THE SIGNATURE OF LONGEVITY IN SICILY

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Ageing is a natural and physiological condition that is the result of compromised stress response, homeostatic imbalance and increased risk of developing diseases. However, if aging with good health and functions (successful ageing) and aging with disease and disability (unsuccessful ageing) depends on a combination of "positive features", including genetic, epigenetic and phenotypic characteristics in combination with favourable environment, economic status and social involvement. In our study, we summarize some key points for the identification of a longevity signature, with a particular focus on long-living Sicilian individuals and centenarians. Analysing three different Sicilian cohorts (young, people with no centenarian parents and long-living individuals (LLI) aged >90) we found APOE ε3\ε3 in our LLIs and no presence of ε4. Regarding FOXO rs2802292 G-allele (G>T) we did not observe an association with longevity, probably because of the small sample of centenarians studied. Regarding haematological and anthropometric results, it is still difficult to point specific longevity features and so far, we cannot specify a single one. On the other hand, we believe that the synergy among genetics and environment might create successful interaction to achieve and obtain effective longevity.

Background

Ageing results in compromised stress response, homeostatic/homeodynamic imbalance, and elevated risk of disease, making organism less, and eventually, non-resilient. All these elements lead people to reach different ages in different conditions (with and without success), and with different life-spans.

Centenarian people represent the best model to study successful ageing and longevity. Demographic selection has permitted to identify centenarians as healthy survivors, offering a "natural" selected population in which studying the effect of specific polymorphisms and genetic loci associated or not with longevity. Undoubtedly, they are able to respond well to the stressors and to repair damages thanks to a combination of "positive features", including genetic, epigenetic and phenotypic characteristics with favourable environment, economic status and social involvement (1). Although many studies exist on centenarians, it has not yet been possible to identify the longevity signature. However, a favourable

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genetic background is essential to live longer. Indeed, siblings and offspring of centenarian (CO), but not their spouses, show an increased odd ratio between 4- and 17-fold for longevity compared with appropriate controls thus they have a good chance to live approximately 100 years or over compared with the average population. Moreover, CO show reduction or delay in cardio-vascular diseases (CVDs) and all-cause morbidity and mortality (2-5).

The aim of this review is to summarize some important key points for the identification of a longevity signature, with a particular focus on Sicilian centenarians.

Genetics of longevity: APOE and FOXO3A

There is growing evidence that genetic component of longevity becomes more influent in the attainment of 100+ years old beyond the age of 90. Accordingly, the heritability of living to at least 100 has been estimated at 0.33 in women and 0.48 in men (6).

There are many possible candidate genes for human longevity but up to now, only two have shown positive results in different studies and populations, including a recent meta-analysis: the APOE and the FOXO3A genes (7-9).

The APOE ε4 allele has been linked to elevated cholesterol, CVDs, age-related cognitive decline, and dementia. It is strongly associated with Alzheimer disease and to a less extent, with CVDs. On the contrary, APOE ε2 allele, encoding a protein with less affinity to cholesterol, has been found more frequently in centenarians, also in Italy, although this datum was not replicated in all studies, as demonstrated by a recent meta-analysis (10, 11).

Our unpublished preliminary results in Sicilian population, including centenarians and their offspring and age-matched people without centenarian parents, showed a frequency of APOE ε3 ranging from 0.81 and 0.90, with a prevalence of ε3/ε3 genotype. This could be lead to hypothesize that in our population there is no specific association of APOE ε2 allele with longevity. But, looking at ε4, the detrimental allele, we found no presence in our long-living individuals (LLIs; ≥90 years old), so confirming the data existing on centenarians about the low frequency of this allele in extremely longevous European people rather than the presence of other specific pro-longevity allele (9,11).

The other replicable association is with FOXO3A rs2802292 G-allele (G>T). Dietary intervention and genetic alterations in gene encoding proteins that take part in metabolic nutrient-sensing pathways can modulate lifespan, as reviewed by Aiello et al. (12). It might depend on the hyper- o hypo-activation of those signalling pathways due to genetic mutations that under or overexpress regulative molecules leading to different expression of homeostatic genes. FOXO3A probably is the gene that more influences longevity in different ethnic populations. FOXO3A might act as a transcription factor on multiple homeostatic genes in response to decreased insulin/insulin growth factor-1 signalling, hence increasing life-span. A meta-analysis of over 7900 cases and 9500 controls confirms the association of the G allele of the single-nucleotide polymorphism (SNP) rs2802292 with exceptional longevity, especially in male (9).

However, in our analysis of this SNP in three different Sicilian cohorts (young, people with no centenarian parents and LLIs) we did not observe association with longevity and we measured G-allele frequencies in all the three cohorts lower that the expected from the data registered in dbSNP (NCBI) from a Tuscany population (expected G frequency 0.5093, measured G frequency: 0.40). In LLIs the frequency of T was double than G (0.66 VS 0.33). This result might be linked to the small sample of centenarians studied.

Longevity in Sicily: overview

Looking at the Italian ratio of centenarians per inhabitants, in some areas of Sicily, in particular in the countryside, there is more than a four-fold increase. In particular, the area of the Sicani Mountain was extensively studied in its dietary habits leading to the conclusion that this high rate of centenarians is strictly related to the adherence to the Mediterranean diet (MD) (13).

New unpublished data obtained from another mountainous population of Sicily (Madonie area), confirmed the results obtained in Sicani Mountain. In this area the probability to reach 90 is 4.6% and

the one to reach 100 is 0.22%. Our preliminary analysis of the nutritional habits confirmed the possible association of longevity phenotype with a Mediterranean lifestyle but not during ageing or extreme longevity, rather during young age. In fact, our LLIs followed MD during life but not during ageing, suggesting an interesting and effective role of epigenetics in the attainment of longevity.

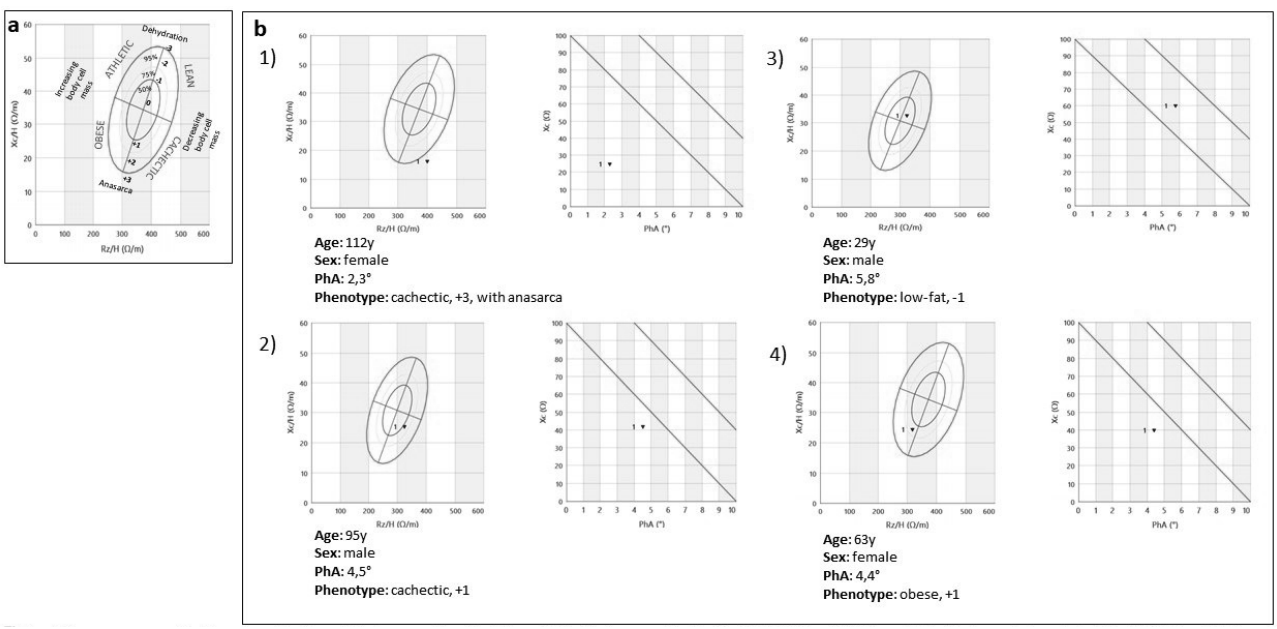
Overall, these data suggest that longevity concerns subjects living in small towns, without pollution, with different working conditions and lifestyles compared to the big towns, and close adherence to MD. Accordingly, these municipalities share low mortality from cancer and CVDs. In addition, old individuals have greater access to social support and family network; hence, they have better health and lower levels of mortality, particularly when living at home with adult daughters.

Phenotypic signature and hematochemical values

Scientific reports show that some values change during ageing, whereas there are some other parameters, which seems do not change significantly with age (14). A study published in 2008, about Italian population, reported laboratory parameters obtained from 120 healthy centenarians and 381 younger subjects (between 65 and 85 years old), to evaluate the possible influence of longevity on these values. Although the majority of values were similar, some differences were identified between the two groups. In particular, these were in urea nitrogen (increased), platelets count (lower in centenarians compared to elderly), ALT, total cholesterol and glycaemia (significantly reduced) (15). These data seem to be confirmed by recent survey on Sicilian centenarians, taking into account the differences with people aged 65+ (Ciaccio and Caruso, unpublished observations).

Body composition

Our study in Sicilian centenarians, with a special focus on anthropometric measurement, are trying to understand the differences in body composition between young-adult and LLIs, to propose reference values of anthropometric measurements and to identify a Longevity Anthropometric Phenotype,



including body mass index (BMI), body composition, body fat distribution, resting metabolic rate and waist-hip ratio. Contemporary, we developed a specific questionnaire to collect anamnestic, lifestyle and nutritional information to try to address the longevity phenotype through a characteristic footprint.

Our preliminary results (unpublished) confirmed the adherence to MD in LLIs, especially during young rather that in old age. Mean BMI was 24.35 Kg/m^2 so not associated with mortality risk. The analysis of body composition demonstrated hyperhydration, especially in extra-cellular compartment and low phase angle (Pa; mean: 3.2°). Mean value of Pa in our LLIs (mean age 101.5) classify them as cachectic (Pa 3.5° or lower). There is a significant difference in Pa between healthy condition and disease. The higher the Pa value, the better is healthy condition (17-19). Pa depends on cell membrane integrity and on body cell mass. Lower Pa appears to be consistent with either cell death or a breakdown in the selective permeability of the cell membrane, in accordance with hyper extracellular hydration (oedema). Although its biological meaning is still not clear, Pa appears to have an important prognostic role for disease, especially for sarcopenia and cachexia (16). One of the problems of its interpretation and use is linked to reference values. In fact, probably it is not possible to use the normal range of values (mean 6.5°) for the oldest old that have their "normal" body composition totally different from young and adults.

Immunophenotype

In aged people, several changes of both innate and acquired immunity have been described and viewed as deleterious, hence the term immunosenescence. On the other hand, centenarian immune system shares characteristics both of young and elderly people. In particular, number and function of natural killer cells are well conserved and comparable to those observed in young, whereas T and B cell number and function are similar to those observed in elderly (20).

We have performed several studies in Sicilian CO with the aim to track their immune signatures to test the hypothesis that these individuals might have immunological advantage, which may explain their longevity. The major finding of our studies is that CO have intermediate features between younger and elderly control groups. In fact, data show that CO retain more youthful immunological parameters and that the exhaustion of the immune system is less evident than in elderly without centenarian parents (references in 21).

CONCLUSION

Human population is very heterogeneous because of the different genetic background and different environmental stimuli, so it has not been yet possible to identify a clear signature of longevity. Longevity is a complex trait influenced by genetic and epigenetic component and other determinants as the environmental conditions during the prenatal and early postnatal period as well as life circumstances at adult and old age. In addition, chance also plays a role (22). Unfortunately, the intrinsic complexity and the heterogeneity among people make studies on ageing and longevity difficult to standardized, also because it is not easy to collect an adequate population in terms of number and information related to previous event happened in life.

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