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Centenarians:

What Can They Teach Us About Lifespan & Healthspan?

ABSTRACT

The human lifespan has increased, and worldwide, the number of centenarians has increased exponentially. In their exceptional longevity, centenarians have a lower incidence of age-related diseases. Researching the underlying protective molecular mechanisms could potentially lead to discovering molecular targets which could be translated into therapeutic and possibly preventive interventions for healthy ageing.

INTRODUCTION

The human lifespan has increased, and worldwide, the number of centenarians has increased exponentially. Classically, 'blue zones' are five places in the world where centenarians and other old people live longest and healthiest. **The blue zones are Sardinia (Italy), Okinawa (Japan), Ikaria (Greece), Loma Linda (California) and Nicoya (Costa Rica).** Here, centenarians form a significant percentage of the population. And researchers have and are studying the local centenarians in order to underline the mechanisms of healthy ageing and longevity. Indeed, using the centenarians as a useful model for ageing, researchers are analyzing and focusing on the environmental and genetic dynamics to unlock the secrets of longevity.

Generally, common people have some form of disease 5 to 8 years before they die. But many centenarians are relatively healthy and keep their abilities up till some months before they die. This means that their debilitating diseases occur in a very compressed time frame. However, this does not imply that their body are young. Indeed, many experience some disability in their mobility, eyesight and hearing. But by and large, they do not suffer from major diseases like diabetes, cardiovascular disease, cognitive decline and cancer that may afflict normal persons that die in their seventies.

One might think that centenarians have these health benefits because they follow a healthy lifestyle. But in many instances, this is not always the case. In the 'The Longevity Genes Project', Dr Nir Barzilai and his team, at the Albert Einstein College of Medicine, US, found that a relatively high percentage were overweight, smoked and did not do moderate exercise. From Dr Barzilai's project, estimates for ageing show that for most people, nurture accounts for 80% and genetics accounts for the rest, whilst for centenarians, genetics accounts for 80% and the environment accounts for 20%. This gives scope to research and look for 'longevity genes'. It is proposed that the knowledge gained will then be used to offer protection from the ageing that centenarians enjoy.

Indeed, research is being directed to decipher how centenarians are living such long lives in such remarkable health. And researchers are using all the -omics (genomics, epigenomics, transcriptomics, proteomics, metabolomics) and more, to unlock the secrets of longevity, which will surely benefit humanity in general.

EPIGENETICS IN CENTENARIANS

Epigenetics is one of the very promising fields in the study of ageing and longevity. And this makes sense because epigenetics is the molecular landscape where genetics and the environment interact with each other. Moreover, this interaction then has ripple effects on the

cellular dynamics, whose alterations are studied through the various –omic fields and more, making the complex puzzle pieces fit together. Furthermore, studies have shown that the epigenetic landscape is dynamic and flexible and certain alterations in it that are conducive to disease are possibly reversible, offering scope for both prevention and treatment.

DNA methylation is a universal epigenetic mechanism controlling chromatin organization and thus its accessibility for transcription. DNA methylation is regularly associated with a compact chromatin status, rendering it less accessible to binding of transcription factors and chromatin remodelling complexes. Generally, epigenetic studies show that normally there is a loss of genome-wide DNA methylation associated with ageing and age-related diseases. However, epigenetic studies in centenarians show that they better preserve their DNA methylation status.[1,2] Furthermore, **centenarians pass this better preservation of their DNA methylation status to their offspring.**

This better preservation of their DNA methylation might imply that transposon sites in the DNA are better kept from being transcribed and thus prevented from causing havoc (see below, transposon theory of ageing).

Dr Steve Horvath, a professor at UCLA, pioneered the first "age clock" based on DNA methylation chemical changes to indicate human ageing. Today, many companies advertise such 'epigenetic clock' tests to distinguish between real age and biological age. Many believe that such information on epigenetic drift with ageing can provide insight into the ageing process that, if used correctly, can help us to make interventions to lengthen our lives. Others rightly debate and believe that ageing is much more complicated than just some DNA alterations. The FDA has not approved any of these tests. And yet researchers continue in their quest to come up with a biological test that will be reliable enough for doctors to base their health recommendations.

GENOMICS

As already said above, genetics plays a significant role in the longevity of centenarians. Thus, some research is focused on looking for 'longevity genes'. Having said this, probably as longevity is a polygenic trait, it depends on the collective small influence of many genes. Genetic studies, like GWAS (Genome Wide Association Studies) of families with long-lived individuals, indicate a longevity locus on chromosome 3, more specifically the 5q33.3 locus,[3] which has been found to be associated with a healthy cardiovascular system and healthy physical functioning.

Other studies point to gene variants of APOE (Apolipoprotein E) and FOXO3A (forkhead box O3A) genes.[4] APOE gene is located on chromosome 18, and FOXO3A is located on chromosome 6. The APOE gene codes for a major apoprotein in chylomicrons and functions in the normal catabolism of lipoproteins rich in triglycerides. FOXO3a is a transcription factor. One function of FOXO3a is to suppress mutations resulting from DNA double-strand breaking. It is proposed that it binds to genes which are then expressed to bring about apoptosis (programmed cell death).

Other genes implicated as candidates for longevity in centenarians are IGF1 (Insulin-Like Growth Factor 1), located on chromosome 12 and CETP (cholesteryl ester transfer protein), located on chromosome 16. IGF1 codes for Insulin-Like Growth Factor 1, which has protective effects against atherosclerosis. CETP codes for a protein which participates in the transfer of cholesteryl ester from HDL (high density lipoprotein) to other lipoproteins.[5]

TRANSCRIPTOMICS

Transcriptomics is the study of the transcriptome, the latter being the collection of RNAs transcribed and that includes tRNA (transfer RNA), rRNA (ribosomal RNA), mRNA (messenger RNA) and ncRNA (noncoding RNA). Its profiling is also providing more insight into longevity.

Karagiannis et al.[6] profiled the transcriptome of mononuclear cells in the peripheral blood of a centenarian cohort. Their study confirmed the established trends with ageing in the immune cell populations, specifically the swing of lymphocytes to myeloid cells, and noncytotoxic cells to cytotoxic cells. Moreover, they found a shift in the population of CD4+ T cells to B cells. Furthermore, they found higher expression of STK17A (Serine/Threonine Kinase 17a) and S100A4 (S100 calcium binding protein A4) genes. STK17A is located on chromosome 7 and codes for a protein that has apoptosis-inducing activity in response to DNA damage. S100A4 is located on chromosome 1 and codes for a protein that belongs to the S100 protein family. These S100 proteins regulate several processes inside cells, like differentiation, cell cycle progression, tubulin polymerization, motility, and invasion. Karagiannis et al. propose that their findings implicate a unique elite immunity in centenarians that remains functional in their exceptional longevity.

Jiang et al.[7] studied the differential expression of long noncoding RNAs (lncRNAs) in peripheral blood samples. They found eight lncRNAs that were differentially expressed and thus proposed them as healthy ageing candidates. Of these eight lncRNAs, two (THBS1-IT1 and THBS1-AS1) were relatively more expressed. THBS1-IT1 (Thrombospondin 1-Intronic Transcript 1) and THBS1-AS1(Thrombospondin 1-Antisense RNA 1) slow down cellular senescence, and so the researchers propose them as prospective molecules involved in the anti-ageing pathways.

In their study, Yang et al.[8] built on the established knowledge that TGF-β (Transforming Growth Factor-beta) superfamily has anti-ageing functions. Indeed, they looked for variants of TGF-β genes. They used a cohort of Ashkenazi Jewish centenarians and found a variant in the SMAD3 (mothers against decapentaplegic homolog 3) gene, which is located on chromosome 15. This gene codes for a protein that participates in the TGF-β pathway, a transduction pathway that passes environmental signals from the cell surface to the nucleus, affecting cellular functions like proliferation, movement and apoptosis (controlled cell death) of cells. From further studies on iPSCs (induced pluripotent stem cells) and a progeroid mouse model, they showed that the SMAD3 variant resulted in decreased senescence and inflammation in endothelial cells. And this, they propose, could be another factor in human longevity. They also suggest that SMAD3 could be a potential molecular target in the quest to develop drugs that could extend longevity.

Micro-RNA (miRNAs) related to longevity have also been studied. Indeed, Serna et al.[9] in their study compared the microRNA signatures in mononuclear cells of centenarians, octogenarians and young individuals. Centenarians showed a microRNA profile that overlapped that of the young individuals but not that of octogenarians. Moreover, 4 miRNAs were significantly overexpressed in centenarians. These miRNAs (Table 1) were miR-21, miR-130a, miR-494 and SCARNA17 (small Cajal body-specific RNA 17).

Table 1. miRNAs which are significantly overexpressed in centenarians.

Up-regulated miRNA	Possible Function
miR-21	Protection from ischemia of neurons[10] and heart[11,12]; Protection from mitochondrial damage[13]
miR-130a	Suppression of myocardial ischemia reperfusion[14]
miR-494	Protection against Ischemia/Reperfusion-Induced Cardiac Injury[15]
scaRNA17	Maintenance of RNA-related metabolic processes and telomere processes[16]

PROTEOMICS

Proteomics is the analytical study of all proteins transcribed by a genome. It varies temporally and spatially according to the distinct needs of the organism. Proteomics is another –omic tool in ageing research. Frankowska et al.[17] did a proteomic study based on their hypothesis that centenarians keep a cellular proteome that is 'young'. Their study confirmed that centenarians have protein dynamics very similar to those of young individuals.

Sebastiani et al.[18] also found similar 'young' signatures in centenarians. Moreover, they also found a list of serum proteins that could be used to estimate cellular senescence. They propose that these signatures could be possible biomarkers of longevity and healthy ageing, and also that they could imply prospective target molecular mechanisms for a long, healthy life.

Santos-Lozano et al.[19] found differential protein expressions in centenarians when compared to controls. Specifically, they found proteins and pathways linked to a healthy immune system (distinctively less inflammation, less autoimmunity and preserved B cell-mediated immune response). They also found that centenarians have a lower protein expression profile than individuals who have cardiovascular disease. Moreover, they found that the studied centenarians have a healthy protein expression involved in angiogenesis and intercellular junctions. They pinpoint the fact that various conditions are associated with cell junction disruptions. Examples include neuro-degeneration[20], cardiovascular disease[21] including cardiomyopathies[22], type 2 diabetes mellitus[23], and autoimmunity[24].

METABOLOMICS

Metabolomics is the process whereby metabolites are profiled in biofluids, cells and tissues. With the advances of informatics and other analytical technologies, metabolomic profile analyses are also being used to understand the physiological and pathophysiological mechanisms of ageing in centenarians.

Collino et al.[25] studied the metabolic profiles of a cohort of Italian centenarians to discover the biological mechanism of longevity. They found that the metabolic longevity phenotype shows an adaptation in the metabolism of lipids, amino acids and microbiota. They propose that this remodelling reflects the ability of centenarians to adapt or respond to the build-up of oxidative and inflammatory insults over their long life.

Cai et al.[26] found similar results (and more) in a cohort of thirty healthy Chinese centenarians. Specifically, they found 69 metabolites that were differentially expressed in the centenarians when compared to controls. From these metabolites, they identified 6 metabolic pathways which are implicated in benefiting cognition, anti-inflammatory processes (phagocytosis, immune response), glucose and lipid metabolism, suppression of oxidative damage, protein glycation, and scavenging of harmful molecules that tend to accumulate with long age. In their paper, they go in depth on specific metabolites and the beneficial pathway/s they are involved in.

TRANSPOSONS

A transposon is a piece of DNA sequence that can move from one position to another position in the genome. The human genome harbours such sequences. Transposons are held in check from doing deleterious effects (like gene mutations) in the genome by several mechanisms, one of which is epigenetic in nature (namely DNA methylation, histone modifications and small non-coding RNAs). Transposons are being implicated to have physiological and pathological functions in the genomes of the cells that host them. Indeed, accumulating evidence is showing that they may be associated with certain human diseases, like rheumatoid arthritis, insulin-dependent diabetes mellitus, neurological diseases (e.g. schizophrenia, multiple sclerosis, motor neuron disease) and cancer. Understanding how these transposons bring about these human diseases could help in their prevention and treatment.[27]

This knowledge begs the question, 'Could centenarians have better systems to keep in check these transposons, that if unleashed could cause havoc in the genome and hence bring on disease?' Research along the hypothesis that transposons might be responsible for ageing (transposon theory of ageing) is ongoing. Simon et al.[28] found a variant of Sirtuin 6 (SIRT6) in Ashkenazi Jewish centenarians. Sirtuin 6 (SIRT6) is implicated in ageing and in the regulation of metabolism. It does so via multiple pathways in the cell. They found that this variant of SIRT6 (centSIRT6) enhances human longevity by maintaining the human genome. Specifically, this variant confers better suppression of LINE1 (long interspersed nuclear elements 1) retrotransposons, better repair of breaks in the DNA double strand, and also offers better protection against cancer cells.

Another mechanism is the preferential insertions of LINE1 (L1) sequences to gene-poor regions like those found in chromosome 13.[29,30] Chromosome 13 (like chromosome 18, 21, and Y) is a gene-poor chromosome. Thus, new insertions of L1s will unlikely disrupt any important gene loci.

STEM CELLS

The functions of stem cells (like repairing or regenerating tissues) deteriorate as humans age.[31] It is believed that this deterioration in stem cell functions plays an important role in the ageing process and its associated disorders. Indeed, in the 'stem cell theory of ageing', it is hypothesized that ageing is due to the failure of stem cells to renew themselves and provide repair.[32,33]

Ingles et al.[34] using peripheral blood mononuclear cells (PBMCs) in centenarians found that they up-regulate the transcription factors called Yamanaka Factors (i.e. OCT3/4, SOX2, and c-MYC) and other genes (VIM, NCAM, BMP4, and BMPR2) that implicate stemness. They also found up-regulation of BCL-xL (B-Cell Lymphoma-extra-large), a mitochondrial protein which promotes cell survival by inhibiting apoptosis. BCL-xL is also involved in senescence and autophagy pathways. The authors propose that their findings might be one mechanism that explains why centenarians have a unique and efficient cell homeostasis, enabling them to live a long and healthy life.

TELOMERES

Telomeres are structures that protect chromosomal ends. Some telomere research is also the focus of ageing research. Specifically, as a chromosome replicates, its telomere shortens, and its length makes it an approximate gauge of biological ageing and mortality.

Tedone et al.[35] compared the frequency of age-related diseases and telomere length in centenarians and their offspring. They discovered that centenarians and their offspring preserve better telomere length. They propose that this superior maintenance might play a part in their longevity and health.

TRANSLATIONAL RESEARCH

The research on centenarians is showing the underlying main hallmarks of ageing, namely inflammation, loss of proteostasis, stem cell exhaustion, metabolic dysregulation, epigenetic aberrations, failure of chromosome maintenance (DNA repair, telomere erosion), cellular senescence, mitochondrial dysfunction, altered intercellular communication and immunity failure. It also shows that there is connectivity between these hallmarks, and it is proposed that one can target one hallmark and also get improvements in the others. The holy grail of gero-therapeutics is targeting such pathway/s to increase human healthspan and lifespan.

One way forward will surely be non-coding RNA-based therapy. Some of the physiological and pathophysiological roles of non-coding RNAs (ncRNAs) associated with ageing have already been discovered. These discoveries have been pivotal in providing insight into the basis of various ageing diseases where ncRNAs are aberrantly expressed. Already, translational

research uses these ncRNAs mechanisms for theranostics. Presently, the main players in non-coding RNA-based therapies include antisense oligonucleotides, locked nucleic acid anti-miRs, miRNA sponges, miRNA mimics, siRNAs and ribozymes. Grech et al.[36] discuss this in their paper.

One gero-therapeutic that is being evaluated for its protective effect against ageing is Metformin.[37] Indeed, studies are showing that it impacts many of the mentioned main hallmarks of ageing and thus has the potential to modulate ageing and treat diseases associated with ageing.

CONCLUSION

Many of the chronic human diseases have ageing as the underlying risk factor. Using centenarians as an ageing model has led to the concerted discovery of conserved molecular pathways associated with extended lifespan and healthspan. Hopefully, these insights will offer molecular druggable targets to develop gerotherapeutic interventions to extend and maximize human healthspan ... some even believe in the possibility of ageing reversal.[38] Moreover, these insights will lead to the discovery of biomarkers that are predictive of healthy ageing, making the latter's medical and socio-economic benefits feasible.

REFERENCES

1. Gentilini D, Mari D, Castaldi D, et al. Role of epigenetics in human aging and longevity: genome-wide DNA methylation profile in centenarians and centenarians' offspring. *AGE* 2013;35:1961–1973.
2. Daunay A, Hardy LM, Bouyacoub Y, et al. Centenarians consistently present a younger epigenetic age than their chronological age with four epigenetic clocks based on a small number of CpG sites. *Aging* 2022;14:7718–7733.
3. Nygaard M, Thinggaard M, Christensen K, et al. Investigation of the 5q33.3 longevity locus and age-related phenotypes. *Aging* 2017;9:247–255.
4. Brooks-Wilson, AR. Genetics of healthy aging and longevity. *Human Genetics* 2013;132:1323–1338.
5. Capri M, Santoro A, Garagnani P, et al. Genes of Human Longevity: An Endless Quest?*Current Vascular Pharmacology* 2014;12(5):707–717.
6. Karagiannis TT, Dowrey TW, Villacorta-Martin C, et al. Multi-modal profiling of peripheral blood cells across the human lifespan reveals distinct immune cell signatures of aging and longevity. *eBioMedicine* 2023;90:104514.
7. Jiang J, Cheng L, Yan L, et al. Decoding the role of long noncoding RNAs in the healthy aging of centenarians. *Briefings in Bioinformatics* 2021;22(5):bbaa439.
8. Yang J, Tare A, Zhang L, et al. Longevity-associated SMAD3 non-coding centenarian variant impairs a cell-type specific enhancer to reduce inflammation. *bioRxiv* 2023:05.17.540984.
9. Serna E, Gambini J, Borras C, et al. Centenarians, but not octogenarians, up-regulate the expression of microRNAs. *Scientific Reports* 2012;2:961.
10. Buller B, Liu X, Wang X, et al. MicroRNA-21 protects neurons from ischemic death. *FEBS* 2010;277(20):4299–4307.
11. Dong S, Cheng Y, Yang J, et al. MicroRNA Expression Signature and the Role of MicroRNA-21 in the Early Phase of Acute Myocardial Infarction. *Journal of Biological Chemistry* 2009;284(43):29514–29525.
12. Cheng Y, Zhu P, Yang J, et al. Ischaemic preconditioning-regulated miR-21 protects heart against ischaemia/reperfusion injury via anti-apoptosis through its target PDCD4. *Cardiovascular Research* 2010;87(3):431–439.
13. Sayed D, He M, Hong C, et al. MicroRNA-21 Is a Downstream Effector of AKT That Mediates Its Antiapoptotic Effects via Suppression of Fas Ligand. *Journal of Biological Chemistry* 2010;285(26):20281–20290.
14. Li Y, Zhang H, Li Z, et al. microRNA-130a-5p suppresses myocardial ischemia reperfusion injury by downregulating the HMGB2/NF-κB axis. *BMC Cardiovascular Disorders* 2021;21:121.
15. Wang X, Zhang X, Ren X-P, et al. MicroRNA-494 Targeting Both Proapoptotic and Antiapoptotic Proteins Protects Against Ischemia/Reperfusion-Induced Cardiac Injury. *Circulation* 2010;122(13):1308–1318.
16. Zhao Y, Abreu E, Kim J, et al. Processive and Distributive Extension of Human Telomeres by Telomerase under Homeostatic and Nonequilibrium Conditions. *Molecular Cell* 2011;42(3):297–307.
17. Frankowska N, Bryl E, Fulop T, et al. Longevity, Centenarians and Modified Cellular Proteodynamics. *International Journal of Molecular Sciences* 2023;24(3):2888.
18. Sebastiani P, Federico A, Morris M, et al. Protein signatures of centenarians and their offspring suggest centenarians age slower than other humans. *Aging Cell* 2021;20(2):e13290.
19. Santos-Lozano A, Valenzuela PL, Llavero F, et al. Successful aging: insights from proteome analyses of healthy centenarians. *Aging* 2020;12(4):3502–3515.
20. Erdık F, Denes L, de Lange E. Age-associated physiological and pathological changes at the blood–brain barrier: A review. *Journal of Cerebral Blood Flow & Metabolism* 2017;37(1):4–24.
21. Li C, Gao M, Zhang W, et al. Zonulin Regulates Intestinal Permeability and Facilitates Enteric Bacteria Permeation in Coronary Artery Disease. *Scientific Reports* 2016;6:29142.
22. Mezzano V, Sheikh F. Cell–cell junction remodeling in the heart: Possible role in cardiac conduction system function and arrhythmias? *Life Sciences* 2012;90(9–10):313–321.
23. Jayashree B, Bibin YS, Prabhu D, et al. Increased circulatory levels of lipopolysaccharide (LPS) and zonulin signify novel biomarkers of proinflammation in patients with type 2 diabetes. *Molecular and Cellular Biochemistry* 2014;388:203–210.
24. Fasano A. Zonulin and Its Regulation of Intestinal Barrier Function: The Biological Door to Inflammation, Autoimmunity, and Cancer. *Physiological Reviews* 2011;91(1):151–175.
25. Collino S, Montoliu I, Martin F-PJ, et al. Metabolic Signatures of Extreme Longevity in Northern Italian Centenarians Reveal a Complex Remodeling of Lipids, Amino Acids, and Gut Microbiota Metabolism. *PLOS ONE* 2013;8(3):e56564.
26. Cai D, Zhao Z, Zhao L, et al. The Age-Accompanied and Diet-Associated Remodeling of the Phospholipid, Amino Acid, and SCFA Metabolism of Healthy Centenarians from a Chinese Longevous Region: A Window into Exceptional Longevity. *Nutrients* 2022;14(20):4420.
27. Grech A, Baldacchino S, HERVs, Transposons and Human Diseases: Part III. *The Synapse* 2013;12(2):9-11.
28. Simon M, Yang J, Gigas J, et al. A rare human centenarian variant of SIRT6 enhances genome stability and interaction with Lamin A. *The EMBO Journal* 2022;41(21):e110393.
29. Bojang Jr P, Anderton MJ, Roberts RA, et al. De novo *LINE-1*retrotransposition in HepG2 cells preferentially targets gene poor regions of chromosome 13. *Genomics* 2014;104(2)96–104.
30. Esnault C, Millet J, Schwartz O, et al. Dual inhibitory effects of APOBEC family proteins on retrotransposition of mammalian endogenous retroviruses. *Nucleic Acids Research* 2006;34(5):1522–1531.
31. López-Otín C, Blasco MA, Partridge L, et al. The Hallmarks of Aging. *Cell* 2013;153(6):1194–1217.
32. Smith JA,& Daniel R. Stem Cells and Aging: A Chicken-Or-The-Egg Issue?*Aging and Disease* 2012;3(3):260–268.
33. Shin J, Mohrin M, & Chen D. Reversing stem cell aging. *Oncotarget* 2015;6:14723–14724.
34. Inglés M, Mas-Barques C, Berna-Erro A, et al. Centenarians Overexpress Pluripotency-Related Genes. *The Journals of Gerontology: Series A* 2019;74(9):1391–1395.
35. Tedone E, Arosio B, Gussago C, et al. Leukocyte telomere length and prevalence of age-related diseases in semisupercentenarians, centenarians and centenarians' offspring. *Experimental Gerontology* 2014;58:90–95.
36. Grech A, West S. Non-Coding RNA-Based Therapy. *The Synapse* 2022;21(3)18–20.
37. Kulkarni AS, Gubbi S, Barzilai N. Benefits of Metformin in Attenuating the Hallmarks of Aging. *Cell Metabolism* 2020;32(1):15–30.
38. Yang J-H, Petty CA, Dixon-McDougall T, et al. Chemically induced reprogramming to reverse cellular aging. *Aging* 2023;15(13):5966–5989.