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LONGEVITY AND STROKE:

a study on 1.176 GeHA 90+ Italian sibs

Dott.ssa Maria Panagiota Panourgia

Coordinatore:

Chiar.mo Prof. Sandro Grilli

Relatore:

Chiar.mo Prof. Claudio Franceschi

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INTRODUCTION

AGING, HEALTHY AGING AND LONGEVITY

Life expectancy rose dramatically in the last century.

The life expectancy at birth, also known as the average lifespan, represents the mean number of years lived by a cohort of individuals and Western world allows ever larger proportions of the population to reach an age that is far beyond that of the reproductive phase (1). Medicine and Health sciences have ameliorate global health both from important drugs discoveries, first of all antibiotics, and from interventions to public health such as massive vaccinations.

Human life expectancy causes can be dived in two categories:

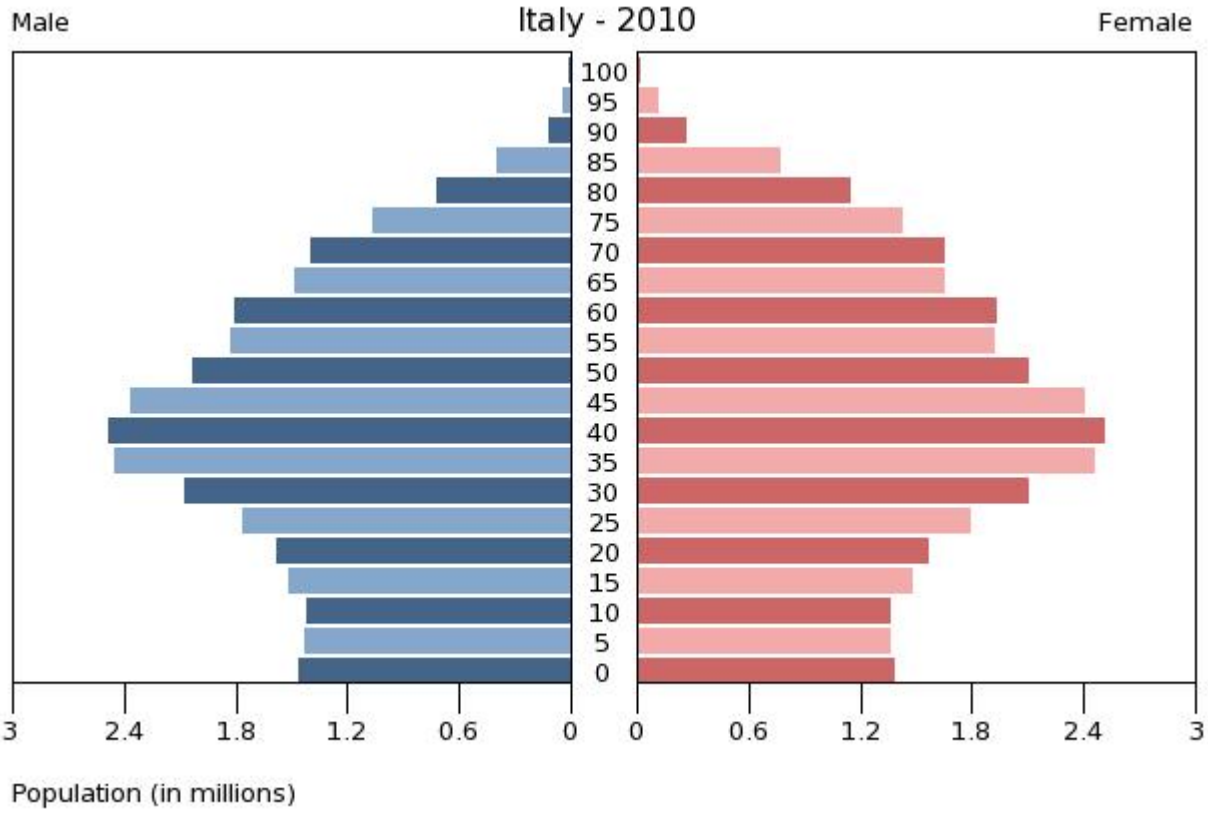
- ✓ Reduction of the infant, childhood and young adult mortality, mostly due the public health interventions and antibiotics and vaccines that reduce deaths from infectious diseases
- ✓ Reduction in old ages mortality, mostly due to prevention and pharmaceutical or procedural interventions for cardiovascular disease and cancer.

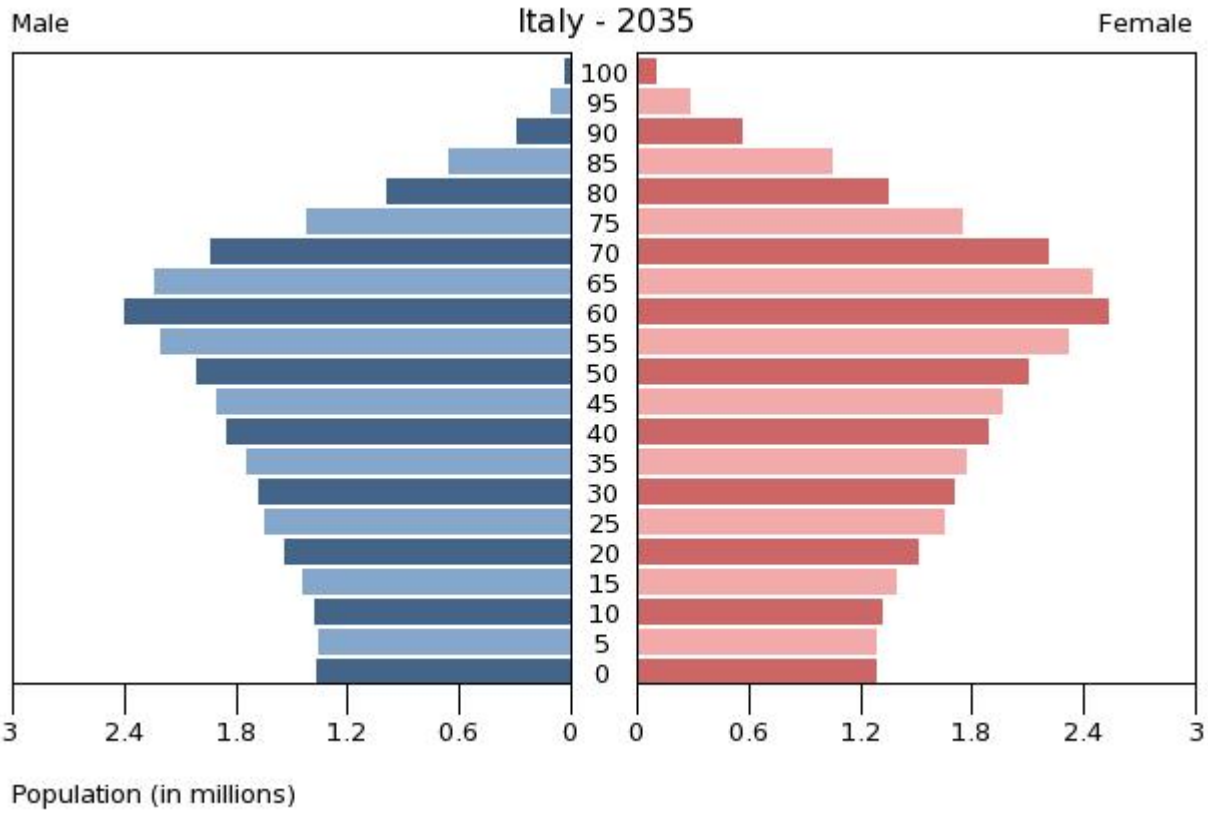
The improvements of the social-environmental conditions, and medical cares and the quality of life caused a general improvement of the health status of the population, with a consequent reduction of the overall morbidity and mortality, resulting in an increase of life expectancy. After the demographic 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. Moreover, the improvement in public health has reduced the principal causes of mortality in the elderly, allowing an increasing number of individuals to reach the maximum lifespan age that represents the age of the longest lived member(s) of the population. Indeed, around the 1950s, in all industrialized countries, the progressive decline of mortality (1–2% per year) in individuals over 80 years old has increased, so that the number of centenarians has augmented about 20 times (2-3).

In Italy, life expectancy is 76,8 years for men and 82,9 years for women (4). According to the U.S. Consensus bureau data, life expectancy in Italy is to increase, while birth rates will remain the same, producing an increasing to the old and very old population (5). (Figure 1)

The progressive increase of oldest old people has 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.

Figure 1: The age Pyramid in Italy in 2010 and 2035





AGING AND HEALTHY AGING

Until some decades ago, it was believed that all the physiological functions of the organism underwent a simultaneous age-related decline (6). 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 (7), whilst for some others, it would become evident even earlier, since the age of sexual maturation (8). Longitudinal studies suggested that the most striking age-related changes occur after the age of seventy (9). An updated vision of the phenomenon proposes that human ageing 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 ageing" (10-11), which is mostly based on evidences obtained from studies on immunosenescence. In particular, these results show that immune functions are differently affected by ageing, being some parameter strongly affected whereas some other remain unchanged or even increased (12-14). The same can be observed in centenarians in good health conditions, who have some types of immune response well preserved (15-20).

Of great interest to gerontologists is that a subset of the exceptionally aged does seem to delay or avoid major clinical diseases and disability into their 90s or 100s (21-23). These subjects are the best example of extreme longevity in our species, and they represent a selected population in which the appearance of major age-related diseases, such as cancer, and cardiovascular diseases among others, has been consistently delayed or escaped (24-26).

Immunosenescence and inflamm-ageing

A decreased ability to maintain homeostasis in response to external stressful stimuli, e.g. physical stress, starvation, can be demonstrated in the elderly. This deficit decreases the ability to maintain homeostasis, with an increased occurrence of diseases and death (27). In Western countries, the mortality of individuals over 60 years old is up to 25 times that of people 25–44 years old by the following factors: 92-fold for heart disease, 43-fold for cancer, 100-fold for stroke, 100-fold for chronic lung disease, and 89-fold for pneumonia and influenza (28). These data suggest a key role for clonotypic and innate immunity in the control of the survival of the elderly, because resistance to these diseases depends, at least in part, on a well-functioning immune system (29).

The ageing of the immune system, immunosenescence, is the consequence of the continuous attrition caused by chronic antigenic overload. Some of the most important characteristics of

clonotypic immunity in ageing are compatible with this assumption since it is characterized by a decrease of virgin T cells and by the filling up of the immunological space by "megaclones" of memory T cells, resistant to apoptosis and capable of exerting negative regulatory functions (30-33). Concomitantly, the antigenic load results in the progressive generation of inflammatory responses involved in age-related diseases, for which the name "inflamm-ageing" has been proposed (27). Most of the parameters influencing immunosenescence appear to be under genetic control, and research is trying to address this point (29, 34). Therefore, immunosenescence fits the basic assumptions of evolutionary theories of ageing, such as antagonistic pleiotropy. In fact, the immune system, by neutralizing infectious agents, plays a beneficial role until the time of reproduction and parenting. Subsequently, determining a chronic inflammation can play a detrimental role late in life, a period largely unforeseen by evolution (35).

Thus, immunological features such as a powerful innate immunity and a high capacity to mount a strong inflammatory response, useful to survive infections at younger age in the past centuries and millennia, can become detrimental later in life in economically developed countries which allow people to survive for several decades after the age of reproduction. Inflamm-ageing can thus be considered the main driving force for major age-related diseases and the evolutionary price to pay for an immune system fully capable of defending against infectious diseases at younger, reproductive age (34, 36-39). These major features of immunosenescence fit the data collected in the framework of the Italian centenarians, as discussed by Sansoni et al. (40).

Besides the pro-inflammatory status, these subjects are characterized by a state of hyper-coagulability. This last point suggest that high plasma levels of the coagulation activation markers in older population do not necessarily mirror a higher risk of arterial or venous thrombosis and it is compatible with the attainment of extreme ages, as described by Mari et al. (41).

Metabolic features

Data collected within the Italian centenarians also suggest that age-related changes in metabolic pathways and endocrine functions may occur. The findings on the higher frequency of the apolipoprotein-E (APOE) ε4 allele in middle-aged subjects than in centenarians were substantially confirmed (42). On the contrary, Italian findings did not confirm previous data on increased prevalence of the high-risk angiotensin I converting enzyme 1 (ACE1) D allele in French centenarians. The variability in the strength of association between ACE1 polymorphism and longevity could be related to regional differences in ACE1*D frequency in Europe, as recently reported for apoE ε2 and ε4 allele in centenarians (43).

Moreover, centenarians appear to have a remarkably low level of insulin resistance and favourable marker of glucose metabolism as discussed by Barbieri et al. (44). Accordingly, the phenomenon of

insulin resistance shows an age-related increase, and it reaches the highest peak in the cohort of 80- to 90-year-old people, but there is a significant reduction of insulin resistance in subjects of 90 and 100 years. The major finding of such studies was that age-related insulin resistance is not an obligatory finding in the elderly. Why oldest old subjects have a lower degree of insulin resistance compared to aged (age>65 years) subjects, is unknown. The lack of insulin resistance in healthy centenarians should be considered as the living evidence that a successful metabolic age-dependent remodelling might provide a consistent contribution for the extreme life span in this special group of subjects.

On the other hand, it results that a severe D hypovitaminosis plays an important role in the oldest old as a factor inducing a vicious circle involving hypocalcaemia, secondary hyperparathyroidism, even in the presence of sufficient renal functions, accompanied by a biochemical situation indicating a consistent loss of bone mass as discussed by Passeri et al. (45).

Furthermore, to better understand the effect of some neuroendocrine changes in ageing process, the adrenocortical, pineal and thyroid secretion in old and very old healthy subjects was studied by Ferrari et al. (46). Their findings suggest the maintenance of a certain circadian organization of melatonin secretion in centenarians and this could be considered as a marker of successful ageing.

Presence of diseases

From a clinical point of view, centenarians appear to be characterized by a large heterogeneity. Indeed their clinical status may vary from people in quite good shape from a physical and a cognitive point of view to subjects in bad shape affected by physical disability and dementia. Two pathologies appear to have a higher frequency in centenarians than in the elderly, i.e. cognitive impairment and respiratory pathologies. The former is about the 82.6% in all the centenarians (but, as pointed out, the tests used to assess cognitive ability have not been validated); the latter strike a percentage of centenarians double with respect to the elderly, basing on autoptic data on broncopneumonias and chronic obstructive pulmonary disease; moreover clinical data indicate that, apart cough and catarrh, respiratory pathologies are present in 26.2% of centenarians (IMUSCE unpublished observations).

Among centenarians, smoking is extremely rare, and even when it occurs among them, it is correlated almost exclusively to bad health conditions and non-autosufficiency, indicating that it compromises health status and the quality of life even in extremely long-living subjects (47).

Moreover, it appears that diseases as Parkinson disease, diabetes, thyreopathies are less frequent in centenarians than in elderly people. In particular, it is interesting to note that diabetes occurs in the oldest old population with a lower frequency than in the normal elderly, and becomes clinically

apparent usually only after 90 years of age. Diabetes in the oldest old persons is paucisymptomatic, and neodiagnosed diabetes is most frequently found in centenarians. The long-lasting diabetes, which starts at an age of about 60 years or earlier, usually does not allow attaining 100 years of age. Another major killer in old age is represented by cardiovascular diseases. It would be expected that centenarians are protected from this type of pathologies (25,26) and indeed the data on 140 autopsies in centenarians compared with the same number of autopsies in elderly subjects suggest that, while ischemic cardiopathies and cerebrovascular pathologies are similar in the two groups, acute myocardial infarction and cardiac amyloidosis are much less and much more frequent in centenarians than in elderly people, respectively.

In conclusion, the best definition for the healthy aging in very old subjects could be *"good healthy status for their age"*. It combines the awareness that centenarians are *de facto* extremely old, and shows the sign of ageing, but at the same time 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, these data suggest that "healthy ageing" 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(48).

LONGEVITY

Human longevity is *a complex trait* resulting from the interaction among environment, genetics and stochasticity, (49,50) which has specific and unusual characteristics (38, 51, 52). Human longevity does not appear to be homogenously distributed from a geographical point of view, (53-55) and it seems to be clustered in families enriched in long-lived parents and ancestors (56). Human studies of longevity face numerous theoretical and logistical challenges, as the determinants of life span are extraordinarily complex (57). Longevity can be achieved by different combinations of genetics, environment and chance, that vary, quantitatively and qualitatively, in different geographic areas according to the population-specific gene pool and to the socioeconomic level of the population. (51)

However, *large-scale* linkage studies of long-lived families, longitudinal candidate-gene association studies and the development of analytical methods provide the potential for future progress, assuming that both public and private genetic variants contribute to such a very complex trait as the healthy aging and longevity in humans (57).

Extreme longevity could be considered as a new phase of life which is characterized by two types of remodelling:

a) immunological remodelling (immunosenescence).

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 procees (inflammaging) (27), which however is compatible with 100 years of age, because centenarians have also high levels of anti-inflammatory markers and protective genotypes of important molecules.

b) genetic remodelling (post-reproductive genetics).

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 (genes involved in IGF-1/Insulin pathway (58)), protective genes become progressively more important with age (like Il-10).

Recent studies on the **genetic of human longevity** have suggest that:

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 cerebrovascular diseases (CVD), diabetes, and cancer, when compared to appropriate controls.(25-26, 59-61). 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 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 (62).
- ✓ 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 (63).
- ✓ Their successful aging seems to be largely influenced by their optimal balance between inflamm-aging and anti-inflammaging (52). 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 (inflamm-aging), 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) (58, 64-72). 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 (73).

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, Islandic, 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 reach extreme longevity (90–99 years old) (59). 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 (60)
- ✓ 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 (61).

(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 (74).
- ✓ 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 (55). 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 (75).

Schoenmaker *et al*. (75) *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 offsring (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*.(76) start from the consideration that although human familiy 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 indiduals) 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.

(4) *OFFSPRING OF CENTENARIANS* presents a lower prevalence of CVD (56%), hypertension (66%), and diabetes (59%) 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 (58).

- ✓ 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 (59).
- ✓ They had a favorable 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) (77), and the −641 C allele in APOC3 gene (60), similar to what has been observed in parents of centenarians.

Christensen *et al*. (57) 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.

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.

At present, it is still unknown how much this familiar component of longevity and successful ageing 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.

STROKE

Symptoms of stroke include numbness, weakness or paralysis, slurred speech, blurred vision, confusion and severe headache (78).

Stroke is defined by the World Health Organization (79) as a clinical syndrome consisting of 'rapidly developing clinical signs of focal (at times global) disturbance of cerebral function, lasting more than 24 h or leading to death with no apparent cause other than that of vascular origin'.

Stroke is divided in **Ischemic** (80% of all stroke) and **Hemorrhagic stroke**.
Ischemic stroke can be subdivided in *major Ischemic stroke* and in *Transient Ischaemic Attack (TIA)*, while **Hemorrhagic stroke** can be caused by an *Intracerebreral* (15-20% of all strokes) or S*ubarachnoid* (3% of all strokes) haemorrhage.

A transient ischemic attack (TIA) is defined as stroke symptoms and signs that resolve within 24 hours. However, there are limitations to these definitions. By conventional clinical definitions, the presence of focal neurological symptoms or signs lasting <24 hours has been defined as a TIA. With more widespread use of modern imaging techniques for the brain, up to one third of patients with symptoms lasting <24 hours have been found to have an infarction (80, 81). This has led to a new tissue-based definition of TIA: *a transient episode of neurological dysfunction caused by focal brain, spinal cord, or retinal ischemia, without acute infarction* (81). The distinction between TIA and ischemic stroke has become less important in recent years because many of the preventive approaches are applicable to both. TIA and ischemic stroke share pathophysiologic mechanisms, but prognosis may vary depending on severity and cause, and definitions are dependent on the timing and extent of the diagnostic evaluation.
The true prevalence of TIA is difficult to measure because a large proportion of patients who experience a TIA fail to report it to a healthcare provider (82). A TIA is an important predictor of stroke. The 90-day risk of stroke after a TIA has been reported as being as high as 17%, with the greatest risk apparent in the first week.(83-84).

The **classification of ischemic stroke** is based on the presumed mechanism of the focal brain injury and the type and localization of the vascular lesion. The classic categories have been defined as (85).

- ✓ large-artery atherosclerotic infarction, (extracranial or intracranial);

- ✓ embolism from a cardiac source;
- ✓ small-vessel disease;
- ✓ other determined cause such as dissection;
- ✓ hypercoagulable states;
- ✓ sickle cell disease;
- ✓ infarcts of undetermined cause.

EPIDEMIOLOGY

According to the WHO Global Burden of disease Project, Cerebrovascular disease represents the 2nd cause for global mortality and according the 2002 data was responsible for the death of 5.5 million people, representing the 9.7% of all deaths.

The influence of Cerebrovascular Disease on global mortality is higher in the high-income countries were is the 3rd cause of burden of disease, measured in measured in disability-adjusted life-years (DALY), whereas in low and middle-income countries corresponds to the 6th cause of DALY (86).

In **Europe**, the stroke incidence is similar in different regions even if it is slightly higher in the Northern area than in the Southern one (87).

In **Italy**, according the Italian guidelines on stoke, stroke represents the 3rd cause of mortality after the cardiovascular diseases and neoplasies, causing the 10-12% of all deaths/year and is the principal factor of invalidity . The prevalence of stroke in the elderly population (>65 y.o.) is of the 6,5% (7,4% of males and 5,9% of females) (88).

Stroke is also the leading cause of serious **long-term disability**. Studies using longitudinal data have determined the influence of older age and sex on disability after stroke (89-90), In the Framingham Study, women were found to be significantly older (75.1 vs 71.1) at the time of their initial stroke, more likely to be dependent in activities in daily living and mobility (91) and more than four times as likely than men to be institutionalized in a nursing home after stroke (92).

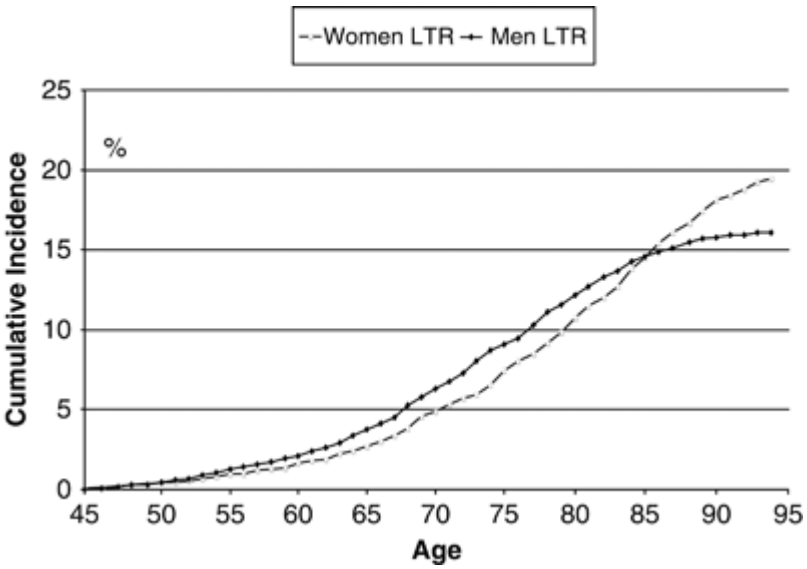
The devastating residual disability associated with stroke, in addition to presence of other chronic illnesses at the time of the stroke, makes stroke one of the most feared consequences of aging.

STROKE IN THE ELDERLY

The risk of stroke increases with age, the incidence doubling with each decade after the age of 45, and more than 70% of all strokes occur in people aged 65 and older. Of the estimated 795,000 new or recurring strokes that occur in the United States each year, approximately 145,000 will result in death (93). For the 6.5 million individuals who survive a stroke and are alive today, nearly half will have moderate to severe neurological deficits, 30% will be unable to walk unassisted, and more than 25% will need assistance in their daily activities.

According Kelly-Hayes recent review on the influence of age on stroke risk, the overall lifetime risk for stroke (LTR) remaines relatively constant until 75 years of age, at 1 in 5 for women (20% to 21%) and approximately 1 in 6 for men (14% to 17%). The LTR at 85 years of age is lower (16% for women and 10% for men; $P<0.05$ compared with participants 55 to 75 years of age). Although the incidence rate of stroke increased with age, this was counterbalanced by a decreasing residual life expectancy (Figure 2) (93).

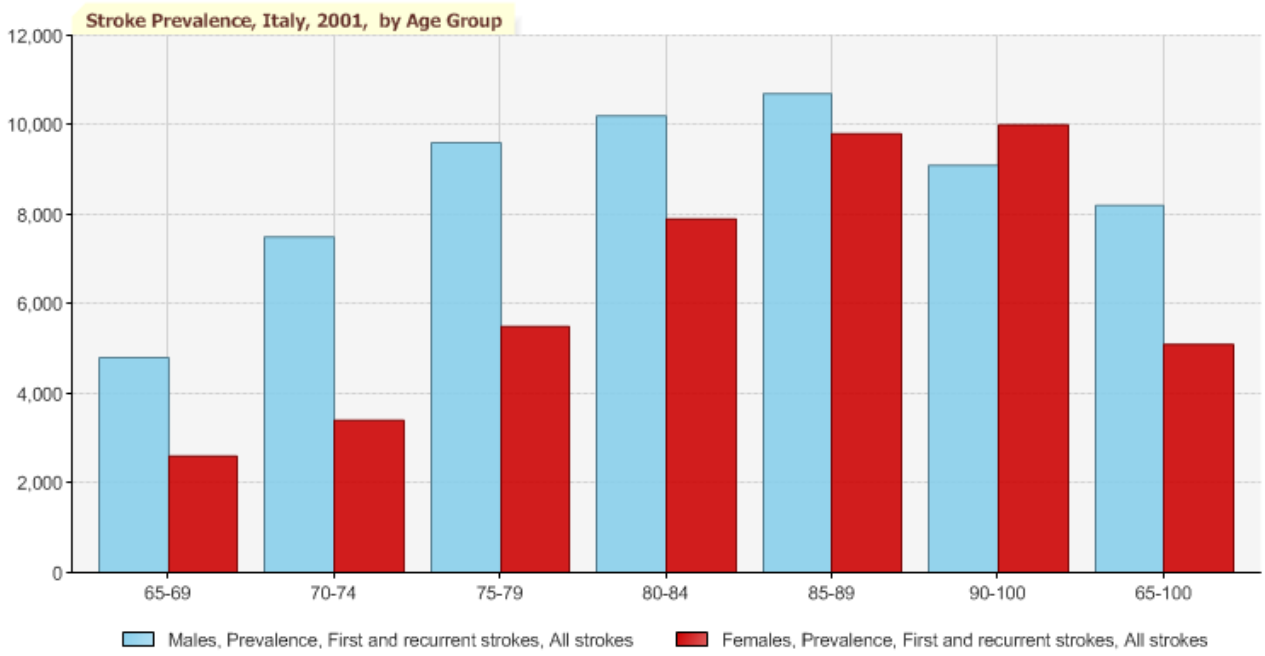
Figure 3. Sex-specific mortality-adjusted cumulative incidence of lifetime risk (LTR) of stroke.



Similar results are reported in the Orlandi et al study (94) performed in an Italian rural population were as old age advances from 65 to 90 years and over, the prevalence of stroke increases a little more than twice in men and up to about fourfold in women, and at the oldest ages it tends to decrease in men whereas it reaches a plateau in women. An excess in mortality and case fatality, especially in males, likely accounts for this trend in the oldest subjects. Moreover, despite the overall prevalence values of 8.2% in males and 5.1% in females, the difference between sexes

decreases with age and becomes lower in men when compared to the women in the oldest range (figure 3).

Figure 3. Stroke prevalence in Italy, 2001



Source: Orlandi G, Gelli A, Fanucchi S, Tognoni G, Acerbi G, Murri L, Prevalence of stroke and transient ischaemic attack in the elderly popualtion of an Italian rural community, 2003

WHO Global Infobase (IBRef: S00959a1)

According the Italian Longitudinal Study on Aging (ILSA), an epidemiological study performed on 5,632 individuals aged 65-84, has demonstrated that incidence for first-ever stroke was 9.51 (95% CI: 7.75-11.27) per 1,000 person years and 12.99 (95% CI: 10.99-14.98) including recurrent stroke (total incidence). Crude mortality resulted 49.2% among first stroke patients and 15% among persons without stroke. The first-ever stroke mortality risk ratio, adjusted for demographics and comorbidity, was 2.40 (95% CI: 1.62-3.54). The first-ever stroke mortality risk ratio, adjusted for demographics and comorbidity, was 2.40 (95% CI: 1.62-3.54). In survivors, impairment of at least one ADL was present in 67.6% of first-ever stroke patients vs. 31.6% of individuals without stroke. The comorbidity-adjusted OR was 2.63 (95% CI: 1.20-5.78) in the total cohort, and 4.00 (95% CI: 1.39-11.46) in individuals without disability at baseline (95).

STROKE RISK FACTORS

The American Heart Association and the American Stroke Association (96, 97) latest version of the for Primary Prevention of Stroke Guidelines, published in 2010, classifies the risk factors for stroke in

- **Nonmodifiable risk factors**
  - ✓ Age
  - ✓ Sex
  - ✓ Low birth weight (<2500 g)
  - ✓ Race/ethnicity
  - ✓ Family history of stroke/TIA
- **Well-Documented and Modifiable Risk Factors**
  - ✓ Hypertension
  - ✓ Cigarette smoking
  - ✓ Diabetes
  - ✓ Dyslipidemia
  - ✓ Atrial Fibrillation
  - ✓ Other Cardiac conditions (i.e. heart failure, valvular heart disease etc)
  - ✓ Asymptomatic Carotid Stenosis
  - ✓ Sickle Cell Disease
  - ✓ Postmenopausal Hormone Therapy
  - ✓ Oral Contraceptives
  - ✓ Diet and Nutrition
  - ✓ Physical Inactivity
  - ✓ Obesity and Body Fat Distribution
- **Less Well-Documented or Potentially Modifiable Risk Factors**
  - ✓ Migraine
  - ✓ Alcohol Consumption
  - ✓ Sleep-Disordered Breathing
  - ✓ Drug abuse
  - ✓ Hyperhomocysteinemia
  - ✓ Hypercoagulability
  - ✓ Inflammation and Infection

Nonmodifiable Risk Factors

Age

Stroke is a disease of the elderly. Although younger age groups (25 to 44 years) are at lower stroke risk (102) the public health burden is high in these populations because of a relatively greater loss of productivity and wage-earning years. The cumulative effects of aging on the cardiovascular system and the progressive nature of stroke risk factors over a prolonged period substantially increase the risks of both ischemic stroke and intracerebral hemorrhage (ICH). The risk of ischemic stroke and ICH doubles for each successive decade after age 55. (97, 103-107)

Sex

Stroke is more prevalent in men than in women (97, 108). Men also generally have higher age-specific stroke incidence rates than women have (based on age-specific rates calculated from strata defined by race/ethnicity), and this is true for ischemic as well as hemorrhagic stroke (97, 103, 107-110) The exceptions are those 35 to 44 years of age and those >85 years of age (110,111).

The earlier cardiac-related deaths (ie, competing causes of death) of men with cardiovascular disease (CVD) may contribute to the relatively greater risk of stroke in older women.

Genetic Factors

A meta-analysis of cohort studies showed that a positive family history of stroke increases risk of stroke by approximately 30% [odds ratio (OR), 1.3; 95% CI, 1.2 to 1.5, $P<0.00001$] (112).

The odds of both monozygotic twins having strokes are 1.65-fold higher than those for dizygotic twins (112).

Cardioembolic stroke appears to be the least heritable type of stroke compared with other ischemic stroke subtypes (113). Women with stroke are more likely than men to have a parental history of stroke (114).

The increased risk of stroke imparted by a positive family history could be mediated through a variety of mechanisms, including:

1. genetic heritability of stroke risk factors,
2. inheritance of susceptibility to the effects of such risk factors,
3. familial sharing of cultural/environmental and lifestyle factors, and
4. interaction between genetic and environmental factors.

Genetic influences on stroke risk can be considered on the basis of individual risk factors, genetics of common stroke types, and uncommon or rare familial stroke types. Many of the established and emerging risk factors described in the sections that follow, such as hypertension, diabetes, and hyperlipidemia, have both genetic and environmental/behavioral components (115-117). Elevations of blood homocysteine occur with 1 or more copies of the C677T allele of the methylenetetrahydrofolate reductase gene (118).

Some apparently acquired coagulopathies, such as the presence of a lupus anticoagulant or anticardiolipin antibody, can be familial in approximately 10% of cases (125,126). Inherited disorders of various clotting factors (ie, factors V, VII, X, XI, and XIII) are autosomal recessive traits and can lead to cerebral hemorrhage in childhood or the neonatal period (119). Arterial dissections, moyamoya disease, and fibromuscular dysplasia have a familial component in 10% to 20% of cases (127,128).

Common variants on chromosome 9p21 adjacent to the tumor suppressor genes *CDKN2A* and *CDKN2B*, which were initially found to be associated with MI (129-131), have been found to be associated with ischemic stroke as well (132). Common variants on 4q25 adjacent to the *PITX2* gene involved in cardiac development were first shown to be associated with atrial fibrillation (133). This locus was subsequently associated with ischemic stroke, particularly cardioembolic stroke (134).

Several rare genetic disorders have been associated with stroke. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is characterized by subcortical infarcts, dementia, and migraine headaches (135). CADASIL can be caused by any of a series of mutations in the Notch3 gene (135,136). Marfan syndrome (caused by mutations in the fibrillin gene) and neurofibromatosis types I and II are associated with an increased risk of ischemic stroke (137).

Fabry disease is a rare inherited disorder that can also lead to ischemic stroke. It is caused by lysosomal α-galactosidase A deficiency, which causes a progressive accumulation of globotriaosylceramide and related glycosphingolipids. Deposition affects mostly small vessels in the brain and other organs, although involvement of the larger vessels has been reported (138).

Intracranial aneurysms tend to be more common within families (139-142).

Intracranial aneurysms are a feature of certain Mendelian disorders, including autosomal dominant polycystic kidney disease (ADPKD) and Ehlers-Danlos type IV (EDS-IV) syndrome (so-called vascular Ehlers-Danlos). Intracranial aneurysms occur in about 8% of individuals with ADPKD and 7% with cervical fibromuscular dysplasia (143,144). EDS-IV is associated with dissection of vertebral and carotid arteries, carotid-cavernous fistulae, and intracranial aneurysms (145).

Well-Documented and Modifiable Risk Factors

Hypertension

Hypertension is a major risk factor for both cerebral infarction and ICH. The relationship between blood pressure (BP) and stroke risk is strong, continuous, graded, consistent, independent, predictive, and etiologically significant (146). Throughout the usual range of BPs, including the nonhypertensive range, the higher the BP, the greater the risk of stroke (147). The risk of stroke increases progressively with increasing BP, and a substantial number of individuals have a BP level below the current drug treatment thresholds recommended in the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure

Cigarette Smoking

Virtually every multivariable assessment of stroke risk factors (eg, Framingham (148), Cardiovascular Health Study [CHS] (105), and the Honolulu Heart Study (149) has identified cigarette smoking as a potent risk factor for ischemic stroke. associated with an approximate doubling of risk for ischemic stroke (after adjustment for other risk factors). Data from studies largely conducted in older age groups also provide evidence of a dose-response relationship. Smoking is also associated with a 2- to 4-fold increased risk for subarachnoid hemorrhage (SAH) (150-153). The data for ICH, however, are inconsistent. A multicenter case-control study found an adjusted odds ratio of 1.58 (95% CI, 1.02 to 2.44) (154) for ICH and analyses from the Physicians' Health Study (153) and Women's Health Study (WHS) (152) also found such an association. But other individual studies, including a pooled analysis of the ARIC and CHS cohorts, found no relationship between smoking and risk of ICH (103,106,155,156). A meta-analysis of 32 studies estimated the relative risk for ischemic stroke to be 1.9 (95% CI, 1.7 to 2.2) for smokers versus nonsmokers; for SAH, 2.9 (95% CI, 2.5 to 3.5); and for ICH, 0.74 (95% CI, 0.56 to 0.98) (155).

There is a definite relationship between smoking and both ischemic and hemorrhagic stroke, particularly at young ages (157,158). The annual number of stroke deaths attributed to smoking in the United States is estimated to be between 21 400 (without adjustment for potential confounding factors) and 17 800 (after adjustment), which suggests that smoking contributes to 12% to 14% of all stroke deaths (159).

Cigarette smoking may also potentiate the effects of other stroke risk factors, including systolic BP (160), vital exhaustion (unusual fatigue, irritability, and feelings of demoralization) (161), and oral contraceptives (OCs) (162,163). The effect of cigarette smoking on ischemic stroke risk may be higher in young adults who carry the apolipoprotein E ε4 allele.

Smoking likely contributes to increased stroke risk through both acute effects on the risk of thrombus generation in atherosclerotic arteries and chronic effects related to increased atherosclerosis (164). Smoking just 1 cigarette increases heart rate, mean BP, and cardiac index and decreases arterial distensibility (165,166). Beyond the immediate effects of smoking, both active and passive exposure to cigarette smoke is associated with the development of atherosclerosis (167). In addition to placing persons at increased risk for both thrombotic and embolic stroke, cigarette smoking approximately triples the risk of cryptogenic stroke among persons with a low atherosclerotic burden and no evidence of a cardiac source of emboli (168).

Diabetes

Persons with diabetes have both an increased susceptibility to atherosclerosis and an increased prevalence of proatherogenic risk factors, notably hypertension and abnormal blood lipids.

Both case-control studies of stroke patients and prospective epidemiological studies have confirmed that diabetes independently increases risk of ischemic stroke with a relative risk ranging from 1.8-fold to nearly 6-fold (169).

In the Euro Heart Survey on Diabetes and the Heart, a total of 3488 patients were entered in the study: 59% without diabetes and 41% with diabetes (170). Evidenced-based medicine was defined as the combined use of renin-angiotensin-aldosterone system inhibitors, β-adrenergic receptor blockers, antiplatelet agents, and statins. In patients with diabetes, evidence-based medicine (RR, 0.37; 95% CI, 0.20 to 0.67; *P*=0.001) had an independent protective effect on 1-year mortality and cardiovascular events (RR, 0.61; 95% CI, 0.40 to 0.91; *P*=0.015). Although stroke rates were not changed, cerebrovascular revascularization procedures were reduced by half.

Dyslipidemia

Total Cholesterol

Most but not all epidemiological studies find an association between higher cholesterol levels and an increased risk of ischemic stroke. In the Multiple Risk Factor Intervention Trial (MRFIT), which included >350 000 men, the relative risk of death from nonhemorrhagic stroke increased progressively for each level of cholesterol. In the Eurostroke Project of 22 183 men and women, there was no relationship between cholesterol with cerebral infarction (171). Overall, epidemiological studies suggest competing stroke risk related to total cholesterol levels in the general population; high total cholesterol may be associated with higher risk of ischemic stroke, whereas lower levels are associated with higher risk of brain hemorrhage (171-186).

HDL Cholesterol

Most but not all epidemiological studies show an inverse relationship between high-density lipoprotein (HDL) cholesterol and stroke (187). HDL cholesterol was inversely related to ischemic stroke in the Copenhagen City Heart Study, the Oyabe Study of Japanese men and women, middle-aged British men, and middle-aged and elderly men in the Israeli Ischemic Heart Disease Study (188-191).

Triglycerides

The results of epidemiological studies that have evaluated the relationship between triglycerides and ischemic stroke are inconsistent, in part because some have used fasting levels and others nonfasting levels. The Copenhagen City Heart Study, a prospective, population-based cohort study composed of approximately 14 000 persons, found that elevated nonfasting triglyceride levels increased the risk of ischemic stroke in both men and women (192). Similarly, the WHS found that in models adjusted for total and HDL cholesterol and measures of insulin resistance, nonfasting triglycerides, but not fasting triglycerides, were associated with cardiovascular events, including ischemic stroke (193).

Atrial Fibrillation

Atrial fibrillation, even in the absence of cardiac valvular disease, is associated with a 4- to 5-fold increased risk of ischemic stroke due to embolism of stasis-induced thrombi forming in the left atrial appendage (194). Embolism of appendage thrombi associated with atrial fibrillation accounts for about 10% of all ischemic strokes and an even higher fraction in the very elderly in the United States (195). The absolute stroke rate averages about 3.5% per year for persons aged 70 years with atrial fibrillation, but the risk varies 20-fold among patients depending on age and other clinical features (see below) (196,197). Atrial fibrillation is also an independent predictor of increased mortality (198). Paroxysmal atrial fibrillation is associated with an increased stroke risk that is similar to that of chronic atrial fibrillation (199).

Other Cardiac Conditions

Cardiogenic embolism is the cause of approximately 20% of ischemic strokes (200). Cardioembolic strokes are relatively severe, are associated with greater neurological deficits at admission, greater residual deficits at discharge, and greater neurological deficits after 6 months compared with noncardioembolic strokes (202). Cardiac conditions associated with a high risk for stroke include atrial arrhythmias (eg, atrial fibrillation/flutter, sick sinus syndrome), left atrial thrombus, primary

cardiac tumors, vegetations, and prosthetic cardiac valves (201). Other cardiac conditions that increase the risk of stroke include dilated cardiomyopathy, coronary artery disease, valvular heart disease, and endocarditis. Stroke may occur in patients undergoing cardiac catheterization, pacemaker implantation, and coronary artery bypass surgery (203,204).

The incidence of stroke is inversely proportional to left ventricular ejection fraction (205-207).

Patients with rheumatic mitral valve disease are at increased risk for stroke (208). Mitral valvuloplasty does not eliminate this risk (209). Thromboembolic events have been reported in association with and attributed to mitral valve prolapse when no other source could be identified (210). Patients with mitral annular calcification are predisposed to embolic phenomena, particularly in older patients with dense calcifications (211). Systemic embolism from isolated aortic valve disease may also occur (211). It is less frequent in the absence of associated mitral valve disease or atrial fibrillation (212). Multiple mechanical prosthetic valves are currently available and deployed (208). The intensity of anticoagulation should be proportional to the thromboembolic risk of the individual mechanical prosthetic valve (208). Ischemic stroke occurs in 15% to 20% of patients with infective endocarditis (213,214). Mitral valve endocarditis carries the greatest stroke risk (213). The management of endocarditis is directed at the underlying etiology.

Cardiac tumors are uncommon and account for a very small minority of embolic events (215,216). Congenital cardiac anomalies, such as patent foramen ovale (PFO), atrial septal defect, and atrial septal aneurysm, can be associated with stroke, especially in younger patients (see sections on migraine and coagulopathy) (217-219). Meta-analysis of case-control studies focused on patients who have had a stroke found an increased risk in those <55 years of age (for PFO: OR, 3.10; 95% CI, 2.29 to 4.21; for atrial septal aneurysm: OR, 6.14; 95% CI, 2.47 to 15.22; and for PFO plus atrial septal aneurysm: OR, 15.59; 95% CI, 2.83 to 85.87) (220). In contrast, population-based studies find no increased risk of a first stroke associated with PFO (221,222).

Asymptomatic Carotid Stenosis

The presence of an atherosclerotic stenotic lesion in the extracranial internal carotid artery or carotid bulb has been associated with an increased risk of stroke.

Assessment of Carotid Stenosis

A "hemodynamically significant" carotid stenosis produces a drop in pressure, a reduction in flow, or both. This generally corresponds to a 60% diameter–reducing stenosis as measured by catheter angiography using the North American Symptomatic Carotid Endarterectomy Trial (NASCET) method (223). The NASCET method measures the minimal residual lumen at the level of the stenotic lesion compared with the diameter of the more distal internal carotid artery, where the walls of the artery become parallel. The following formula is used: stenosis=(1–R/D)x100%.

Sickle Cell Disease

Sickle cell disease (SCD) is an autosomal recessive inherited disorder in which the abnormal gene product is an altered hemoglobin β-chain. Although the clinical manifestations are highly variable, SCD typically manifests early in life as a severe hemolytic anemia with painful episodes involving the extremities and bones ("vaso-occlusive crises"), bacterial infections, and organ infarctions, including stroke. Other effects include cognitive deficits related to MRI-demonstrated strokes and otherwise asymptomatic white matter hyperintensities (224,225).

Postmenopausal Hormone Therapy

The Women's Health Initiative (WHI), a randomized trial of conjugated equine estrogens (CEE) combined with medroxyprogesterone acetate (MPA) versus placebo in women 55 to 79 years of age (226), has had a profound impact on the practice of prescribing these therapies to postmenopausal women (227). Additional analyses of the WHI focused on specific subgroups of women to determine those at particularly high risk (228). The risk of stroke with CEE was limited to ischemic (HR, 1.55; 95% CI, 1.19 to 2.01) and not hemorrhagic stroke (HR, 0.64; 95% CI, 0.35 to 1.18). There was no difference based on stroke etiologic subtype, severity, or mortality (228).

An analysis of the Nurses' Health Study reported similar findings: women using hormone therapy had an increased risk of stroke regardless of age at initiation or years since menopause (229).

Oral Contraceptives

The risk of stroke associated with use of OCs is low. Certain women, particularly those who are older; who smoke cigarettes; and who have hypertension, diabetes, obesity, hypercholesterolemia, and prothrombotic mutations may be at higher risk. Estimates are based primarily on case-control studies and a smaller number of cohort studies, both of which are limited by small numbers of women with stroke events. The incremental risk of stroke associated with use of low-dose OCs in women without additional risk factors, if one exists, appears to be low (230,231)

Diet and Nutrition

A large and diverse body of evidence has implicated several aspects of diet in the pathogenesis of high BP, the major modifiable risk factor for ischemic stroke. A recent AHA scientific statement

concluded that several aspects of diet lead to elevated BP (232), specifically, excess salt intake, low potassium intake, excess weight, high alcohol consumption, and suboptimal dietary pattern.

In observational studies, several aspects of diet are associated with risk of stroke. A meta-analysis found a strong, inverse relationship between servings of fruits and vegetables and subsequent stroke (233).

The effects of sodium and potassium on stroke risk are likely mediated through direct effects on BP, as well as mechanisms that are independent of BP (234).

Physical Inactivity

Physical inactivity is associated with numerous adverse health effects, including an increased risk of total mortality, cardiovascular mortality, cardiovascular morbidity, and stroke. The 2008 Physical Activity Guidelines for Americans provides an extensive review and concludes that physically active men and women generally have a 25% to 30% lower risk of stroke or death than the least active people (235).

The protective effect of physical activity may be partly mediated through its role in reducing BP (236) and controlling other risk factors for CVD (237,238), including diabetes (236), and excess body weight. Other biological mechanisms have also been associated with physical activity, including reductions in plasma fibrinogen and platelet activity and elevations in plasma tissue plasminogen activator activity and HDL-cholesterol concentrations (239-241).

Obesity and Body Fat Distribution

A large number of prospective studies have examined the relationship between weight (or measures of adiposity) and incident stroke. A meta-analysis found a nonlinear association between BMI and mortality (242). In the BMI range of 25 to 50 kg/m^2, each 5 kg/m^2 increase in BMI was associated with a 40% increased risk of stroke mortality; in the lower BMI range (15 to 25 kg/m^2), there was no relationship between BMI and stroke mortality, even after excluding smokers.

The effect of BMI on stroke risk is in part mediated by the effect of adiposity on other stroke risk factors.

Less Well-Documented or Potentially Modifiable Risk Factors

Migraine

Migraine headache has been most consistently associated with stroke in young women (<55 years of age), especially those with migraine with aura (243).

Alcohol Consumption

Excessive consumption of alcohol can lead to multiple medical complications, including stroke. Strong evidence exists that heavy alcohol consumption is a risk factor for all stroke subtypes (244-248). Most studies suggest a J-shaped association between alcohol consumption and the risk of total and ischemic stroke, with a protective effect in light or moderate drinkers and an elevated risk with heavy alcohol consumption (101,244,245,249-256). In contrast, a linear association exists between alcohol consumption and risk of hemorrhagic stroke (103,151,257,258).

Light to moderate alcohol consumption, particularly in the form of wine, is associated with reduced risk of total and ischemic stroke since determines greater levels of HDL cholesterol (259-260), reduced platelet aggregation (262,263), lower fibrinogen concentrations (264,265), and increased insulin sensitivity and glucose metabolism (266). Heavy alcohol consumption increases risk of stroke given that can result in hypertension, hypercoagulability, reduced cerebral blood flow, and increased risk of atrial fibrillation (245,250,252,265,267).

are no controlled trials demonstrating a reduction in stroke risk with abstinence.

Sleep-Disordered Breathing

Epidemiological studies suggest that habitual snoring is a risk factor for ischemic stroke, independent of confounding factors such as hypertension, ischemic heart disease, obesity, and age (268,269). Loud snoring is associated with an increased risk of carotid compared with femoral atherosclerosis (OR, 10.5; 95% CI, 2.1 to 51.8; P=0.004) independent of other risk factors, including measures of nocturnal hypoxia and severity of obstructive sleep apnea (270). Consistent with these observations, a 10-year observational study of 1651 men found that severe obstructive sleep apnea-hypopnea (according to the apnea-hypopnea index, >30 occurrences per hour of sleep) increased the risk of fatal (OR, 2.87; 95% CI, 1.17 to 7.51) and nonfatal (OR, 3.17; 95% CI, 1.12 to 7.52) cardiovascular events (MI, acute coronary insufficiency requiring coronary artery bypass surgery and/or percutaneous transluminal angioplasty, and stroke) as compared with healthy participants (271). A 6-year longitudinal prospective study of 394 noninstitutionalized, initially

event-free subjects (70 to 100 years of age, median 77.28 years, 57.1% male) found that severe obstructive sleep apnea-hypopnea (defined as apnea-hypopnea index ≥30) increased the risk of ischemic stroke independent of known confounding factors (272).

sleep-disordered breathing (SDB) SDB can increase stroke risk by leading to or worsening hypertension and heart disease and possibly by causing reductions in cerebral blood flow, altered cerebral autoregulation, impaired endothelial function, accelerated atherogenesis, hypercoagulability, inflammation, and paradoxical embolism in patients with patent foramen ovale (272-275).

Hyperhomocysteinemia

Homocysteine is an amino acid that is derived from the metabolism of the essential amino acid methionine. Increased plasma levels of homocysteine are often a consequence of reduced enzymatic activity in its metabolic pathways. This may be caused by genetic defects in the enzymes involved in homocysteine metabolism, sed by nutritional deficiencies of pyridoxine (vitamin B_6), of folic acid and cobalamin (vitamin B_{12}). Decreased renal clearance of homocysteine in patients with chronic renal failure may contribute to hyperhomocysteinemia.

Elevated levels of plasma homocysteine are associated with a 2- to 3-fold increased risk for atherosclerotic vascular disease, including stroke (276-282).

Inflammation and Infection

Stroke risks is associated with several inflammatory conditions and markers. Inflammation affects the initiation, growth, and destabilization of atherosclerotic lesions (283). A number of serum markers of inflammation, including fibrinogen, serum amyloid A, Lp-PLA2, and interleukin 6 have been proposed as risk markers. Several studies suggest a relationship between Lp-PLA2 and stroke risk (approved by the US Food and Drug Administration as a predictor of ischemic stroke and coronary artery disease), (284-286) with high-sensitivity C-reactive protein (hs-CRP) being the most commonly used (287). In addition to numerous epidemiological studies and randomized clinical trials with coronary disease end points, several epidemiological studies have identified associations between hs-CRP and stroke, including the Physician's Health Study (288), the WHS (289), and the Framingham Heart Study (290).

Another way to evaluate the role of inflammation as a risk factor for stroke is to examine the incidence of vascular disease in persons with systemic chronic inflammatory diseases, such as rheumatoid arthritis (RA) and SLE. A large number of prospective cohort studies have identified increased risks for CVD (including stroke) in persons with RA, with odds ratios consistently in the

1.4 to 2.0 range compared with persons without RA (292-296). Excess risk was especially apparent in women with RA who were 35 to 55 years of age (292). This association remained after adjustment for other cardiovascular risk factors. Similarly, patients with SLE had very elevated relative risks for CVD in the 2- to 52-fold range (297). Although stroke rates were not assessed, several studies have identified a higher prevalence of atherosclerotic plaque in the carotid arteries of patients with RA or SLE compared with control subjects (298-300).

A final issue in the role of infection and inflammation in stroke deals with the role of acute infectious diseases (eg, influenza) inducing a cerebrovascular event (TIA or stroke). Possible mechanisms include the induction of procoagulant acute phase reactants (eg, fibrinogen) or the destabilization of atherosclerotic plaques. An increase in cardiovascular deaths has long been observed in association with influenza (301,302).

Psychosocial Behaviors and Risk

It has been suggested that psychosocial well-being interacts with biological factors to influence risk of disease. Longitudinal studies provide an appropriate venue to follow the association between psychosocial well-being and health behaviors over a sufficient length of time to observe the relationship, interactions, and effect on health and disease. Evidence derived from longitudinal studies has demonstrated that marital strain, job stress, and depression are associated with coronary heart disease, mortality, and stroke (303-305). Depression has been associated with unhealthy behaviors such as inactivity, change in diet, and smoking (306,307), and starting in middle age, its presence has been associated with risk of stroke in men and women (306).

A new model for novel risk factor interaction is currently evolving around the association between social connections and health behaviors.

THE GEHA PROJECT

The origins of the GEHA Project

Europe is the oldest continent and in the last decade the number of people aged more than 90 years is rapidly rising. The actual proportion of people with more than 90 years is about 50% of the total population. There is a small proportion of elders that apparently undergoes an aging process and they surprisingly appear deprived of the most common age-related disease (cardiovascular disease, stroke, II type diabetes, cancer and dementia). In this scenario it is important to study causes and mechanisms of the aging, that in 2001 the **5-year European Union Integrated Project GEnetics of Healthy Aging (GEHA)** born. The most important aim of this study is to identify genes involved in healthy aging and longevity, which allows individuals to reach advanced old age in good cognitive and physical conditions, without the major age-related diseases.

The GEHA Project represents the strongest and the most competitive consortium ever realized in Europe to investigate genetic bases of human aging process, capable of reaching results that is impossible to obtain in a single European country.

The 5-year GEHA Project is supported through Priority 1 (Life Sciences, Genomics and Biotechnology for Health) of EU's FP6 (Project Number LSHM-CT-2004-503270) and approved by European Commission. The project started on May 1, 2004 and ended ion April 30, 2010 (with a 1-year delay).

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);

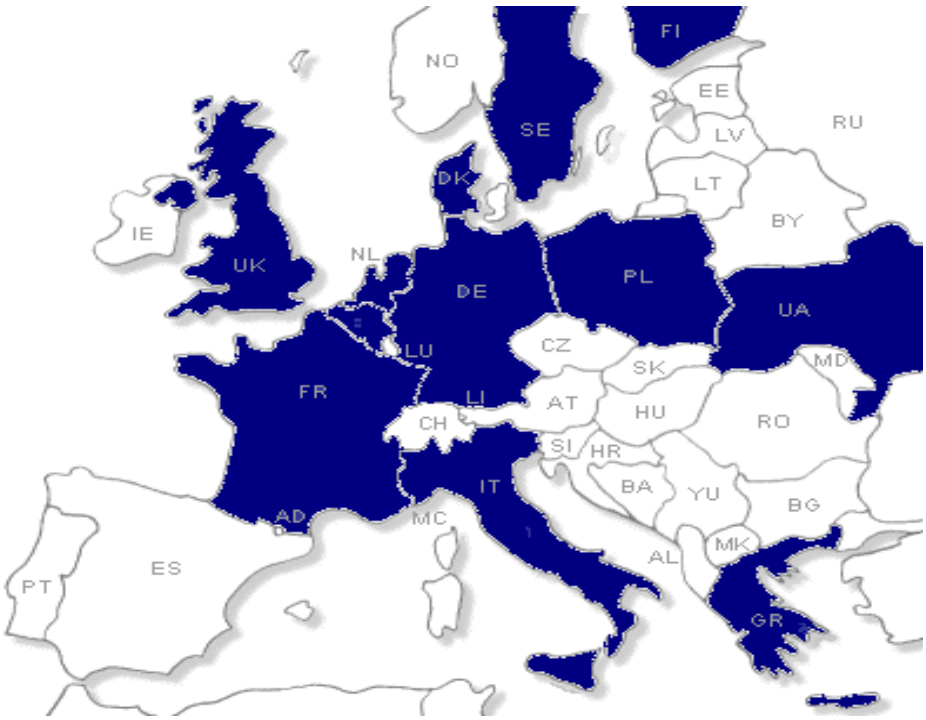
Steering Committee (SC) composed by 9 members (i.e., the leaders of the 12 Work Packages);

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)

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, camcer 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.

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) is localized in **Odense (Denmark)**;
- the **Genotypic Database** (containing genotyping data) is localized in **Kiel (Germany)**;
- the **mtDNA Database** (containing data related to mtDNA) is 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.

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.

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 5. These possibilities (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.

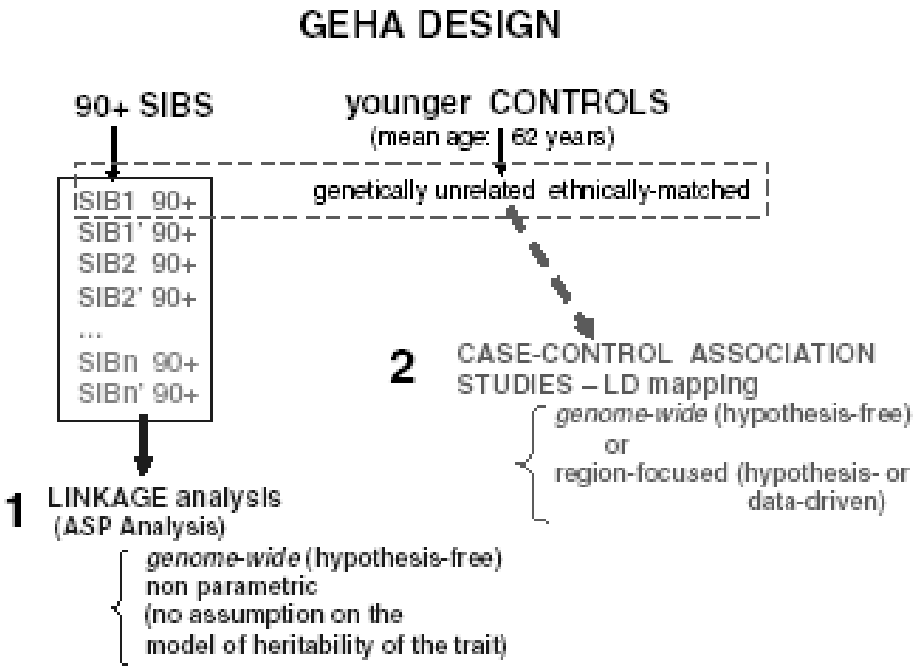


Figure 5. The design of the GEHA project allows to perform either genome-wide linkage studies, using the DNA collected from the 2650 90+ sibpairs, or association studies using the DNA collected from one (or both) member of each sibship and the DNA collected from the unrelated, ethnically matched younger 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.

The 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.

GEHA QUESTIONNAIRES

a) 90+ siblings questionnaire

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 (27).

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***) (306) 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, ***light daily exercise*** and going outside with or without anyone's help) (307).

Cognitive function is assessed by the ***standardized Mini Mental State Examination (SMMSE*)** (309).

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.

b) Controls Questionnaire

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.

c) Family Questionnaire

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.

SURVIVAL ANALYSIS

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.

MATERIALS AND METHODS.

POPULATION

Our sample consists of the 90+ sibs (Total 1176, 801 females, 375 males) collected from the 3 Italian Recruiting Units of the GeHA project.:

- University of Bologna (UNIBO) (549 subjects, 385 females and 164 males): mainly collected subjects from North Italy, mainly from Bologna area;
- Istituto Superiore di Sanità (ISS) (216 subjectes, 158 females and 58 males): collected subjects from Rome area (Central Italy)
- University of Calabria (RC) (411 subjects, 258 females and 153 males): Collected samples from the Calabria Region (South Italy)

This population contains all 90+ siblings that were interviewed and whose phenotype data were entered in the GEHA Phenotypic Database (localised in Odense, Denmark).
The **local Ethical Committees** approved the study.

CLASSIFICATION OF THE 90+ SIBS IN THE STUDY

1) PROXY INTERVIEW

A proxy-responder was encouraged to participate in the interview if the nonagenarian was unable to participate due to mental or physical handicaps.
Proxy interviews have been performed for the reason that in some families one of the siblings had poor cognitive and/or functional status, even if it's sibling(s) were in a good status.
Proxy interview data are reported to the "Results" section, even if we decide to not use these data for the rest of the analysis, in order to avoid bias from the extreme disability (cognitive and functional deficit) that the proxy interview 90+ sib is affected.

2) STROKE

We have divided pour sample in three main categories according their answer in the **55c question of the 90+ sibs questionnaire** ("Have you ever had one or more of the following diseases? Stroke, cerebral thrombosis/haemorrhage. If "yes", report age of first time").

a) ***Stroke free***: negative answer in the 55c question (960 subjects, 88.72%).
b) ***Young age stroke***: positive answer in the 55c question (42 subjects, 3.88%). Reported age of Stroke incidence **< *85 y.o.***
c) ***Old age stroke***: positive answer in the 55c question (80 subjects, 7.39%). Reported age of Stroke incidence **≥ *85y.o.***

3) COGNITIVE STATUS

Cognitive function was measured using the Standardized Mini-Mental State Examination (SMMSE) (310).

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, as reported in Table 1, and our sample has been classified in two groups:

i) Normal cognitive status (MMSE ≥24)
ii) Cognitive Impairment (MMSE < 24)

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) (309).

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

Moreover we took under consideration the items regarding the cognitive efficiency like the ability to understand the questions or the presence of serious memory impairments like dementia

4) DISABILITY

Questions in this area covered the Katz Index of activities of daily living (ADL) - bathing, dressing, toileting, transfer, feeding and continence.

Using the ADL scale we have divided our sample in:

- **ADL independent** (ADL score 5-6).
- **ADL moderate dependent** (ADL score 3-4).

Furthermore, we examine the presence of arthritis, capability to walk 500m without any help, daily exercise or daily light housework to assess the disability status of our population.

5) MEASURES OF PHYSICAL PERFORMANCE

Ability to perform a five times chair stand test and Handgrip strength and were included in the study.

In the chair stand test, participants were divided in two groups (able to complete the test and unable to complete the test).

Handgrip strength was measured using a hand-held dynamometer (SMEDLYS' dynamometer, Scandidact, Kvistgaard, Denmark) for two performances with each hand. Also in this test participants were divided in two groups (able to complete the test and unable to complete the test).

6) LIFE STYLE 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.

7) SURVIVAL ANALYSIS

Vital status for the total cohort was assessed at **January 1st 2009**.

NOTE: Differences in the number of cases are due to the presence of missing values.

STATISTICAL ANALYSIS

Univariate analysis.

ii) **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, ADL scale categories).

iii) **Student *t* test** was used to compare scores of continuous variables (SMMSE, Hand Grip).

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

Multivariate analysis

ii) **Logistic regression** was performed in order to evaluate the risk of disability (low MMSE score, low ADL score, incapacity to perform hand grip or chair standing test) and occurrence of stroke at a certain age.

Survival Analysis

i) Cox regression-based test for equality of survival curves was used to compare the mortality risk in different groups of subjects.

All the analysis were performed using Stata version 9.0 (Stata Corp., College Station, TX).

RESULTS

Preliminary remarks:

In this statistical analysis we considered the age of the interview, the reported age of the first stroke event and some additional variables. These variables have been selected because they could represent ***cerebrovascular disease risk factors*** *(Sex, Age, Smoke habit, Alcohol consumption, lack of exercise),* ***cognitive and disability indicator*** *(ability to understand the questions, presence of serious memory impairments like dementia, presence of arthritis, capability to walk 500m without any help, daily exercise or daily light housework).*

Moreover, we considered the age of ***85 y.o*** *as the cut-off for the reported age of the first stroke event since from this age on many population and aging studies, have demonstrated a shift in the importance of the classic metabolic risk factors.*

Our sample consists of 1176 subjects: 801females (68,11%) and 375 males (31,89%), between 90-105 years old recruited in three centres (Table 1 : Sex Distribution). Applying the Two-sample t test no differences in the age distribution have been found between the centres. (Table 1 : Age Distribution).

Table 1 : Sex Distribution

Sex	Center			Total
	UNIBO	*ISS*	*UNICAL*	
Male	**164**	**58**	**153**	**375**
	29,87 %	***26,85 %***	***37,23 %***	***31,89 %***
Female	**385**	**158**	**258**	**801**
	70,13 %	***73,15 %***	***62,77 %***	***68,11 %***
Total	**549**	**216**	**411**	**1.176**
	100,00 %	***100,00 %***	***100,00 %***	***100,00 %***

Table 1 : Age Distribution

Group	Obs	Mean	Std. Err.	Std. Dev.	95% Conf. Interval	
UNIBO	**549**	93,91075	**,1269501**	**2,974535**	**93,66138**	**94,16012**
ISS	**216**	93,48148	**,1900817**	**2,793619**	**93,10682**	**93,85614**
UNICAL	**411**	93,34793	**,1382179**	**2,80211**	**93,07623**	**93,61964**

Of these subjects, 13 are part of the *"never completed trios"* group 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. Thus, in total 554 families of 2 or more siblings have been collected, consisting of 509 (91,88%) families of two siblings, 35 (6,32%) of three siblings, 9 (1,63%) of four and 1 (0,18%) of five siblings (Table 2). 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.

Table 2: Family and number of members

Members of the family	Centre			Total
	UNIBO	ISS	UNICAL	
2	**215**	**102**	**192**	**509**
3	**24**	**4**	**7**	**35**
4	**8**	**0**	**1**	**9**
5	**1**	**0**	**0**	**1**
Total	**258**	**106**	**203**	**554**

Remarkable is the fact that in our sample we collected 48 **centenarians** (100-105 y.o), of whom 1 subject has reported a stroke episode in young age and 7 in old age (just one of these 90+ siblings was 102 y.o while the other six were 100 y.o. at the time of the interview).

Proxy interview

In proxy interviews the items in the questionnaire are responded by the 90+ sib care giver, given that the 90+ sib is not able to participate actively to the study. Proxy interviews have been performed for the reason that in some families one of the siblings had poor cognitive and/or functional status, even if it's sibling(s) were in a good status.

Proxy interviews have been collected from 87 (25 male representing the 6,68% of the male population and 62 females, representing the 7,74% of the population) 90+ sibs, representing the 7,40% of the whole sample. From these 51 proxy interview have been collected from UNIBO, 13 from ISS and 23 from UNICAL, representing respectively the 9,29%, the 6,02% and the 9,29% of the total interviews collected from each centre

Exists a statistically significant difference in the age between proxy interview and non-proxy interview. Proxy interview has been collected from older subjects (mean age 95.39 y.o.) confronting with the non-proxy interview subjects (mean age 93.49, p>0.00).

In Table is reported the distribution of proxy interviews according to the reported age of the first stroke event (cut-off = 85 y.o.). 60 proxy interviews are from free stroke subjects (5,14% of the total interviews), 5 are from the young age stroke group (0,43%) and 21 from the old age stroke group (1,8%).

Table 3 : Distribution of proxy interviews according to the reported age of the first stroke event

Proxy Interview	Stroke Status and Age Onset			Total
	Free	41-84	85-99	
Yes	**60**	**5**	**21**	**86**
	5,14 %	***0,43 %***	***1,80 %***	***7,37 %***
No	**960**	**42**	**80**	**1.082**
	82,20 %	***3,60 %***	***6,83 %***	***92,63 %***
Total	**1.020**	**47**	**101**	**1.168**
	87,33 %	***4,02 %***	***8,65 %***	***100,00 %***

In Table 3 we can see that 1 proxy interview (0,09% of the total) is from an ADL independent 90+ sib (ADL score 5-6), in 6 (0,51) proxy interviews 90+ sibs belong to ADL moderate dependent group (ADL score 3-4) and 80 (6,81%) to the ADL completely dependent group.

Only 4 subjects of the proxy interviews were able to perform the chair standing test (0,34%) (Table 5) and 29 (2,47%) were able to perform the hand grip test (Table 6).

Table 3 : Proxy interview and ADL score

Proxy Interview	ADL score			Total
	5 - 6	3 - 4	0 - 2	
Yes	**1**	**6**	**80**	**87**
	0,09 %	***0,51 %***	***6,80 %***	***7,40 %***
No	**654**	**117**	**317**	**1.088**
	55,66 %	***9,96 %***	***26,98 %***	***92,60 %***
Total	**655**	**123**	**397**	**1.175**
	55,74 %	***10,47 %***	***33,79 %***	***100,00 %***

Table 4 : Proxy interview and chair standing

Proxy Interview?	Chair Standing Performed		Total
	Able	Unable	
Yes	**4**	**83**	**87**
	0,34 %	***7,07 %***	***7,41 %***
No	**642**	**445**	**1.087**
	54,69 %	***37,90 %***	***92,59 %***
Total	**646**	**528**	**1.174**
	55,02 %	***44,98 %***	***100,00 %***

Table 5 : Proxy interview and hand grip

Proxy Interview?	Hand Grip Performed		Total
	Yes	No	
Yes	**29**	**58**	**87**
	2,47 %	***4,93 %***	***7,40 %***
No	**1.058**	**30**	**1.088**
	90,04 %	***2,56 %***	***92,60 %***
Total	**1.087**	**88**	**1.175**
	92,51 %	***7,49 %***	***100,00 %***

From this point data from **proxy interview** questionnaires (87 subjects) will not be considered for the analysis. We decide to not use these data, in order to avoid bias from the extreme disability (cognitive and functional deficit) that the proxy interview 90+ sib is affected.

Stroke Incidence

We have divided our sample in three main categories according their answer in the **55c question** ("Have you ever had one or more of the following diseases? Stroke, cerebral thrombosis/haemorrhage. If "yes", report age of first time").

a) ***Stroke free***: negative answer in the 55c question (960 subjects, 88.72%).

b) ***Young age stroke***: positive answer in the 55c question (42 subjects, 3.88%). Reported age of Stroke incidence **< *85 y.o.***

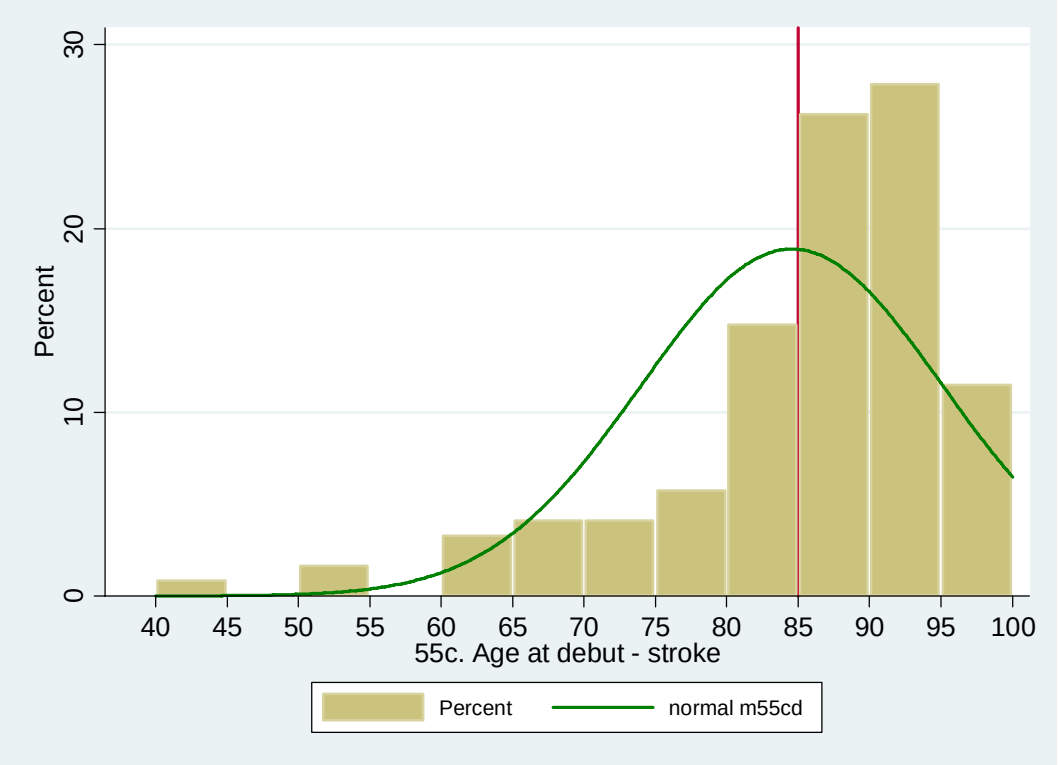
c) ***Old age stroke***: positive answer in the 55c question (80 subjects, 7.39%). Reported age of Stroke incidence **≥ *85y.o.***

Free stroke group is considered the reference category for all the following analysis.

In Figure we can see the distribution of the reported stroke incidence in different ages.

It is remarkable the fact that incidence is low before 85 y.o and then increase sharply.

Figure 1 : Distribution of stroke incidence in different ages



Regarding stroke incidence within the same **family**, our data report that in the 1.71% of the families composed of two 90+ siblings, both siblings have reported a stroke episode, while in 5.71% of the families composed by three 90+ siblings two siblings have reported a stroke episode (Table 6). However in this group as well as in the four 90+ siblings family, the small amount of families (just 9 families are composed of four 90+ siblings and 35 of three 90+ siblings) does not allow any conclusion regarding the possible inheritance of the cerebrovascular disease within siblings.

Table 6 : Incidence of stroke within the 90+ siblings family

Members of the family	Members affected by ictus 0	1	2	Total
2	**388**	**113**	**8**	**509**
	76,23 %	***22,20 %***	***1,57 %***	***100,00 %***
3	**19**	**14**	**2**	**35**
	54,29 %	***40,00 %***	***5,71 %***	***100,00 %***
4	**5**	**3**	**1**	**9**
	55,56 %	***33,33 %***	***11,11 %***	***100,00 %***
5	**1**	**0**	**0**	**1**
	100,00 %	***0,00 %***	***0,00 %***	***100,00 %***
Total	**424**	**132**	**11**	**567**
	74,78 %	***23,28 %***	***1,94 %***	***100,00 %***

In Table 7 is represented the statistical relationship between the presence or not and the age of reported stroke with:

- Age at interview
- sex
- ability to understand the questions
- can the participant walk 500 m without help
- smoke habit
- Alcohol daily consumption
- Presence of serious memory impairments (e.g. dementia)
- Daily Exercise or daily light housework
- History of arthritis

It is interesting that there is a correlation between stroke incidence age, walking without any help for 500 mt, the daily exercise and alcohol consumption. On the contrary and quite surprising, no correlation has been found between stroke status and sex of the participant, as well as with the smoke habit, even when we analysed the sample combining the categories of “actual” and “ex smoker”.

Table 7 : Stroke and variables

Characteristics	Stroke Status and Age Onset						Total		
	Free*		41-84		85-99				
Subjects	960		42		80		1082		
Age at interview									
	mean	*st.dev*	*mean*	*st.dev*	*mean*	*st.dev*	*mean*	*st.dev*	*p (ttest)*
Years	93,46	2,83	92,83	2,50	94,26	2,68	93,49	2,81	0,08/0,99
Centre									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
UNIBO	426	44,4	24	57,2	44	55,0	494	45,7	
ISS	185	19,3	4	9,5	13	16,3	202	18,7	
RC	349	36,3	14	33,3	23	28,7	386	35,6	0,154
Sex									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
male	315	32,8	10	23,8	22	27,5	347	32,1	
female	645	67,2	32	76,2	58	72,5	735	67,9	0,313
Is the participant able to understand?									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	737	76,8	31	73,8	55	68,8	823	76,1	
Little or Great difficulties and No	218	22,7	11	26,2	25	31,2	254	23,5	0,215
Can you WITHOUT difficulty walk 500 m WITHOUT help?									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	403	42,0	15	35,7	14	17,5	432	39,9	
No	557	58,0	27	64,3	66	82,5	650	60,1	**<0,001**
Smoke habit									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Never	694	72,3	34	81,0	64	80,0	792	73,3	
Smoke History *(Actual/Ex-Smoker)*	265 *(35/230)*	27,6	8 *(0/8)*	19,0	16 *(2/14)*	20,0	289 *(37/252)*	26,7	0,173
Daily Alcohol Consumption									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	523	54,5	21	50,0	40	50,0	624	57,7	
No	396	41,3	21	50,0	40	50,0	457	42,3	0,186
Presence of serious memory impairments (e.g. dementia)									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
No	895	93,2	38	90,5	71	88,8	1.004	92,8	
Yes	65	6,8	4	9,5	9	11,2	78	7,2	0,277
Daily light exercise									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	539	56,1	19	45,2	28	35,0	586	54,2	
No	421	43,9	23	54,8	52	65,0	496	45,8	**<0,001**
History of Arthritis									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
No	493	51,4	20	47,6	48	60,0	561	51,8	
Yes	467	48,6	22	52,4	32	40,0	521	48,2	0,283

Afterwards the **logistic regression test** has been performed, to analyse the global effect of the variables in the 90+ siblings population.

In the old age stroke group the age at interview, the inability to walk without any help and the absence of daily exercise represent risk factors for stroke incidence. (Table 8)

Table 8 : Stroke and variables logistic regression

Characteristics	Stroke Status and Age Onset							
	Free* vs 41-84				Free* vs 85-99			
	OR	**p**	**lower**	**upper**	**OR**	**p**	**lower**	**upper**
Age at interview								
years	0,91	0,155	0,81	1,03	1,10	**0,006**	1,03	1,17
centre								
UNIBO	1,00	*reference category*						
ISS	0,38	0,079	0,13	1,11	0,68	0,225	0,37	1,27
RC	0,71	0,322	0,36	1,39	0,64	0,103	0,37	1,10
sex								
male	1,00	*reference category*						
female	1,56	0,227	0,76	3,23	1,29	0,327	0,78	2,14
Is the participant able to understand?								
Yes	1,00	*reference category*						
Little or Great difficulties and No	1,20	0,614	0,59	2,43	1,54	0,084	0,94	2,50
Can you WITHOUT difficulty walk 500 m WITHOUT help?								
Yes	1,00	*reference category*						
No	1,30	0,408	0,70	2,43	3,41	**0,000**	1,91	6,09
Smoke habit								
Never	1,00	*reference category*						
Act/Ex-Smoker	0,62	0,229	0,28	1,36	0,65	0,145	0,37	1,16
Daily Alcohol Consumption								
Yes	1,00	*reference category*						
No	1,42	0,249	0,78	2,59	1,42	0,119	0,91	2,12
Serious memory impairments (e.g. dementia)								
No	1,00	*reference category*						
Yes	1,45	0,489	0,51	4,15	1,75	0,134	0,84	3,62
Daily light exercise								
Yes	1,00	*reference category*						
No	1,55	0,170	0,83	2,90	2,38	**0,000**	1,47	3,86
History of Arthritis								
No	1,00	*reference category*						
Yes	1,16	0,623	0,64	2,11	0,70	0,136	0,44	1,12

Cognitive status

The SMMSE was used as measure of the **cognitive status** of 90+ siblings and it was calculated in a total of 1084 subjects. Four (n.4) subjects have not complete the SMMSE because of physical disability (i.e. Visually impaired, Hearing impaired, Paralyzed in the arms, etc).

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 and our sample has been classified in two groups:

iii) Normal cognitive status (MMSE ≥24): 446 of 90+ sibs (41.37%)

iv) Cognitive Impairment (MMSE < 24): 637 of 90+ sibs (58.76%)

Since the 58.76% of our sample was cognitively impaired, a further analysis of this group has been performed, dividing the subjects in those with MMSE score between 20 - 24, 15 - 19 and ≤ 14, according to the Scott et al classification, and the presence and age of stroke incidence. (Table 9)

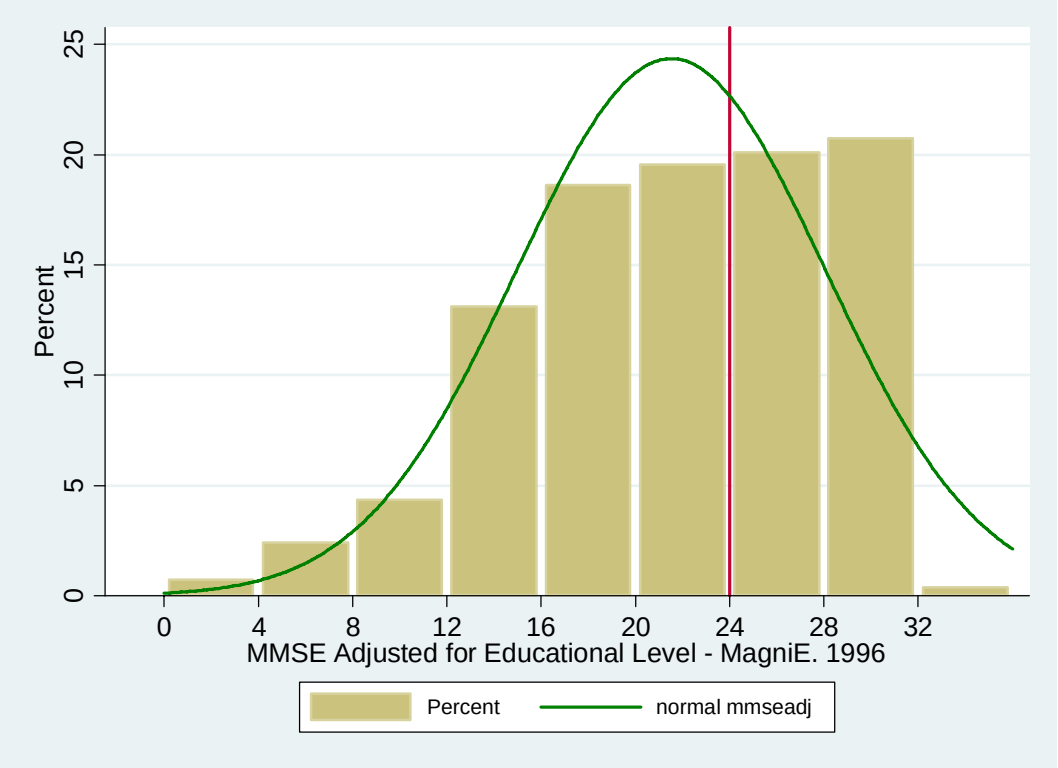
Table 9 : MMSE score and stroke incidence

MMSE Score	Ictus free	41-84	85-99	Total
≤ 14	**152**	**9**	**19**	**180**
Severe Cognitive Impairment	***15,90 %***	***21,43 %***	***23,75 %***	***16,70 %***
15-19	**215**	**8**	**18**	**241**
Moderate Cognitive Impairment	***22,49 %***	***19,05 %***	***22,50 %***	***22,36 %***
20-24	**182**	**13**	**16**	**211**
Mild Cognitive Impairment	***19,04 %***	***30,95 %***	***20,00 %***	***19,57 %***
≥24	**407**	**12**	**27**	**446**
Normal Cognitive Status	***42,57 %***	***28,57 %***	***33,75 %***	***41,37 %***

As we can see almost 42% of our population belongs to the mild and moderate cognitive impairment groups, and just the 16.7% of the sample is affected by a severe cognitive impairments. This correspond to the GeHA spirit, according to which 90+ relatively healthy subjects were supposed to participate to the project.

In Figure 1 is represented the MMSE score distribution, adjusted for educational level.

Figure 1 : MMSE score distribution, adjusted for educational level



In Table 10 we can see that MMSE score adjusted for educational level is strongly correlated with the majority of the variables considered to perform this analysis: interview age of the participant, sex of the participant, ability to understand the questions, can the participant walk without help, smoke habit, Alcohol daily consumption, Presence of serious memory impairments (e.g. dementia), Daily Exercise or daily light housework, at the exception of History of Arthritis.

Table 10 : MMSE score adjusted for educational level and variables

Characteristics	MMSE Adjusted for Educational Level						
	mmse>=24		mmse<24		totale		
Subject	447		637		1084		
Stroke Status and Age Onset							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Free*	407	91,1	549	86,2	956	88,2	0,07
41-84	12	2,7	30	4,7	42	3,9	
85-99	27	6,0	53	8,3	80	7,4	
Age at interview							
	mean	*st.dev*	*mean*	*st.dev*	*mean*	*st.dev*	*p (ttest)*
years	92,9	2,43	93,9	2,95	93,5	2,80	**<0.001**
Centre							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
UNIBO	259	57,9	236	37,0	495	45,7	
ISS	133	29,8	69	10,8	202	18,6	
RC	55	12,3	332	52,1	387	35,7	**<0,001**
Sex							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
male	165	36,9	182	28,6	347	32,0	
female	282	63,1	455	71,4	737	68,0	**0,004**
Is the participant able to understand							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	417	93,3	409	64,2	826	76,2	
Little or Great difficulties and No	27	6,0	226	35,5	253	23,3	**<0,001**
Can you WITHOUT difficulty walk 500 m WITHOUT help?							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	227	50,8	207	32,5	434	40,0	
No	220	49,2	430	67,5	650	60,0	**<0,001**
Smoke habit							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Never	296	66,2	495	77,7	791	73,0	
Act/Ex-Smoker	151	33,8	140	22,0	291	26,8	**<0,001**
Daily Alcohol Consumption							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	280	62,6	346	54,3	626	57,7	
No	166	37,1	291	45,7	457	42,2	**<0,001**
Serious memory impairments (e.g. dementia)							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
No	442	98,9	564	88,5	1006	92,8	
Yes	5	1,1	72	11,3	77	7,1	**<0,001**
Daily Alcohol Consumption							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	338	75,6	249	39,1	587	54,2	
No	109	24,4	388	60,9	497	45,8	**<0,001**
History of Arthritis							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
No	251	56,2	309	48,5	560	51,7	
Yes	196	43,8	327	51,3	523	48,2	0,014

Performing the **logistic regression test** we have obtain some interesting results:

There is a major risk to have a cognitive impairment (MMSE < 24) if the 90+ sib belongs to the Young age stroke, if she is a female, leaves in Calabria, drinks moderate quantity of alcohol, doesn't perform any kind of light exercise and is affected by arthritis.

Unexpectedly, smoke habit results a slight protective factor! (OR 0.55, p<0.001).

These results are reported in Table 11

Table 11 : MMSE score and variables Logistic regression

Characteristics	MMSE Adjusted for Educational Level			
	mmse<24 vs mmse>=24			
	OR	*p*	*lower*	*upper*
Stroke Status and Age Onset				
free *	*reference category*			
41-84	1,85	0,077	0,94	3,67
85-99	1,46	0,124	0,90	2,35
Age at interview				
years	1,16	**<0,001**	1,10	1,22
Centre				
UNIBO	*reference category*			
ISS	0,57	**0,004**	0,39	0,84
RC	6,62	**<0,001**	4,60	9,55
Sex				
male	*reference category*			
female	1,46	**0,004**	1,13	1,89
Is the participant able to understand?				
Yes	*reference category*			
Little or Great difficulties and No	8,53	**<0,001**	5,61	12,99
Can you WITHOUT difficulty walk 500 m WITHOUT help?				
Yes	*reference category*			
No	2,14	**<0,001**	1,66	2,76
Smoke habit				
Never	*reference category*			
Act/Ex-Smoker	0,55	**<0,001**	0,42	0,72
Daily Alcohol Consumption				
Yes	*reference category*			
No	1,42	**0,007**	1,10	1,83
Serious memory impairments (e.g. dementia)				
No	*reference category*			
Yes	11,29	**<0,001**	4,56	27,92
Daily light exercise				
Yes	*reference category*			
No	4,83	**<0,001**	3,64	6,42
History of Arthritis				
No	*reference category*			
Yes	1,36	0,024	1,04	1,76

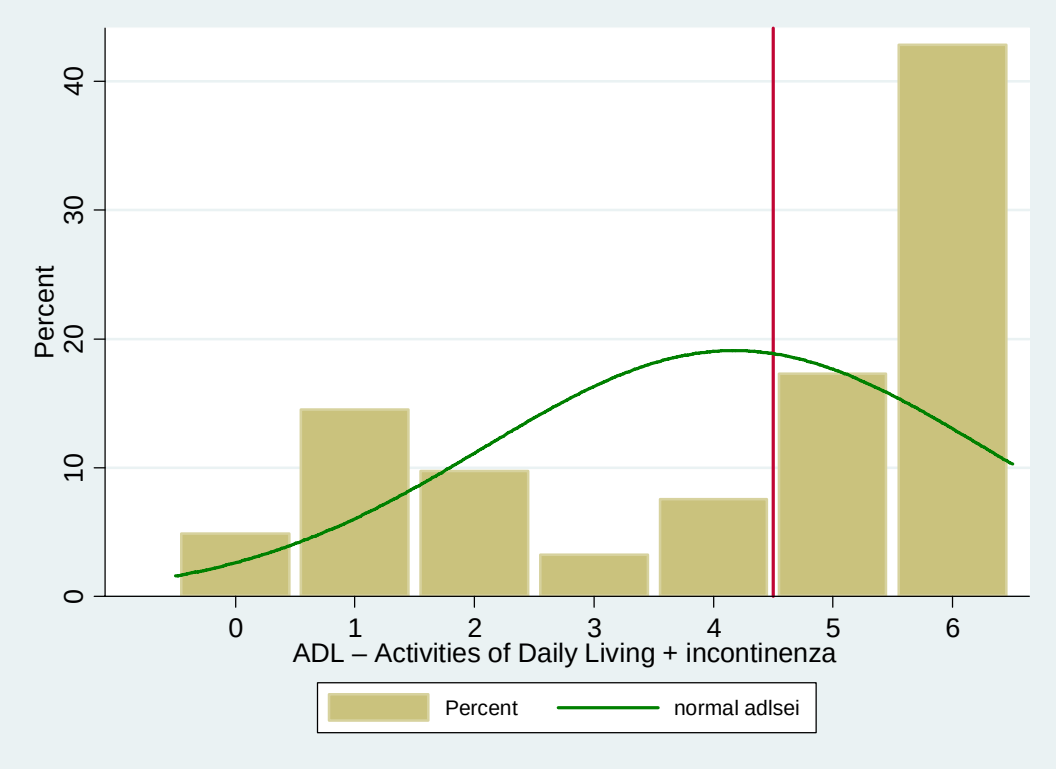
Presence of daily disability

Using the ADL scale we have divided our sample in:

- **ADL independent** (ADL score 5-6). In this category belongs the **60.11%** of our sample (654 90+ sibs). These subjects have been interviewed in relatively younger age comparing with the other two categories (Mean age at interview 92,95 y.o.)
- **ADL moderate dependent** (ADL score 3-4). In this category belongs the **10.75%** of our sample (117 90+sibs, Mean age at interview 94,15 y.o.)
- **ADL completely dependent** (ADL score 0-3). In this category belongs the **29.14%** of our sample (317 90+sibs, Mean age at interview 94,59.o.)

In Figure 2 is represented the ADL score distribution

Figure 2 : ADL score distribution



ADL score resulted statistically associated with all the different variables, as reported in Table 12.

Table 12 : ADL score and variables

Characteristics	ADL Status								
	5-6		3-4		0-2		Totale		
Subject	654		117		317		1088		
Stroke Status and Age Onset									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*

free*	601	91,9	103	88,0	256	80,8	960	88,2	
41-84	17	2,6	7	6,0	18	5,7	42	3,9	
85-99	32	4,9	7	6,0	41	12,9	80	7,4	**<0,001**
Age at interview									
	mean	st.dev	mean	st.dev	mean	st.dev	mean	st.dev	p (ttest)
years	92,95	2,48	94,15	2,94	94,59	3,19	93,90	2,69	
Centre									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
UNIBO	307	46,9	70	59,8	121	38,2	498	45,8	
ISS	134	20,5	29	24,8	40	12,6	203	18,7	
RC	213	32,6	18	15,4	156	49,2	387	35,6	**<0,001**
sex									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
male	245	37,5	32	27,4	72	22,7	349	32,1	
female	409	62,5	85	72,6	245	77,3	739	67,9	**<0,001**
Is the participant able to understand									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	574	87,8	73	62,4	180	56,8	827	76,0	
Little or Great difficulties and No	79	12,1	41	35,0	136	42,9	256	23,5	**<0,001**
Can you WITHOUT difficulty walk 500 m WITHOUT any help?									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	382	58,4	29	24,8	24	7,6	435	40,0	
No	272	41,6	88	75,2	293	92,4	653	60,0	**<0,001**
Smoke habit									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Never	445	68,0	85	72,6	264	83,3	794	73,0	
Ex-Smoker+Actual	208	31,8	32	27,4	52	16,4	292	26,8	**<0,001**
Daily Alcohol Consumption									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	408	62,4	64	54,7	157	49,5	629	57,8	
No	245	37,5	53	45,3	160	50,5	458	42,2	**<0,001**
Serious memory impairments (e.g. dementia)									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
No	635	97,1	103	88,0	271	75,7	1009	92,7	
Yes	18	2,9	14	12,0	46	23,8	78	7,2	**<0,001**
Daily light exercise									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	510	78,0	54	46,2	25	7,9	589	54,1	
No	144	22,0	63	53,8	292	92,1	499	45,9	**<0,001**
History of Arthritis									
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
No	355	54,3	66	56,4	142	44,8	563	51,7	
Yes	298	45,6	51	43,6	175	55,2	524	48,2	**0,011**

Performing the **logistic regression test** the results are analogous as the ones we have seen for the MMSE score. Once more there is a major risk to be completely dependent in the ADL if the 90+ subjects is a female, leaving in Calabria, drinks moderate quantity of alcohol, doesn't perform any kind of light exercise and is affected by arthritis. In addition smoke habit is a risk factor for low ADL scores.

Table 13 : ADL score and variables, Logistic regression

Characteristics	ADL Status							
	3-4 vs 5-6				0-2 vs 5-6			
	OR	*p*	*lower*	*upper*	*OR*	*p*	*lower*	*upper*
Stroke Status and Age Onset								
Free*	*reference category*				*reference category*			
41-84	2,40	0,061	0,96	6,00	2,49	**0,006**	1,30	4,78
85-99	1,28	0,574	0,55	2,99	3,00	**0,000**	1,85	4,88
Age at interview								
years	1,17	**0,000**	1,10	1,25	1,20	**0,000**	1,14	1,27
centre								
UNIBO	*reference category*				*reference category*			
ISS	0,95	0,848	0,56	1,62	0,76	0,235	0,48	1,20
RC	0,37	**0,000**	0,22	0,64	1,86	**0,002**	1,36	2,55
sex								
male	*reference category*				*reference category*			
female	1,59	**0,035**	1,03	2,45	2,04	**0,000**	1,50	2,77
Is the participant able to understand								
Yes	*reference category*				*reference category*			
Little or Great difficulties and No	4,08	**0,000**	2,53	6,58	5,49	**0,000**	3,96	7,61
Can you WITHOUT difficulty walk 500 m WITHOUT any help?								
Yes	*reference category*				*reference category*			
No	17,15	**0,000**	10,81	27,20	4,26	**0,000**	2,76	6,59
Smoke habit								
Never	*reference category*				*reference category*			
Act/Ex-Smoker	0,81	0,336	0,52	1,25	0,42	**0,000**	0,30	0,59
Daily Alcohol Consumption								
Yes	*reference category*				*reference category*			
No	1,38	0,101	0,94	2,02	1,70	**0,000**	1,30	2,22
Serious memory impairments (e.g. dementia)								
No	*reference category*				*reference category*			
Yes	4,80	**0,000**	2,26	10,17	5,99	**0,000**	3,35	10,70
Daily light exercise								
Yes	*reference category*				*reference category*			
No	4,13	**0,000**	2,72	6,28	41,37	**0,000**	26,36	64,92
History of Arthritis								
No	*reference category*				*reference category*			
Yes	0,92	0,683	0,62	1,37	1,47	**0,006**	1,12	1,93

Functionality measures

Two functionality test have been carried out: The chair standing test that provides information about the lower limbs functionality and the hand grip test for the upper limbs.

A. CHAIR STANDING.

In our population the 59.06% of the 90+ subject were able to perform the chair standing test, the 6.53% had to stop after one to four attempts and the 34.41% were unable to complete the test. Therefore the 40.94% were incapable to accomplish test. These 90+ sibs are older (mean age 94.30 y.o., $p<0,001$) than the ones that were able to perform the chair standing test (mean age 92.93 y.o.).

More over the capacity to carry out the chair test is strongly correlated with the majority of the variables except, alcohol consumption and presence of arthritis. Slight correlation exists also with smoke. (Table 14)

Table 14 : Chair Standing test correlation with variables

Characteristics	Perform Chair Standig						
	Able		Unable		Totale		
Subject	642		445		1087		
Stroke Status and Age Onset							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
free	593	92,4	366	82,2	959	88,2	
41-84	17	2,6	25	5,6	42	3,9	
85-99	*30*	4,7	*50*	11,2	80	7,4	**<0,001**
Age at interview							
	mean	*st.dev*	*mean*	*st.dev*	*mean*	*st.dev*	*p (ttest)*
years	92,93	2,51	94,30	3,02	93,50	2,81	**<0,001**
centre							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
UNIBO	239	37,2	259	58,2	498	45,8	
ISS	90	14,0	113	25,4	203	18,7	
RC	313	48,8	73	16,4	386	35,5	**<0,001**
sex							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
male	238	37,1	110	24,7	348	32,0	
female	404	62,9	335	75,3	739	68,0	**<0,001**
Is the participant able to understand							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	550	85,7	276	62,0	826	76,0	
Little or Great difficulties and No	90	14	165	37,1	256	23,6	**<0,001**
Can you WITHOUT difficulty walk 500 m WITHOUT any help?							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	376	58,6	58	13,0	434	39,9	
No	266	41,4	387	87,0	653	60,1	**<0,001**
Smoke habit							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Never	455	70,9	339	76,2	794	73,0	
Ex-Smoker+Actual	186	28,9	105	23,6	291	26,8	***0,05***
Daily Alcohol Consumption							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	383	59,7	245	55,1	628	57,8	
No	258	40,2	200	44,9	458	42,1	0,123
Serious memory impairments (e.g. dementia)							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
No	619	96,4	389	87,4	1008	92,7	
Yes	23	3,6	55	12,4	78	7,2	**<0,001**
Daily light exercise							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	440	68,5	148	33,3	588	54,1	
No	202	31,5	297	66,7	499	45,9	**<0,001**
History of Arthritis							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
No	339	52,8	223	50,1	562	51,7	
Yes	303	47,2	222	49,1	524	48,2	0,403

Performing the **logistic regression test** we notice that the reported age of first stroke episode, age at interview, female sex, presence of cognitive impairment and functional inability represent risk factors for the capacity to perform the chair standing test.

No statistically significant correlation have been found with the chair test performance and alcohol consumption, history of arthritis or smoke habit. (Table 15)

Table 15 : Chair Standing test and variables: Logistic regression

Characteristics	Perform Chair Standig Unable vs Able			
	OR	p	lower	upper
Stroke Status and Age Onset				
free	*reference category*			
41-84	2,38	**0,005**	1,30	4,38
85-99*	2,70	**0,000**	0,70	4,28
Age at interview				
years	1,20	**0,000**	1,14	1,26
centre				
UNIBO	*reference category*			
ISS	1,16	0,428	0,81	1,67
RC	0,22	**0,000**	0,16	0,30
sex				
male	*reference category*			
female	1,79	**0,000**	1,37	2,34
Is the participant able to understand				
Yes	*reference category*			
Little or Great difficulties and No	3,61	**0,000**	2,68	4,87
Can you WITHOUT difficulty walk 500 m WITHOUT any help?				
Yes	*reference category*			
No	9,43	**0,000**	6,77	13,14
Smoke habit				
Never	*reference category*			
Act/Ex-Smoker	0,76	0,056	0,57	1,01
Daily Alcohol Consumption				
Yes	*reference category*			
No	1,21	0,124	0,95	1,55
Serious memory impairments (e.g. dementia)				
No	*reference category*			
Yes	3,81	**0,000**	2,24	6,45
Daily light exercise				
Yes	*reference category*			
No	4,37	**0,000**	3,38	5,65
History of Arthritis				
No	*reference category*			
Yes	1,11	0,42	0,86	1,42

B HAND GRIP TEST.

From our sample the 97,33% of the 90+ subject was able to perform the hand grip test, while the 2,67% was unable to complete or perform the test. These 90+ sibs are older (mean age 95,03 y.o., p=0,0014) than the ones that were able to perform the chair standing test (mean age 93,45 y.o.).

Table 16 reports the descriptive analysis for the hand grip test.

More over the capacity to carry out the chair test is strongly correlated with the majority of the variables except, alcohol consumption and smoke habit.

Table 16 : Hand grip test and vorrelation with variables

Characteristics	Perform Hand Grip Test						
	No		Yes		Total		
Subject	29		1059		1088		
Stroke Status and Age Onset							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
free*	21	72,4	939	88,7	960	88,2	
41-84	2	6,9	40	3,8	42	3,9	
85-99	*4*	13,8	*76*	7,2	80	7,4	**<0,001**
Age at interview							
	mean	*st.dev*	*mean*	*st.dev*	*mean*	*st.dev*	*p (ttest)*
years	95,03	3,10	93,45	2,79	93,49	2,81	**0,0014**
centre							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
UNIBO	20	69,0	478	45,1	498	45,8	
ISS	5	17,2	198	18,7	203	18,7	
RC	4	13,8	383	36,2	387	35,5	**0,023**
sex							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
male	2	6,9	347	32,8	349	32,1	
female	27	93,1	712	67,8	739	67,9	**0,003**
Is the participant able to understand							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	9	31,0	818	77,2	827	76,0	
Little or Great difficulties and No	20	69,0	236	22,3	256	23,5	**<0,001**
Can you WITHOUT difficulty walk 500 m WITHOUT any help?							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	2	6,9	433	40,9	435	40,0	
No	27	93,1	626	59,1	653	60,0	**<0,001**
Smoke habit							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Never	25	86,2	769	72,6	794	73,0	
Ex-Smoker+Actual	3	10,3	289	27,3	292	26,8	0,051
Daily Alcohol Consumption							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	12	41,4	617	58,3	629	57,8	
No	17	58,6	441	41,6	458	42,1	0,068
Serious memory impairments (e.g. dementia)							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
No	20	69,0	989	93,4	1009	92,7	
Yes	8	27,6	70	6,6	78	7,2	**<0,001**
Daily light exercise							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
Yes	4	13,8	585	55,2	589	54,1	
No	25	86,2	474	44,8	499	45,9	**<0,001**
History of Arthritis							
	n	*%*	*n*	*%*	*n*	*%*	*p (chi2)*
No	5	17,2	558	52,7	563	51,7	
Yes	23	79,3	501	47,3	524	48,2	**<0,001**

Performing the **logistic regression test** we notice that the age at interview, female sex, presence of cognitive impairment and functional inability and history of arthritis represent risk factors for the capacity to perform the chair standing test.

Unexpectedly no statistically significant correlation has been found with the first stroke event reported age.

Again, no correlation resulted with smoke habit and alcohol consumption. (Table 17)

Table 17 : Hand grip and variables logistic regression test

Characteristics	Perform Hand Grip Test No vs Yes			
	OR	*p*	*lower*	*upper*
Stroke Status and Age Onset				
free *	*reference category*			
41-84	2,24	0,291	0,50	9,96
85-99	2,35	0,131	0,77	7,14
Age at interview				
years	1,18	**0,002**	1,06	1,31
centre				
UNIBO	*reference category*			
ISS	0,60	0,105	0,23	1,162
RC	0,25	**0,012**	0,08	0,74
sex				
male	*reference category*			
female	6,58	**0,011**	1,54	28,06
Is the participant able to understand?				
Yes	*reference category*			
Little or Great difficulties and No	7,07	**0,000**	3,41	17,40
Can you WITHOUT difficulty walk 500 m WITHOUT any help?				
Yes	*reference category*			
No	9,34	**0,003**	2,19	39,76
Smoke habit				
Never	*reference category*			
Act/Ex-Smoker	0,32	0,065	0,95	1,07
Daily Alcohol Consumption				
Yes	*reference category*			
No	1,98	0,080	0,92	4,26
Serious memory impairments (e.g. dementia)				
No	*reference category*			
Yes	5,65	**0,000**	2,38	13,40
Daily light exercise				
Yes	*reference category*			
No	7,71	**0,000**	2,65	22,43
History of Arthritis				
No	*reference category*			
Yes	5,12	**0,001**	1,93	13,62

MULTIVARIATE ANALYSIS

When the multivariate analysis is performed in the total of the variables and the tests that we consider to describe the cognitive and functional status of the 90+ sibs some we obtain some very interesting results.

In figure 4, we can notice that in all the tests the old age stroke group Odds ratio are lower than the young age stroke group.

This result is very important since we can assume that young age stroke subjects are functionally and cognitively in a worst status comparing with the old age stroke!

Figure 4: Stroke groups versus stroke free odds ratio

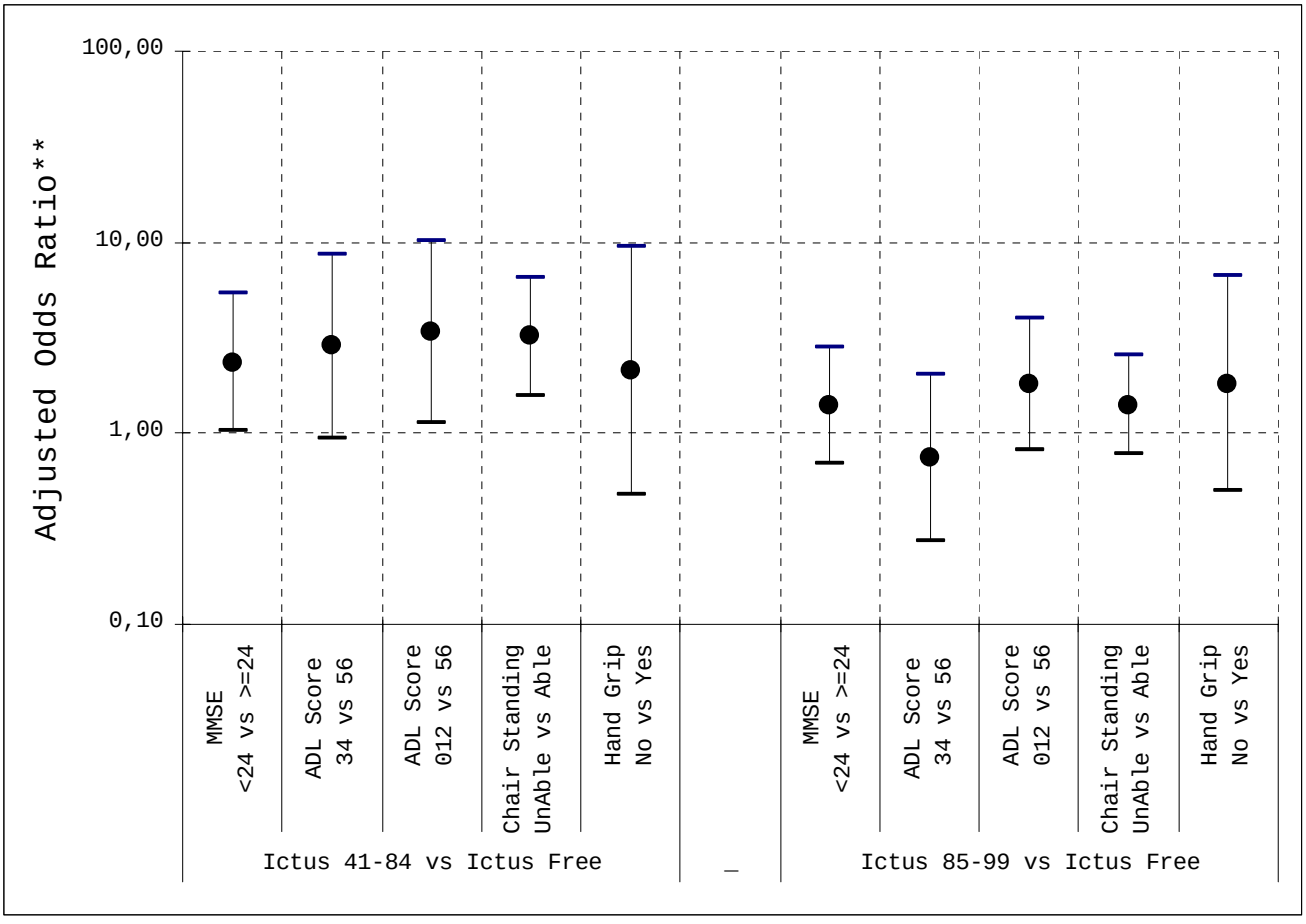


Table 18 reports the summary of the values of the multivariate analysis.

Table 18 : Multivariate analysis, summary

Characteristics	Stroke Status and Age Onset			Crude		Adjusted**	
	Free*	41-84	85-99	41-84	85-99	41-84	85-99
	N	*N*	*N*	*OddsRatio*	*OddsRatio*	*OddsRatio*	*OddsRatio*
	(%)	*(%)*	*(%)*	*(95%CI)*	*(95%CI)*	*(95%CI)*	*(95%CI)*
MMSE Adjusted for Educational Level							
mmse>=24*	407	12	27	*Reference Category*		*Reference Category*	
	(42,57)	(28,57)	(33,75)	*Reference Category*		*Reference Category*	
mmse<24	549	30	53	1,9	1,5	2,4	1,4
	(57,43)	(71,43)	(66,25)	(0,99-4,53)	(0,59-2,49)	(1,02-5,46)	(0,69-2,83)
ADL Status							
5 - 6*	601	17	32	*Reference Category*		*Reference Category*	
	(62,6)	(40,48)	(40)	*Reference Category*		*Reference Category*	
3 - 4	103	7	7	2,4	1,3	2,9	0,7
	(10,73)	(16,67)	(8,75)	(0,96-6,01)	(0,55-2,99)	(0,95-8,6)	(0,28-2,04)
0 - 2	256	18	41	2,5	3,0	3,4	1,8
	(26,67)	(42,86)	(51,25)	(1,29-4,78)	(1,85-4,88)	(1,12-10,2)	(0,82-3,99)
Perform Chair Standig							
Able*	593	17	30	*Reference Category*		*Reference Category*	
	(61,84)	(40,48)	(37,5)	*Reference Category*		*Reference Category*	
Unable	366	25	50	2,4	2,7	3,2	1,4
	(38,16)	(59,52)	(62,5)	(1,30-4,38)	(1,70-4,28)	(1,57-6,55)	(0,78-2,53)
Perform Hand Grip Test							
No*	21	2	4	*Reference Category*		*Reference Category*	
	(2,19)	(4,76)	(5)	*Reference Category*		*Reference Category*	
Yes	939	40	76	2,2	2,4	2,1	1,8
	(97,81)	(95,24)	(95)	(0,50-9,96)	(0,78-7,14)	(0,48-9,53)	(0,5-6,62)

MORTALITY AND STROKE

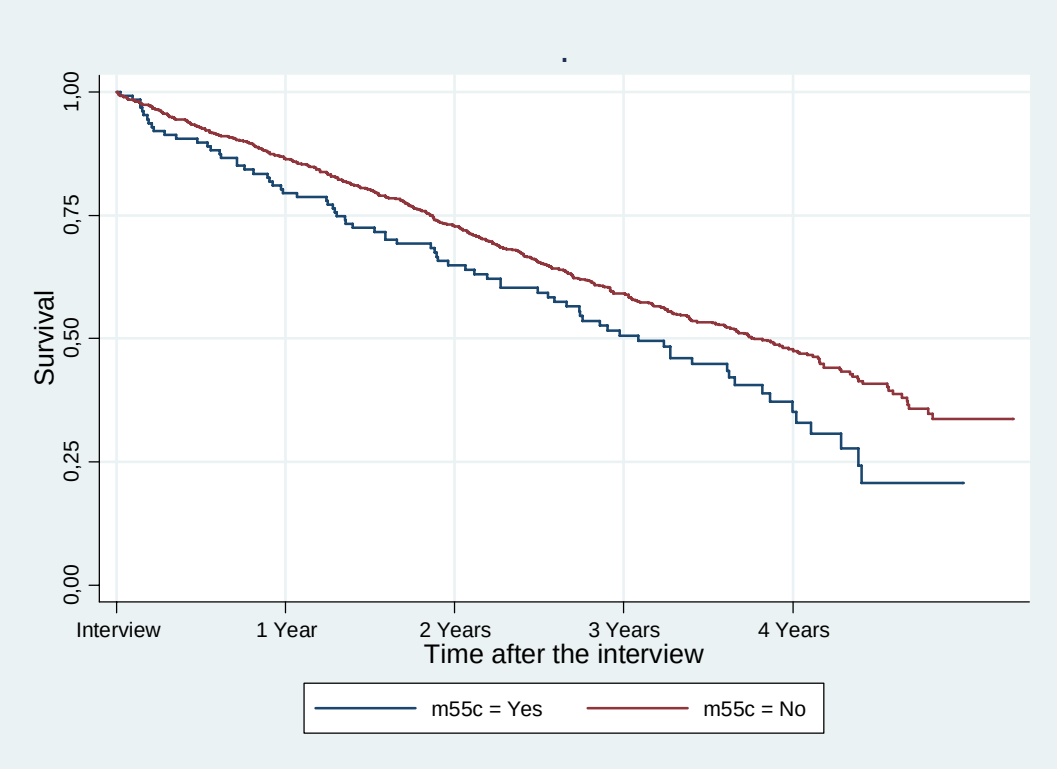
The vital status of GEHA 90+ siblings was checked at **January 1st, 2009** and the results of the mortality analysis, as reported in Table 19, indicate that **538 out of 1081 subjects died** (**49,77%**) during the follow-up . Mortality was higher in the ***old age stroke group*** **(65%, p < 0,05)** while was similar in the ***stroke free*** **(48,59%)** and ***young age stroke group*** **(47,62%)**. *p* values were calculated according to Cox regression-based test for equality of survival curves.

Table 19 : Survival and stroke in January 1st 2009

Status	Stroke Status and Age Onset			Total
	Free	41-84	85-99	
Not Alive	**466**	**20**	**52**	**538**
	48,59 %	***47,62 %***	***65,00 %***	***49,77 %***
Alive	**493**	**22**	**28**	**543**
	51,41 %	***52,38 %***	***35,00 %***	***50,23 %***
Total	**959**	**42**	**80**	**1.081**
	100,00 %	***100,00 %***	***100,00 %***	***100,00 %***
Pearson chi2(2) = 8,0325	***Pr = 0,018***	***Pr = 0,018***		

In Figure 5 we can see that stroke affected group survive less in comparison with the stroke free group. The survival curves are almost parallel from 1 year until near the 3 and a half years after the interview. Following the 3 and a half years, survival curve of the stroke affected group becomes more abrupt.

Figure 5: Stroke free and stroke affected subjects survival curves



m55c	Events observed	Events expected	Relative hazard
Yes	**75**	**56,66**	**1,3308**
No	**466**	**484,34**	**0,9671**
Total	**541**	**541,00**	**1,0000**

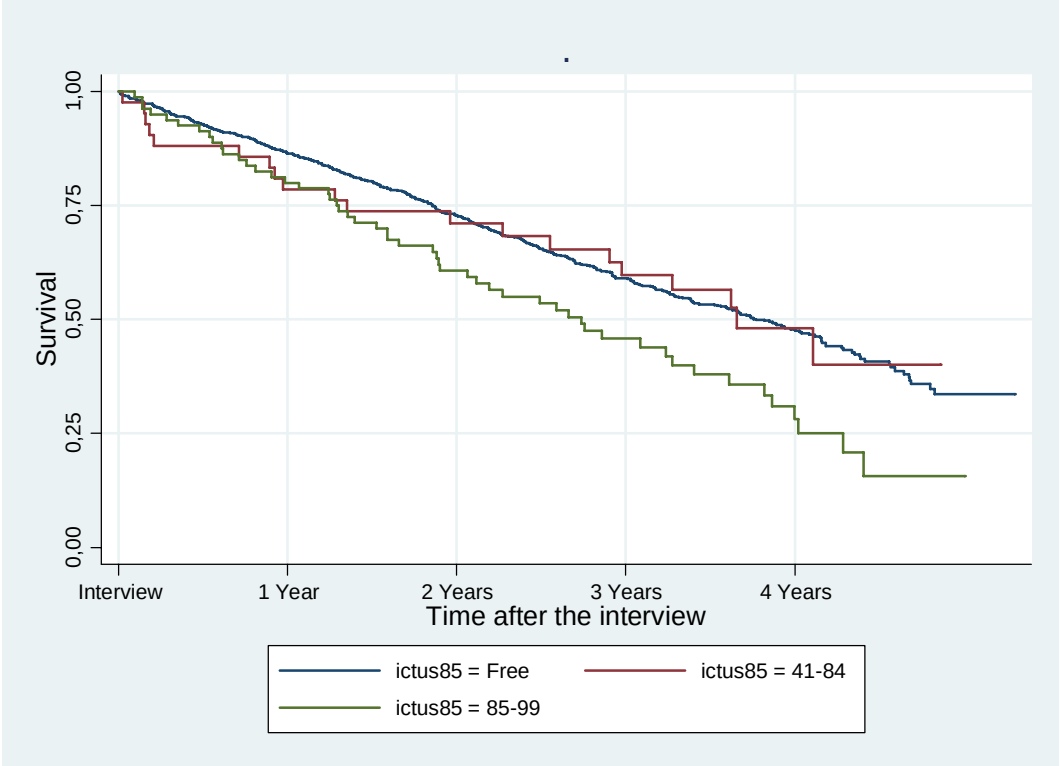
LR chi2(1) = 6,09 ***Pr>chi2 = 0,0136***

In Figure 6 we can see the differences in the survival curves between the stroke free group, the old age stroke and the young age stroke groups.

We can notice that survival curves of the old age stroke and the stroke free group almost overlap in the first half year and then the old age stroke group has a sharper form.

Curiously the young age stroke group survival curve follows the old age stroke group until the year and a half from the interview and then follows the stroke free group survival curve.

Figure 6: Stroke free, old and young age stroke group survival curves



Stroke 85	Events observed	Events expected	Relative hazard
Free	**466**	**483,76**	**0,9704**
41-84	**20**	**20,47**	**0,9839**
85-99	**52**	**33,77**	**1,5525**
Total	**538**	**538,00**	**1,0000**

LR chi2(2) = 9,12 ***Pr>chi2 = 0,0105***

Finally, the Cox regression-based test for equality of survival curves has been performed subdividing our population in 4 categories according to the presence or not of the following variables: MMSE score ≥ 24, ADL score 5-6, Chair standing test performed.

We considered these variables to investigate the presence and degree of disability/frailty and it's influence to the survival.

Fom this analysis we have not use the hand grip data, since just 29 subjects could not complete the test.

Category 0: All the variables are present (excellent performance group)

Category 1: One of the variables is lacking

Category 2: Two of the variables are lacking

Category 3: All the variables are lacking (worst performance group)

In Table 22 and 23 are reported the distribution of not alive and alive subjects subdivided by the four categories and the distribution of the four categories within the stroke age and stroke free groups, respectably.

Table 22. Alive and not alive subjects in the 4 categories

Status	somm3				Total
	0	1	2	3	
Not Alive	**133**	**144**	**176**	**88**	**541**
	74,30 %	***59,26 %***	***46,32 %***	***30,88 %***	***49,77 %***
Alive	**46**	**99**	**204**	**197**	**546**
	25,70 %	***40,74 %***	***53,68 %***	***69,12 %***	***50,23 %***
Total	**179**	**243**	**380**	**285**	**1.087**
	100,00 %	***100,00 %***	***100,00 %***	***100,00 %***	***100,00 %***

Pearson chi2(3) = 94,3481 ***Pr = 0,000***

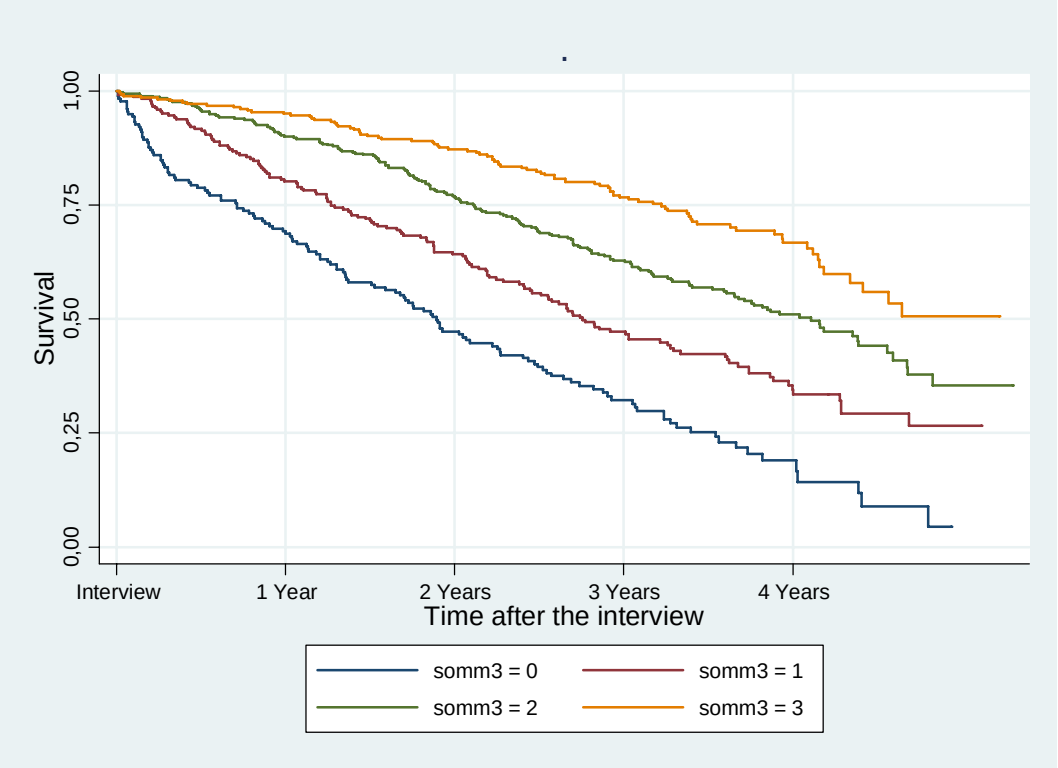
Table23. Stroke status and 4 categories

somm3	Status and Age Onset			Total
	Free	41-84	85-99	
0	**137**	**15**	**25**	**177**
	14,27 %	***35,71 %***	***31,25 %***	***16,36 %***
1	**208**	**7**	**27**	**242**
	21,67 %	***16,67 %***	***33,75 %***	***22,37 %***
2	**349**	**14**	**15**	**378**
	36,35 %	***33,33 %***	***18,75 %***	***34,94 %***
3	**266**	**6**	**13**	**285**
	27,71 %	***14,29 %***	***16,25 %***	***26,34 %***
Total	**960**	**42**	**80**	**1.082**
	100,00 %	***100,00 %***	***100,00 %***	***100,00 %***

Pearson chi2(6) = 41,1510 ***Pr = 0,000***

In 7 we can see the survival curves of the four categories. As expected the subjctes of category 0 survive more than the other groups. Worst survivals are the Category 4 subjects (more fragile).

Figure 7 : Survival curves of the four categories



. sts test somm3, coxSomm3	Events observed	Events expected	Relative hazard
0	**133**	**58,63**	**2,5947**
1	**144**	**107,56**	**1,5113**
2	**176**	**205,82**	**0,9579**
3	**88**	**169,00**	**0,5820**
Total	**541**	**541,00**	**1,0000**
LR chi2(3) =	***133,98***	***Pr>chi2 =***	***0,0000***

DISCUSSION

Stroke represents the 3rd cause of deaths for the industrialized countries, and the most important factor of invalidity. One of the non modifiable risk factors of stroke is the age.

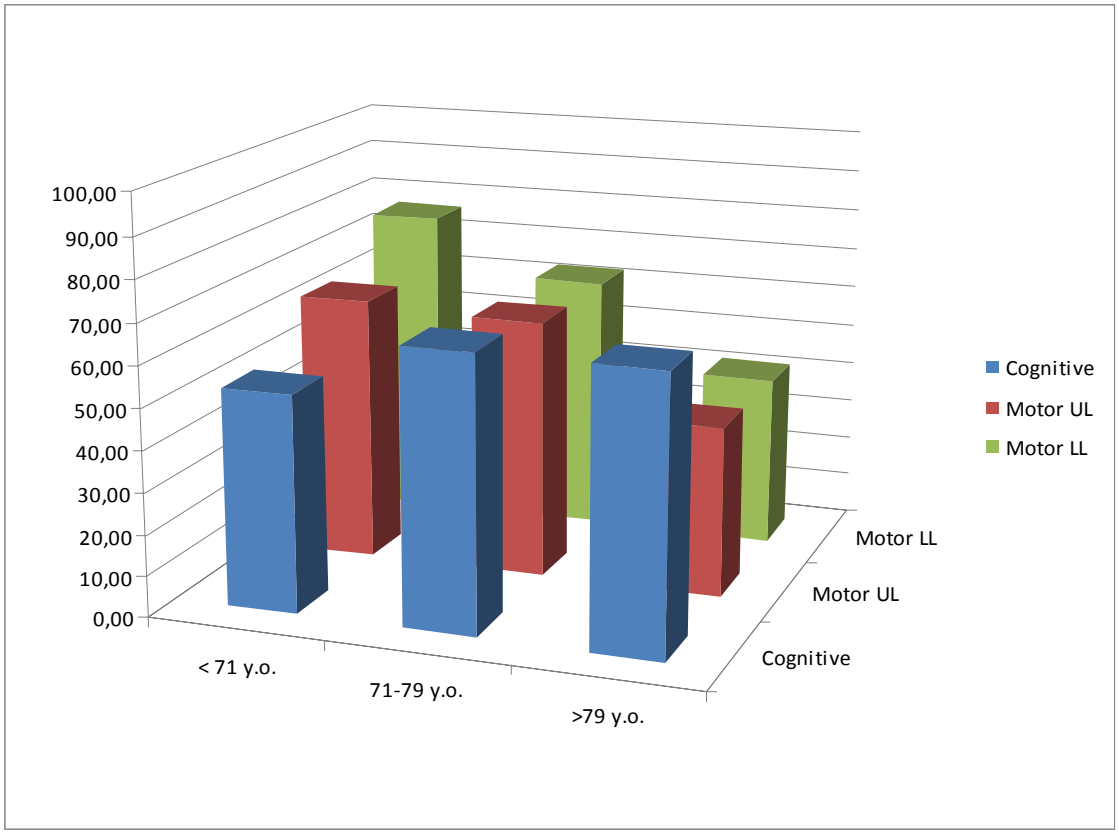
On the other hand, exists a well documented change on the life expectancy, with the important increase of the old and very old subjects.

During the last years of the PhD course, I had the opportunity to work in an Intensive Rehabilitation Unit specialized on Neurological Disease, where the majority of the patients was affected by ischemic or hemorrhagic stroke, as a Clinician specialized in Geriatric Medicine. I had the chance to follow a various number of stroke affected patients from their admission (a week after the acute stroke episode until) until their discharge (60 days later). During this period patients are treated for their disability according to a specific intense motor and (when necessary) neuropsychological treatment, consisting of 2 hours/day of motor rehabilitation and 1 hour/day of neuropsychological treatment.

With my Colleagues we notice that geriatric patients were less disable in admission and they recovered better than the younger patients. In fact, when we analysed the outcomes after specific rehabilitation treatment of ***Unilateral spatial neglect*** **(USN)** (a neuropsychological disorder whereby patients fail to detect objects, or execute movements in the portion of space controlateral to the side of brain lesion) we notice that aged patients (>80 y.o) had best performance in admission and high percentages of recovery, on cognitive status. (Data presented as Poster presentation in the IAAG World Congress, Paris 2009).

Comparing with the motor recovery and increasing the sample, we obtain similar results (data submitted in the IAAG European Congress, Bologna April 2011). (Figure A)

Figure A. Outcomes after intensive rehabilitation in USN affected patients



At this point we thought that it would be interesting to see in the GeHA subjects if the age incidence of stroke is lower than the general population, classify them according to their functional and cognitive status and compare the stroke GeHA subjects mortality rates with the stroke free GeHA subjects.

Our analysis have shown that just the 3,88% of the population belongs to the young age stroke, which could be explained from the fact that individuals with less than 85 y.o. affected by stroke have higher mortality rates than the general population. There for, these subjects are to be considered "survivors". The prevalence of stroke in the old age stroke is 7,9%, slightly higher than the one in the Italian population (6,5%, (88)), probably due to the very old age.

Regarding stroke incidence within the same **family**, our data report that in the 1.71% of the families composed of two 90+ siblings, both siblings have reported a stroke episode, while in 5.71% of the families composed by three 90+ siblings two siblings have reported a stroke episode (Table 6). However in this group as well as in the four 90+ siblings family, the small amount of families (just 9 families are composed of four 90+ siblings and 35 of three 90+ siblings) does not allow any conclusion regarding the possible inheritance of the cerebrovascular disease within siblings.

As expected GeHA stroke affected subjects resulted disable to walk or to perform a light daily exercise. Quite surprising, no correlation has been found between stroke status and sex of the participant, as well as with the smoke habit, even when we analysed the sample combining the categories of "actual" and "ex smoker".

Regarding the Cognitive performance, there is a major risk to have a cognitive impairment (MMSE < 24) if the 90+ sib belongs to the Young age stroke, if she is a female, leaves in Calabria, drinks moderate quantity of alcohol, doesn't perform any kind of light exercise and is affected by arthritis.

Performing the **logistic regression test** the results regarding the ADL score are analogous as the ones we have seen for the MMSE score. Once more there is a major risk to be completely dependent in the ADL if the 90+ subjects is a female, leaving in Calabria, drinks moderate quantity of alcohol, doesn't perform any kind of light exercise and is affected by arthritis.

Regarding chair standing test we notice that the reported age of first stroke episode, age at interview, female sex, presence of cognitive impairment and functional inability represent risk factors for the capacity to perform the chair standing test.

More over the capacity to carry out the chair test is strongly correlated with the majority of the variables except, alcohol consumption and smoke habit.

It is of particular interest the fact that when we performed the multivariate analysis, old age stroke patients resulted to be in a better status both cognitive and functional, comparing to the young age stroke group!

One of the hypothesis for the reason of this results could stand in the pathogenic moment of the acute stroke. Stroke determines a tissue necrosis, which activates the inflammatory factors. In young age the inflammatory respond is very strong, because is useful to survive infections. Probably in the old and very old subjects, the inflammatory respond is less important and consequently provoke a lighter cerebral tissue damage. This theory concords with the fact that inflammatory diseases can be a risk factor for stroke episode and an antinflammatory medicine is used worldwide to prevent stroke (acetylsalicylic acid).

An other important finding of these analysis is the fact that even though old age stroke 90+ sibs resulted in a better condition, they leave less than the younger age stroke subjects. This could be for the following reasons:

a) are older individuals and in this age frame one year of difference has an important weight in the mortality risk.
b) Young age stroke group have been undergone at least for minimum four years in a secondary prevention treatment and have uphold life-style changes. In epidemiological studies there is a reduction on stroke re-incidence and a positive effect on the mortality after

life style changes and pharmacological treatment for hypertension, diabetes and hyperlipidemia (96, 97). So, these individuals have a worst outcome, but they survive more than the old age stroke group.

CONCLUSIONS

These data suggest that in the Italian GeHA population, a stroke episode in young age represents a risk factor for cognitive impairment, ADL disability and upper and/or lower limb disability, but secondary prevention is important to increase survival.

Even though these findings are very interesting and confirms the fact that nonagenarians have singular performances, comparing to the younger ages, further studies (also within the GeHA project), should be conducted in order to validate these analysis.

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