself?

Number: _____

If the participant lives alone:

For how long have you lived alone? Number of years: _____ Go to Q.16

---

15. Do you live together with the following? (several answers possible)

	Yes	No
Spouse or partner	☐1	☐2
Siblings	☐1	☐2
Child/children	☐1	☐2
Other relatives.....	☐1	☐2
Friend/friends.....	☐1	☐2
Others.....	☐1	☐2

Text 3: ADL – Activities of Daily Living

Now I will ask you some questions about your ability to carry out daily chores.

For the interviewer:

These questions (16-21) aim to evaluate what the participant ACTUALLY DOES and not what he/she is able to do.

16. Eating

a. Do you usually feed yourself without anyone's help?

Yes ..... ☐1 Go to Q.17

No ..... ☐2

b. For how long have you needed help with feeding yourself?

Less than a year ago..... ☐1

1-4 years ago..... ☐2

5-9 years ago..... ☐3

10 years ago or more ..... ☐4

---

17. Getting out of and into bed

a. Do you usually get out of and into bed without anyone's help?

Yes ..... ☐1 Go to Q.18

No ..... ☐2

b. For how long have you needed help to get out of and into bed?

Less than a year ago..... ☐1

1-4 years ago..... ☐2

5-9 years ago..... ☐3

10 years ago or more ..... ☐4

---

18. Undressing and dressing

a. Do you usually undress and dress without anyone's help?

Yes ..... ☐1 Go to Q.19

No ..... ☐2

b. For how long have you needed help to undress and dress?

Less than a year ago..... ☐1

1-4 years ago..... ☐2

5-9 years ago..... ☐3

10 years ago or more ..... ☐4

---

19. Going to the toilet

a. Do you usually go to the toilet without anyone's help?

Yes ..... ☐1 Go to Q.20

No ..... ☐2

b. For how long have you needed help to go to the toilet?

Less than a year ago..... ☐1

1-4 years ago..... ☐2

5-9 years ago..... ☐3

10 years ago or more ..... ☐4

---

20. Washing all over

a. Do you usually wash yourself all over without anyone's help?

Yes ..... ☐1 Go to Q.21

No ..... ☐2

b. For how long have you needed help to wash yourself all over?

Less than a year ago ..... ☐1

1-4 years ago ..... ☐2

5-9 years ago ..... ☐3

10 years ago or more ..... ☐4

---

21. Continence

a. Do you ever leak urine when you don't want to?

Yes ..... ☐1

No ..... ☐2

b. Do you have urethal catheter or do you use incontinence pads?

Yes ..... ☐1

No..... ☐2 Go to Q.22

c. For how long have you had a urethal catheter or used incontinence pads?

Less than a year ago ..... ☐1

1-4 years ago ..... ☐2

5-9 years ago ..... ☐3

10 years ago or more ..... ☐4

For the interviewer:

The next questions (22-26) aim at evaluating whether the participant IS ABLE TO do something, even though he/she actually does not do it in normal everyday life.

22. CAN you read or clearly see ordinary newspaper print WITHOUT glasses or other aids?

Yes ..... ☐1

No ..... ☐2

IP is blind or almost blind..... ☐3 Go to Q.24

If "No", CAN you read or clearly see ordinary newspaper print WITH glasses or other aids?

Yes ..... ☐1

No ..... ☐2

I have no glasses or other aids ..... ☐3

---

23. CAN you, WITHOUT glasses or other aids, clearly see (recognize) the face of someone 4 metres away (in the other end of the room)?

Yes ..... ☐1

No ..... ☐2

If "No", CAN you clearly see (recognize) the face of someone 4 metres away (in the other end of the room) WITH glasses or other aids?

Yes ..... ☐1

No ..... ☐2

I have no glasses or other aids ..... ☐3

---

24. In a quiet room, CAN you, WITHOUT hearing aid or other aids, distinctly hear what is being said in a conversation with ONE other person?

Yes ..... ☐1

No ..... ☐2

If "No", CAN you in a quite room, WITH hearing aid or other aids, distinctly hear what is being said in a conversation with ONE other person?

Yes ..... ☐1

No ..... ☐2

I have no hearing aid or other aids..... ☐3

---

25. CAN you walk about half a kilometer/a quarter of a mile WITHOUT a cane or other walking aids or anyone's help?

Yes ..... ☐1

No ..... ☐2

If "No", CAN you walk about half a kilometer/a quarter of a mile WITH a cane or other walking aids, but WITHOUT anyone's help?

Yes ..... ☐1

No ..... ☐2

I have no cane or other walking aids ..... ☐3

---

26. CAN you go up and down the stairs, e.g. a flight of stairs or one floor WITHOUT anyone's help (you may use a cane …)?

Yes ..... ☐1

No ..... ☐2

---

27. Do you do any kind of light housework or exercise (e.g. vacuuming, sweeping, mopping floors, ironing, gardening, gymnastics or short walks)?

Yes ..... ☐1

No ..... ☐2

If "Yes", how often?

Every day, or almost every day ..... ☐1

Several times a week ..... ☐2

Approx. once a week ..... ☐3

Approx. 2-3 times a month ..... ☐4

Approx. once a month ..... ☐5

---

28. How often do you get outside (with or without anyone's help)?

Every day, or almost every day ..... ☐1

Several times a week ..... ☐2

Approx. once a week ..... ☐3

Approx. 2-3 times a month ..... ☐4

Approx. once a month ..... ☐5

Couple of times a year ..... ☐6

Never..... ☐7

If Proxy interview, go to Question 44.

Text 4: SMMSE – Standardized Mini –Mental State Examination

Now I am going to ask you some questions and give you some problems to solve. You may think that they are difficult or you may think that they are very simple.

For the interviewer

*It is **not** permitted to help the participant by suggesting options for the answer.*

For each SMMSE question, tick of:
1 *for correct answer*
0 *for incorrect answer and do not know*
88 *no answer – due to physical disability*
99 *no answer – did not wish to answer*

29. Time orientation

Allow 10 seconds for each reply.

	Correct	Incorrect		
a. What year is this? *(accept exact answer only)*	☐1	☐0	☐88	☐99
b. What season is this? *(during last week of the old season or first week of a new season, accept either season)*	☐1	☐0	☐88	☐99
c. What month of the year is this? *(on the first day of the new month, or last day of the previous month, accept either)*	☐1	☐0	☐88	☐99
d. What is today's date? *(accept previous or next date, e.g. on the 7th accept the 6th or 8th)*	☐1	☐0	☐88	☐99
e. Whath day of the week is this? *(accept exact answer only)*	☐1	☐0	☐88	☐99

30. Place orientation

Allow 10 seconds for each reply.

	Correct	Incorrect		
a. What country are we in? ..... *(accept exact answer only)*	☐1	☐0	☐88	☐99
b. What province/state/county are we in? ..... *(accept exact answer only)*	☐1	☐0	☐88	☐99
c. What city/town are we in? ..... *(accept exact answer only)*	☐1	☐0	☐88	☐99
d. *(in clinic)* **What is the name of this hospital/building?** ..... *(accept exact name of hospital or institution only)*	☐1	☐0	☐88	☐99
(in home) **What is the street address of this house?** ..... *(accept street name and house number or equivalent in rural areas)*	☐1	☐0	☐88	☐99
e. *(in clinic)* **What floor of the building are we on?** ..... *(accept exact answer only)*	☐1	☐0	☐88	☐99
(in home) **What room are we in?** ..... *(accept exact answer only)*	☐1	☐0	☐88	☐99

---

31. I am going to name 3 objects. After I have said all three objects. I want you to repeat them. Remember what they are because I am going to name them again in a few minutes.

For the interviewer:
Say them slowly at approximately 1 second intervals:

Ball ..... ☐
Car ..... ☐
Man ..... ☐

Please repeat the 3 items for me.

For the interviewer:

Allow 20 seconds for reply, if participant did not repeat all three, repeat until they are learned or up to a maximum of 5 times.

Note the number of correct answers in the first attempt: _____ ☐88 ☐99

---

32. Now I will ask you to spell "WORLD" backwards.

For the interviewer:

Spell the word "World" (you may help participant to spell "World" correctly).

D L R O W The participant's answer: _____

Allow 30 seconds to spell backwards.

Score 1 point for each correctly placed letter. If the participant cannot spell "World" even with assistance – score 0.

Score: _____ ☐88 ☐99

---

33. Now, what were the three objects that I asked you to remember?

For the interviewer:

Ball ☐
Car ☐
Man ☐

Score 1 point for each correct response regardless of order, allow 10 seconds.

Number of correct responses: _____ ☐88 ☐99

---

34. Now I will show you two objects. Then I will ask you to tell me their names:

For the interviewer:

Show a wristwatch and a pencil. Ask the participant to tell you their names.

Wristwatch ☐
Pencil ☐

Allow 10 seconds.

Score 1 point for each correct response. Accept "wristwatch" or "watch", do not accept "clock", "time", etc. Accept "pencil" only – score 0 for "pen".

Number of correct answers: _____ ☐88 ☐99

---

35. I'd like you to repeat a phrase after me: "No if's, and's or but's".

For the interviewer:

Read the following sentence: "No if's, and's or but's".

Ask the participant to repeat. Allow 10 seconds for response.
Score 1 point for a correct repetition. Must be exact, e.g. "No if's, or but's" – score 0.

Score .... ☐1 ☐0 ☐88 ☐99

---

36. I will now ask you to read the words on this page and then do what it says.

For the interviewer:

Hand participant Card A with text "Close your eyes".
If participant just reads and does not then close eyes – you may repeat: "Read the words on this page and then do what it says" to a maximum of 3 times.
Allow 10 seconds.
Score 1 point only if participant closes eyes.
Participant does not have to read aloud.

Score: ..... ☐1 ☐0 ☐88 ☐99

For the interviewer:

Ask if the participant is right or left handed.

37. Now I will now ask you to carry out a small practical task, but first I will give you instructions. Take this paper in your RIGHT/LEFT hand, fold the paper in half once with both hands, and put the paper down on your lap.

For the interviewer:

Alternate right/left hand in statement, e.g.:
- if the participant is right-handed say "Take this paper in your left hand….";
- if the participant is left-handed say "Take this paper in your right hand….".
Allow 30 seconds.
Score 1 point for each instruction correctly executed.
1. Take the paper in correct hand ☐
2. Folds it in half ☐
3. Puts it on the lap ☐

Number of correct movements: _____ ☐88 ☐99

---

38. Now I will ask you to write a complete sentence on that piece of paper.

For the interviewer:

Hand participant a pencil and a paper.
Allow 30 seconds.
Score 1 point if the sentence makes sense – ignore spelling errors.

Write the participant's sentence here: _____

Score: ..... ☐1 ☐0 ☐88 ☐99

---

39. I will now ask you to copy this figure I now show you.

For the interviewer:

Place Card B, pencil, eraser and paper in front of the participant.
Allow multiple tries until participant is finished and hands it back.
Score 1 point for correctly copied diagram. The subject must have drawn a 4-sided figure between two 5-sided figures.
Maximum time – 1 minute.

Score: ..... ☐1 ☐0 ☐88 ☐99

---

40. Total test score: _____

---

41. Did the participant complete all the tests?

Yes ..... ☐1 Go to Q.43

No ..... ☐2

---

42. Why did the participant not complete all the tests?

		Yes	No
a.	Visually impaired	☐1	☐2
b.	Hearing impaired	☐1	☐2
c.	Paralysed in the arms/Paralysed	☐1	☐2
d.	Speech impaired	☐1	☐2
e.	Did not wish to participate/Didn't want to	☐1	☐2
f.	Other reason	☐1	☐2

Other observations:

_____

_____

_____

---

43. Was the participant nervous or anxious about carrying out the tests?

Not at all ..... ☐1

A little bit..... ☐2

Quite a lot ..... ☐3

So much that it impeded the participant
or made the participant stop the tests..... ☐4

Text 5: The next questions are about your smoking and drinking habits

44. Do you smoke at present?

Yes ..... ☐1 Go to Q.46

No ..... ☐2

---

45. Did you previously smoke?

Yes ..... ☐1

No ..... ☐2 Go to Q.48

If "Yes", when did you quit smoking? Year: _____

---

46. For how many years have you smoked/did you smoke?

Number of years: _____

---

47. Have you ever smoked more than 10 cigarettes/cigars/pipes a day?

Yes ..... ☐1

No ..... ☐2

---

48. Do you drink beer, wine, or alcohol almost every day?

Yes ..... ☐1

No ..... ☐2

If proxy interview, go to Question 51.

Text 6: HEALTH AND MORBIDITY

Now I will ask you some questions about your health.

49. How is your health in general?

Very good ..... ☐1

Good..... ☐2

Fair ..... ☐3

Poor..... ☐4

Very poor ..... ☐5

---

50. How is your attitude towards life?

Optimistic ..... ☐1

Neither optimistic nor pessimistic ..... ☐2

Pessimistic ..... ☐3

---

51. For the past 6 months or more, have you been limited in activities, which people usually do, because of a health problem?

Yes ..... ☐1

No..... ☐2

---

52. Do you use any "prescribed" medicine?

Yes ..... ☐1

No..... ☐2 Go to Q.53

If "Yes", fill in the following scheme on use of prescription medicine and count how many prescribed drugs the proband uses:

a. Number of prescribed drugs: _____

b. Number of <u>different</u> diseases treated with prescribed drugs? _____

Name of medicine?	For which disease?

For the interviewer:

All prescribed medicine, which is taken on a regular basis, should be taken into account, e.g.:

Digoxin *For the heart*

If the participant cannot state the name of the medicine, but take "2 of the small red pills" every day for the stomach, you note e.g.:

Unknown *For the stomach*

Usually you may get information on the medicine by looking in the respodent's dosage box or in the contact book between the participant and the community's home care.

53. Which of the following health problems/diseases do you have?

For the interviewer:
All lines beginning with a letter should be filled in. If the participant has diseases related to the heart, the lines with numbers should also be filled in.

		Yes	Age first time	No
a.	Vision impairment	☐1	_____	☐2
b.	Hearing impairment	☐1	_____	☐2
c.	Neurological diseases (e.g. Parkinson's disease)	☐1	_____	☐2
d.	Diseases related to the heart	☐1	_____	☐2
	1. Angina pectoris	☐1	_____	☐2
	2. Irregular heart rhythm	☐1	_____	☐2
	3. Heart failure	☐1	_____	☐2
e.	High blood pressure (hypertension treated with prescribed drugs)	☐1	_____	☐2
f.	Venous insufficiency in legs/leg ulcers	☐1	_____	☐2
g.	Cancer (excluding skin cancers)	☐1	_____	☐2
h.	Chronic respiratory diseases (bronchitis/asthma)	☐1	_____	☐2
i.	Chronic renal failure	☐1	_____	☐2
j.	Diabetes	☐1	_____	☐2
k.	Arthritis, including ostearthritis or rheumatism	☐1	_____	☐2
l.	Osteoporosis (brittle bones)	☐1	_____	☐2
m.	Serious memory impairments (e.g. dementia)	☐1	_____	☐2
n.	Other mental health problems	☐1	_____	☐2

---

54. Do you currently have any other diseases which have not been mentioned?

Yes ..... ☐1

No..... ☐2

If "Yes", specify which: _____

_____

_____

---

55. Have you ever had one or more of the following diseases?

		Yes	Age first time	No
a.	Pneumonia	☐1	_____	☐2
b.	Myocardial infarction (AMI)	☐1	_____	☐2
c.	Stroke, cerebral thrombosis/haemorrhage	☐1	_____	☐2
d.	Cancer (except skin cancer)	☐1	_____	☐2
e.	Hip fracture	☐1	_____	☐2

---

56. Have you fallen within the last year?

Yes ..... ☐1

No..... ☐2

If "Yes", how many times? _____

---

57. Have you been hospitalized within the last year?

Yes ..... ☐1

No..... ☐2

If "Yes", how many times? _____

If "Yes", have you undergone major surgery?

Yes ..... ☐1

No..... ☐2

---

58. Have you lost weight during the past year?

Yes ..... ☐1

No..... ☐2

If "Yes", how much? _____ kg

Text 7: PHYSICAL TESTS

As you know, some things get more difficult to do as you grow older.
Now I will ask you to do a couple of exercises.

First I will describe to you and show you an exercise,
then I will ask you to do the same exercise.

Hand grip test

The first exercise tests the strength in your forearms and your hands. I will now ask you to squeeze the handle of this instrument as hard as possible – two times with each hand.

59. Did the participant complete the test?

Yes ..... ☐1

No..... ☐2 Go to Q.62

---

60. Right hand: _____ kg _____ kg

Left hand: _____ kg _____ kg

---

61. How was the test carried out?

Standing ..... ☐1

Sitting..... ☐2

Lying down ..... ☐3

Other remarks: _____ Go to Q.62

---

62. The test was not carried out because the participant is:

Visually impaired..... ☐1

Hearing impaired ..... ☐2

Paralyzed..... ☐3

Could not understand the instructions ..... ☐4

Confined to bed..... ☐5

Will not ..... ☐6

Other reason ..... ☐7

If "Other reason", which? _____

Chair stand test

63. Please, could you now stand up and sit down again from your chair 5 times in a row?

For the interviewer:

The participant is to stand up from an ordinary dining room chair 5 times in a row without breaks and as fast as he/she is able to. The hands should be folded across the chest. Time is to be measured by a stop watch.

Pulse before starting (resting pulse), pulse beat per 60 seconds: __ __ __

Number of seconds used for the exercise: __ __

Pulse at the end of the exercise, pulse beat per 60 seconds: __ __ __

(Stop the exercise after 60 seconds)

---

64. a. Was the participant able to carry out the exercise?

Yes ..... ☐1 Go to Q.65

No, had to stop after 1-4 attempts ..... ☐2

No, not at all ..... ☐3

b. If no, why did the test have to be stopped?

The participant used more than one minute..... ☐1

The participant used his/her arms ..... ☐2

The interviewer felt the situation to be unsafe..... ☐3

The participant did not understand the instructions. ☐4

The participant did not want to participate ..... ☐5

The participant was too tired or too weak..... ☐6

Other reason ..... ☐7

If "Other reason", which? _____

c. Is the participant able to stand up at all with the help of his/her arms?

Yes ..... ☐1

No ..... ☐2

---

65. Height.

Measured knee height: _____ cm

(Distance from the upper edge of the knee cap to the floor with a 90 degree angle in knee and foot joint)

How tall are you? _____ cm

---

66. Weight.

Measured weight: _____ kg

How much do you weigh? _____ kg

---

67. May we take a blood sample?

Yes ..... ☐1

No..... ☐2

If "No", may we take a cheek swab sample

Yes ..... ☐1

No..... ☐2

Text 8: For the interviewer

The circumstances/conditions of the interview (to be filled in by the interviewer):

68. Finishing time of the interview: **Time:** __ __:__ __

---

69. Who participated in the interview?

The participant alone ..... ☐1 Go to Q.73

The participant and the proxy ..... ☐2

The proxy alone ..... ☐3

---

70. How is the Proxy related to the participant?

Spouse ..... ☐1

Child ..... ☐2

Grandchildren ..... ☐3

Brother or sister ..... ☐4

Other relatives ..... ☐5

Nursing staff ..... ☐6

Home care assistant ..... ☐7

Friend/acquaintance ..... ☐8

Other ..... ☐9

---

71. How often does the Proxy see the participant?

Daily ..... ☐1

Weekly ..... ☐2

Monthly ..... ☐3

More seldom ..... ☐4

---

72. Who answered the questions?

The participant alone ..... ☐1

Mainly the participant ..... ☐2

The participant as much as the proxy ..... ☐3

Mainly the proxy ..... ☐4

The proxy alone ..... ☐5

---

73. Was the interview:

Easy to perform ..... ☐1

Sometimes difficult to perform ..... ☐2

Difficult to perform ..... ☐3

Family information.

If information about the family already has been collected and verified through archival resources, please tick here: ☐

Otherwise please complete the family questionnaire with information about the participant's parents and siblings.
