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Review

Centenarians as a model to discover genetic and epigenetic signatures of healthy ageing

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ABSTRACT

Centenarians are a model of successful ageing. The data favours the theory that, in order to live to 100, it is mandatory to inherit the right genetic variants from parents or acquire epigenetic variants through the environment. Therefore, the study of epigenetic signatures of healthy ageing is becoming an important aspect to identify the role of chromatin modification in ageing and understand how manage this fine-tuning system. So, according to the concept of developmental plasticity, establishment of a longevity phenotype requires a combination of stochastic and non-stochastic events that modulate the genetic substrate and leads to a different outcome. It can be concluded that centenarians have a more powerful "engine" shaped by evolution, and that the environment, through epigenetic system, is a component influencing outcome.

1. Introduction

Centenarians are a model of successful ageing. They have reduced mortality since childhood and are less susceptible to the diseases of ageing, with some even being disease free, the so called "escapers". Others are frailer, meaning that they have survived diseases contracted earlier in life, the so called "survivors". Furthermore, centenarians have lived on average almost twice as long as their cohort of the beginning of the 20th century, when the survival rate was on average 53 years for males and 58 years for females (Christensen et al., 2009; Terry et al., 2008).

2. The interaction between genes and the environment in longevity

There is a strong familial trait for exceptional longevity, as demonstrated by families containing a number of centenarians so high that clustering by chance can be excluded (Perls et al., 2000). In addition, sibling relative risk studies to survival to extreme age also suggest an influential genetic component (Perls et al., 2002; Sebastiani et al., 2016b; Willcox et al., 2006). Furthermore, centenarian offspring have clear reductions or delay in cardiovascular diseases and all-cause morbidity and mortality (Andersen et al., 2012; Balistreri et al., 2014; Ismail et al., 2016; Perls et al., 2002). Genetic studies have also shown that centenarian sibling pairs share loci, such as a locus at q25 on Chromosome 4 (Puca et al., 2001), and that centenarians have a genetic profile that differs from the general population, being enriched in protective alleles. For example, the polymorphic variant *rs2070325* (Ile229Val) of the bactericidal/permeability-increasing fold-containing family B member 4 (BPIFB4) gene associates with exceptional longevity under a recessive genetic model (Villa et al., 2015a); on the other hand, centenarians are not necessarily devoid of detrimental loci (Beekman et al., 2010; Freudenberg-Hua et al., 2014; Sebastiani et al., 2012), with the notable exception of the epsilon 4 allele of apolipoprotein E (APOE) (Schachter et al., 1994). BPIFB4 levels classify the health status of centenarians, being increased in healthy as compared to frail ones. Experimental studies on animal models have shown that some of the genetic variants found associated with exceptional longevity, such as those of the calcium/calmodulin dependent protein kinase 4 (CAMK4) and BPIFB4 genes, induce changes in blood pressure levels. Indeed, the longevity-associated variant (LAV) of BPIFB4 has been reported to reduce blood pressure levels when overexpressed in old mice, activating vascular rejuvenation and promoting reparative processes (Villa et al., 2015a, 2015b). Other genetic variants associated with longevity can be found in nutrient-sensing pathways (NSPs) in animal models as well as in humans (see below) (Aiello et al., 2017). To be noted, the *meta*-analysis of four different Genome-Wide Association Study (GWAS) identified an enrichment in centenarians of a rare mutation in ELOVL6

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that reduces gene expression and bringing to accumulation of protective palmitoleic acid (C16:1) (see below) (Sebastiani et al., 2017)

Apart from the inheritance of genetic variants, longevity may be subject to the acquisition of epigenetic modifications induced by the environment. To this end, methylation analysis has identified genes that are hypo- or hyper-methylated during ageing and in centenarians as compared to newborns (Heyn et al., 2012). Thus, genetic/epigenetic variations, possibly inherited from long-living parents, may predispose cells to better adapt to cellular stress and to better survive an adverse environment. This may make, for example, individuals more resistant not only to diseases associated with ageing, but also to diseases, such as infections, that occur during early life.

In addition, genetic modifications enriched in the centenarian genome are subject to evolutionary forces. For example, the LAV of BPIFB4, which is enriched in centenarians, has been conditioned by balancing and positive selections, two evolutionary forces that have allowed us to fight disease and to adapt to environmental changes (Andrés et al., 2009; Jónsson et al., 2014). So, are centenarians the "best shaped" by evolutionary forces?

An additional striking overlap between evolution and survival concerns oxygen and nitric oxide (NO), two archaic molecules that regulate the production of free radicals and cell survival. Calorie restriction (CR, see below) (Most et al., 2016) is incapable of prolonging the life-span of mice deficient in endothelial NO synthase (eNOS), the main enzyme of NO production. Moreover, the response to hypoxia is crucial in keeping organs well fed. BPIFB4 is crucial for eNOS activation and the migration of endothelial progenitor cells, important for keeping tissues well vascularised. The presence of LAV-BPIFB4 potentiates eNOS-dependent NO release, activating endothelial function when expressed in young and old mice, and reduces blood pressure. Its protective role is exerted through improved revascularization (Puca et al., 2012; Villa et al., 2015a).

3. Lifestyle and diet in longevity

An excessive intake of calories is fuelling today's epidemics in type II diabetes mellitus, obesity, and cancer in industrialized populations because this change in behaviour is too recent to have been able to shape our genome in any favourable way. Moreover, any rapid change may promote deleterious effects in terms of health by interacting negatively with a genomic milieu that has been shaped by slow evolutionary forces. For example, the sympathetic nervous system was of great importance during pre-history because it activated the flight-or fight response in our ancestors during moments of danger, such as when being faced with a big predator, but in the modern era it has become an unfavourable phenomenon, especially with regards to cardiovascular disease, which has increased in prevalence in parallel with the rapid increase in human life span we have witnessed (approximately 3 months for every year during the last 160 years). Indeed, beta-blockers are now adopted to increase survival in patients with cardiac diseases.

Despite much evidence in favour of a strong genetic component for exceptional longevity, environmental factors may represent a main cause for the increased average survival rate, and a parallel progressive increase in height, in industrialized countries over the last century and a half or so. Moreover, average life span has increased in the general population over the last seventy years thanks to the development of health care systems, a lack of wars and epidemics, and a more balanced diet; this is different from the concept of exceptional longevity, and has led also to an increase even in the number of centenarians, probably on account of fewer impediments to their way of life (Crimmins and Finch, 2006; Christensen et al., 2009).

Why are there so many centenarians in some isolated Italian villages? (Bifulco and Pisanti, 2017; Poulain et al., 2004; Vasto et al., 2012a, 2012b) It is possible that some villages have been enriched in centenarians with a high degree of consanguinity, such as occurring in big families. The fact that most centenarians are female, the female to male ratio is 5:1, with the exception of Sardinian villages, where the ratio is 1:1 (Caselli et al., 2006; Poulain et al., 2004), is an additional evidence in favour of biological and cultural factors being behind exceptional longevity (Candore et al., 2010; Caruso et al., 2013).

So, what is the role of diet in becoming a centenarian? There is no doubt that an inappropriate diet, in terms of quantity and/or quality of food, is a risk factor for disease, especially in the elderly. Moreover, gerontologists have demonstrated that a healthy diet, with a daily consumption of phytochemicals, i.e. plant chemicals that have biological effects, can improve health status and contribute to successful ageing. Similar results were obtained in model organism exposed to a specific dietary regimen (Aiello et al., 2016, 2017; Caruso et al., 2012, 2013; Fontana et al., 2010; Vasto et al., 2014a, 2014b).

CR also exerts beneficial effects on health, improving important parameters, such as blood pressure and fat and sugar levels, and improving cancer outcomes (Most et al., 2016). However, is there any evidence on specific diets or on diet supplements in determining an increase in the probability of reaching 100 years? Although studies on humans are needed, models can be adopted for getting a quicker answer. Recent research showed that the natural polyamine spermidine, contained in cereals and pulses, extends lifespan and health in yeast, flies, worms, and mice. In particular, oral supplementation of spermidine in old mice has cardio-protective effects, reducing cardiac hypertrophy and preserving diastolic function. It acts by enhancing autophagy, mitophagy, and mitochondrial respiration in cardiomyocytes (Eisenberg et al., 2016). Another example is palmitoleic acid (C6:1), present in small doses in macadamia nuts and in buckthorn oil. Palmitoleic acid treatment in mice increases the insulin sensitivity of adipose tissue by enhancing fatty-acid oxidation and glucose uptake, and reducing local inflammation (Bolsoni-Lopes et al., 2014; Cao et al., 2008; Lima et al., 2014). In longest-lived *C. elegans* mutants, the abundance of this fatty acid is positively correlated with the increased lifespan of the worms (Shmookler Reis et al., 2011). In humans, the gene coding ELOVL6, the main enzyme responsible for elongation of C6:1, is located in the 4q25 longevity locus (Puca et al., 2001). Furthermore, centenarians' offspring have an increased level of palmitoleic acid (Puca et al., 2008).

But, is it sufficient to take supplements or to change diet to become a centenarian? Intuitively, we could imagine that the probability of becoming a centenarian can be improved by virtuous behaviours, such as having a balanced diet and doing physical exercise, even if we do not have any scientific data to support this. In fact, it is not possible or easy to conduct such a study in humans, although the identification and the analysis of well-defined geographical areas with higher numbers of centenarians than the average of the country, suggest that a specific nutritional pattern may favour longevity. Several papers have been written on the special diet of isolated populations harbouring a high degree of centenarians, such as Okinawa (Willcox et al., 2014). However, this was no scientific association between diet components and more or less survival among its population. The same is true for the Mediterranean diet (MedDiet) – which, more than a simple dietary pattern, is a lifestyle – typically followed in some Mediterranean islands, such as Ogliastra in Sardinia and Ikaria in Greece (Poulain et al., 2004; Vasto et al., 2014b). MedDiet, the dietary pattern based on a balanced diet from the Mediterranean basin, has shown to be protective towards cancer and cardiovascular diseases. It is one of the most studied diets worldwide. It is characterized by low a glycaemic index (GI) and a low animal protein intake. In addition, it contains phytochemical compounds found in vegetables, fruit, red wine, olive oil, and nuts with anti-inflammatory and antioxidant effects. Moreover, studies on Sicilian centenarians have demonstrated that this population undergoes a sort of self-administrated CR, with an intake of only about 1200–1300 kilocalories per day. The Asiatic diet, such as that of Okinawa, is also characterized by low GI, legumes, fruit, and vegetables, with moderate fish and marine food consumption, similar to the MedDiet (Aiello et al., 2016, 2017; Caruso et al., 2012; Fontana et al., 2010; Vasto et al., 2014a).