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Gender and longevity

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Summary. At present, women show a higher life expectancy than men and this gender difference is becoming a world-wide phenomenon indicating the central demographic role of gender in ageing and longevity. A combination of genetic, environmental, historical, anthropological, socio-economic and cultural aspects as well as the geographical origin is at the basis of the longer survival of women. Maternal transmission is determining in all the three pillars of human genetics of ageing and longevity (nuclear genetics, mitochondria genetics, and microbiome genetics). Moreover, mothers and grandmothers play a key role in the offspring and grandchildren care/survival by contributing to their wellbeing and transmitting traditional and hygienic habits able to modulate life-long health status and lifespan. Finally, women live longer than men but they show a worse quality of life in advanced age indicating that gender is probably the most significant variable for the lifelong health status.

Genere e longevità

Riassunto. Attualmente, le donne mostrano un'aspettativa di vita maggiore degli uomini e questa differenza di genere sta diventando un fenomeno diffuso in tutto il mondo indicando il ruolo demografico centrale del genere nel processo di invecchiamento e nella longevità. La combinazione di aspetti genetici, ambientali, storici, antropologici, socioeconomici, culturali nonché l'origine geografica è alla base della maggiore aspettativa di vita delle donne. La trasmissione materna è determinante in ognuno dei tre pilastri della genetica umana dell'invecchiamento e della longevità (genetica nucleare, genetica mitocondriale e genetica del microbioma). Inoltre, le madri e le nonne giocano un ruolo chiave nella cura e nella protezione dei figli/nipoti contribuendo al loro benessere e trasmettendo loro abitudini tradizionali e igieniche in grado di modulare lo stato di salute e l'aspettativa di vita. Infine, le donne vivono più a lungo degli uomini ma mostrano una peggiore qualità della vita in età avanzata, indicando che il genere è probabilmente la variabile più significativa nel determinare lo stato di salute per tutta la durata della vita.

Introduction

Among humans, it is well known that at present women live longer than men, and gender difference in life expectancy is becoming a world-wide phenomenon both in industrialized nations and in low-income countries. Therefore, gender plays a central demographic role in ageing and longevity. In the biology of ageing the impact of gender difference has been extensively evaluated, but it still remains largely unexplored territory, particularly regarding the interconnection between a series of fundamental aspects such as hormonal, immunological and metabolic pathways. All these themes will be extensively addressed in the next issues of this journal. In the current article, we will concentrate on topics that have generally received less attention in previous literature, and we will focus on some genetic and cultural specificities regarding women in order to help explain some of the reasons that allow them to live longer.

It is now clear that life span as well as longevity are complex and multifactorial traits resulting from an intriguing combination of "Nature" and "Nurture", the unique reciprocal interaction between genetics and environment which characterizes every human being. Thus, we can start from the assumption that a peculiar combination of genetic, environmental, historical, anthropological, socio-economic and cultural factors as well as geographical origin contributes to longer female life expectancy worldwide.

Genetic aspects of longevity

The complex role of the double X chromosome in ageing and longevity

In the mammalian cells of a young woman, one of the two X chromosomes is epigenetically and randomly inactivated in early embryonic life. Females are therefore a mosaic of cells in which either the maternal or the paternal X chromosome is inactivated[1], and the ratio is close to 50% for each chromosome. Several studies have shown that skewed X chromosome inactivation (XCI) occurs with age in blood cells[2,3]. We investigated this phenomenon, employing an experimental model of longevity/healthy ageing consisting

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of female centenarians, their female offspring, sex- and age-matched controls (offspring born from shorter-lived parents) and young women. The model of centenarians and their offspring has allowed us to demonstrate the better health status and significant survival advantage of centenarian offspring, i.e. reduced prevalence of several age-associated diseases (cardiovascular, respiratory, osteoarticular diseases and cancer) and improved functional fitness (handgrip strength, chair stand, etc.)[4]. We confirmed that XCI skewing is an age-dependent phenomenon and demonstrated that this process was significantly less severe and frequent in centenarian offspring compared to their age-matched controls[5]. The underpinning mechanisms and biological consequences of XCI skewing with age still remain unclear. Within this scenario, our results highlight a possible detrimental link between the rate of XCI skewing and healthy ageing/longevity, fitting the hypothesis that the female mosaic is a winning strategy, sustaining a cooperative adaptive mechanism with possible advantages in pathological and physiological conditions. Conversely, the absence of a similar mosaic strategy in men might contribute to their shorter lifespan[6,7].

Mitochondria, ageing and longevity: the case of fibroblasts from centenarians

Mitochondria are fundamental organelles for cellular energy/ATP production and are actively involved in a variety of cellular processes, including urea cycle, heat production, apoptosis, inflammasome activation and cell senescence, among others. Mitochondria are also the main producers of reactive oxygen species (ROS), the most important by-product of OXPHOS. It has been suggested that, besides their physiological role in cell signalling, they may play a role in the ageing process as well as in age-related diseases. Data from primary culture of fibroblasts from long-lived individuals, including female centenarians, indicated that such cells displayed a lower complex I-driven ATP synthesis and a higher production of H_2O_2 compared with fibroblasts from old and young subjects. However, the bioenergetics of centenarian cells was well preserved and similar to that of younger subjects owing to a compensatory response provided by increased mitochondrial mass and hyperfused and elongated mitochondria. Elongated mitochondria, such as those found in centenarian fibroblasts, are largely spared from autophagic degradation, show increased levels of dimerization and ATP synthase activity, and maintain ATP production more efficiently than fragmented mitochondria. This longevity-associated mitochondria "hypertrophy" can be considered a "remodelling" mechanism capable of compensating for age-related functional defects and preserving bioenergetic functionality even at an extreme age, which likely contributes to longevity[8].

The intriguing role of maternally-inherited mitochondrial DNA (mtDNA) in human longevity

In the context of complex biological role of mitochondria, mitochondrial DNA (mtDNA) variability represents another aspect which merits particular attention in the study of longevity. Human mtDNA is a constitutive part of the genetic machinery of each cell, within the framework of continuous crosstalk between mtDNA and the nuclear genome. mtDNA (16,569 bp) encodes a few qualitatively and quantitatively relevant genes, being a constitutive component of OXPHOS and owing to the high copy number of mtDNA in each cell. mtDNA is only inherited through the mother and its germline variants (haplogroups) have been found to be associated with longevity in several populations, suggesting the presence of a maternal component of human longevity. In particular, the large EU project GEHA (Genetics of Healthy Ageing) studied 2,200 ultra-nonagenarians (90+) from different European countries belonging to 90+ sib-pairs together with the same number of sex- and geographically-matched younger controls, and was able to identify different haplogroups related to longevity in both males and females. The J2 haplogroup was associated with male longevity while H2 and T2 haplogroups were associated with female longevity[9]. Taking advantage of the complete sequencing of a high number of mtDNA molecules, it was also possible to evaluate for the first time the cumulative effect of specific and concomitant mtDNA mutations, including those that have a low or very low impact *per se*. The analysis of the mutations occurring in different OXPHOS complexes showed an intricate scenario with a different mutation burden in nonagenarian subjects *versus* controls. In particular, mutations in subunits of OXPHOS complex I had a beneficial effect on longevity, while the simultaneous presence of mutations in complexes I and III and in complexes I and V appeared to be detrimental[9]. The final conclusion was that "particular rare mtDNA mutations present only in specific populations might be beneficial (or detrimental) for longevity and may explain part of the genetic component of longevity in that population, similarly to what has been suggested for private nuclear DNA polymorphisms"[9].

MtDNA mutations are transmitted from centenarian mothers to their progeny

One of the factors that can contribute to ageing and longevity is the accumulation with age of mtDNA mutations. mtDNA heteroplasmy, *i.e.* the presence in

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the same cell of wild-type and mutated mtDNA molecules, has been supposed to have a Janus role, fuelling mitochondrial dysfunction, but also serving as a reservoir of genetic variability, helping the cells to cope with environmental and physiological stressors during life[10,11]. We were interested in testing the hypothesis that mtDNA heteroplasmy could play a role in human ageing and longevity. To this aim, we adopted two different approaches:

1. the previously described informative model, *i.e.* 31 centenarian families comprising a centenarian mother plus female offspring, in comparison with 28 female offspring of shorter-lived parents;
2. The most recent technology of ultra-deep mtDNA sequencing (average coverage of 49,334 fold for each 853bp mtDNA fragment examined).

This approach allowed us to detect 119 heteroplasmic positions with a minor allele frequency of ≥0.2%. The results indicate that low-level heteroplasmies are transmitted and maintained within families until extreme age. However, we did not observe any heteroplasmic variants associated with longevity and healthy ageing, but we identified a particular and unique heteroplasmy profile for each family. Therefore, mtDNA heteroplasmy appears to be a familial trait transmitted by the mothers which can contribute to healthy ageing and longevity[12].

Circulating mtDNA and inflammaging

Mitochondrial components, including mtDNA, can be released extracellularly, entering the blood flow and acting as damage-associated molecular patterns (DAMPs) capable of activating an innate immune response and triggering inflammation[13]. We recently observed that this phenomenon is particularly relevant in ageing because circulating mtDNA was significantly increased after the fifth decade of life, reaching the maximum value in nonagenarians. Moreover, the highest concentration of circulating mtDNA was associated with elevated levels of pro-inflammatory cytokines, thus identifying mtDNA as a new contributor to inflammaging[14]. Despite strong inter-individual variability, plasma levels of mtDNA were highly correlated in two members of the same family (90+ siblings), indicating that the mechanism regulating the levels of some inflammatory stimuli is genetically determined[14]. Inflammaging refers to the low-grade chronic, sterile inflammatory process which progressively develops with age and contributes to the pathogenesis of major age-related diseases[15,16].

Microbiomes and the role of maternal microbiomes

To this scenario, we must add a further genetic factor capable of deeply influencing human ageing and longevity. Humans must be considered as metaorganisms due to the symbiotic relationships between numerous microbial communities ("microbiota") present in various anatomical locations of the human body. Several hundreds of individual bacterial species colonize the mouth, upper airways, skin, vagina, and intestinal tract, constituting a complex and dynamic eco-system which crosstalks with the environment as well as the rest of the body, including the liver and brain. Each bacterial species has its own genome and the overall genome of each microbiota is called the "microbiome". It is worth noting that the gastrointestinal tract of neonates is colonized with microorganisms immediately after birth, mainly from the mother. The composition of the mother's vaginal tract microbiota, the mode of delivery (natural or caesarean) and breast or formula feeding have a deep impact on the gut microbiota (GM) of human offspring from the very beginning of life. Strong evidence suggests that early composition of neonates' microbiota plays an important role in the postnatal development and functionality of the immune system. The importance of GM on human health and ageing is becoming dramatically apparent. This endogenous ecosystem, together with the external antigenic load, appears to be a crucial driving force in homeostasis of the immune system, and GM lifelong changes, from neonates to centenarians, can represent an important source of inflammatory stimuli. Our group has shown that female centenarians have a different composition of GM in comparison with sex-matched younger subjects, which is associated with an increase of inflammaging (high plasma levels of proinflammatory cytokines such as IL-6 and IL-8). In general, a decrease with ageing in the biodiversity of the composition of GM is observed, with a trend towards an increase in

potentially pathogenic bacteria (pathobionts) with respect to beneficial ones (symbionts producing butyrate and other short chain fatty acids)[17].

On the whole, these considerations indicate that genetics of human ageing and longevity is dependent on three pillars (nuclear genetics, mitochondria genetics, and microbiome genetics)[18] that must be evaluated together. It is worth noting that maternal transmission plays a key role in all three areas of genetics and in their relationship with human longevity.

Maternal cultural habits, offspring survival and longevity

Socio-economic and cultural/anthropological components are fundamental contributors to human longevity and are likely related to offspring care/protection as well as transmission of behavioural habits whose influence can have a lifelong duration. A woman who protects her own health and survival through her behaviour will also protect the health of her offspring, both directly (as she is in a better state of health at the time of childbearing) and indirectly (as she is more mindful about her children's health)[19].

Healthy ageing could be advantageous and therefore positively selected for, if parents can influence improved fertility and/or survival of their children, grandchildren and/or other family members. The "Grandmother hypothesis" argues that caregiving by older women who underwent menopause could improve grandchildren's survival by alleviating the child burden of their daughters, who in turn could have more successful/numerous pregnancies and offspring. Therefore, mothers and grandmothers have assumed and continue to play a key role in the care/survival of offspring and grandchildren, by contributing to their health, by providing nutrition and by passing on traditional habits and hygienic guidelines. These practices and life-style behaviours have lifelong effects starting from intrauterine life and may modulate health status and lifespan in advanced age. However, it is also important to consider that mothers and grandmothers may also have a negative influence on offspring (by not breastfeeding, smoking and drinking alcohol, drug abuse, unbalanced diets, among other factors).

In any case, mothers do have the role and responsibility of providing the first nine months of environment to human beings. A uterine environment is sensitive to any type of metabolic, infectious and stressful event affecting the mothers, and is now recognized to be developmentally crucial in establishing a variety of set points (metabolic and epigenetic among others) in the offspring, which in turn may have long-term biological effects, as predicted by the hypothesis of the foetal origin of many diseases in adulthood and old age.

Conclusions

All the existing data suggests that one of the most powerful strategies for reaching exceptional longevity is to be female. Women live longer than men because they die at lower rates[20], but they pay for their survival advantage with a worse quality of life due to the increased prevalence of a variety of non-lethal pathological conditions. Old women display a higher overall rate of physical disability and are more likely than men to report difficulties in walking, climbing stairs and other common activities[20]. This gender dichotomy in ageing is confirmed by the centenarian model, of which females form the majority, but are characterized by poorer health as well as more impaired functional and cognitive status indicating that, even in the last phase of human life, gender is probably the most important variable of lifelong health status. This final consideration is in our opinion the most robust scientific justification for starting a new journal specifically focused on gender-specific medicine, an emerging, current and somewhat neglected topic which we are beginning to appreciate in its fascinating complexity and which deserves much more attention than we previously thought.

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