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REVIEW ARTICLE

ROLE OF GENETICS IN AGEING, LONGEVITY AND WAYS TO REVERSE AGEING

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ABSTRACT

Ageing is a normal process in all living organisms including human. With the progress of ageing physiological functions of different organs of the body become slower leading to many age-related diseases that are responsible for shortening of longevity. But this reduction of ageing varies from individual to individual. This diversity of changes in ageing process has come to the mind of scientists to find out some factors that regulate ageing both at the cellular and genetic levels. All these improvements may be due to the advancement of medical research as well as the development of new drugs in relation to different types of diseases starting from infant mortality to age related ailments. Still demographic data of increase in longevity in different countries have opened new ideas to the scientists that the identification of factors or genes that regulate ageing is urgently needed. Demographic studies show that human life span has no sign of fixed limit imposed by biology or other factors. It has been noted that both the average and maximum life span have increased steadily over time for more than a century. Thus the research on Ageing , its genetics and the application of modern technique of Genetic engineering and other methods to reverse the ageing has gained its importance to enjoy healthy and active life even in old ages.

INTRODUCTION

Life span of Human is correlated with a process called Ageing. This is followed by the gradual changes in the body systems. With the progress of ageing physiological functions of different organs of the body become slower and slower leading to different types of diseases that are responsible for the shortening of life span. But some people can evade or delay all these age related problems leading to the increase of their life-span or longevity. In some individuals ageing appears first in their appearances like wrinkled skin, grey hairs and others may affect first in the function of different organs like heart, kidney, liver, brain etc and decrease in muscular strength without appearing major changes in external appearance. This diversity of changes in ageing process has come to the mind of scientists to find out some factors that regulate ageing both at the cellular and genetic levels. It has also been noted that average life-span has increased at the rate of three months per year in both males and females since 1840 (Oeppen and Vaupel 2002 ; Wheeler and Kim 2011). The assessment of life-expectency was based on the Demographic data on age-specific morality and the incidence of age-related diseases. During demographic survey it has been noted that in Japan the longevity of women have increased from the age of 65 to 100 from less than 1 in 1000 in 1950 to 1 in 20 in 2002 (Oeppen and Vaupel 2002).

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In United States also the centenarian population is showing the increase of longevity from 3700 in 1940 to about 61000 in 2006 (Sonnega 2006; Wheeler and Kim 2011). All these improvements may be due to the advancement of medical research as well as the development of new drugs in relation to different types of diseases starting from infant mortality to age related ailments. Still demographic data of increase in longevity in different countries have opened new ideas to the scientists that the identification of factors or genes that regulate ageing is urgently needed. Demographic studies show that human life span has no sign of fixed limit imposed by biology or other factors. It has been noted that both the average and maximum life span have increased steadily over time for more than a century (Wilmoth 2000). Thus the research on Ageing , its genetics and the application of modern technique of Genetic engineering and other methods to reverse the ageing has gained its importance to enjoy healthy and active life even in old ages. In this context, one ancient story of Yajati in Mahabharata may be mentioned here. Yajati , known as World Emperor in Mahabharata, was once cursed by sage Shukra due to his some misdeeds . He married Devjani, the daughter of Shukra and also married Sharmistha, daughter of King Vrishaparvana. When Devjani complained to his father that Yajati had another relationship with Sharmistha , then Shukra cursed Yajati to attain old age in his young age. Later Shukra gave Yajati some relaxation of being able to transfer his old age to his willing son. The two sons of Devjani refused the offer. Of the three sons of Sharmistha , the last son Puru accepted the offer.

Then Yajati got his youth and Puru got the old age of his father. Later Yajati transfers his old age to his son Puru and in return he got his old age. Then Puru became the first king of Pourava Dynasty and the ancestors of Dushyanta, Kauravas and Pandavas. This old story of Mahabharata might have some relationship with GENE TRANSFER TECHNOLOGY of Modern science as it is known that longevity (duration of human life) and ageing is determined by genes. Similar story was also noted in Greek mythology. Here Tithonus was the lover of Eos, Goddess of the Dawn. Eos has taken Tithonus from the Royal house of Troy for her lover. Eos asked Zeus to make Tithonus immortal and Titonus lived forever. Zeus is considered the father of all Gods and humans. But Eos forgot to ask eternal youth for him and so he became older and finally faced death. This myth also showed that ageing might be controlled or manipulated.

Diversity of Longevity and Ageing

The diversity of longevity (life-span) and ageing is found in many animals which may be due to different factors like genes, environment and lifestyle. It has been noted that some turtles and rockfishes (Marine ray-finned fishes) do not show any sign of ageing except little senescence. But certain fishes like African annual fishes do not live more than 12 weeks in the laboratory showing sign of fast ageing processes showing diversity in ageing (de Magalhaes 2012). Generally mammals show wide differences in longevity. The mouse is generally short lived of about 4 years while bow-headed whale can live for 211 years. The second longest mammal is human maximum up to 122 years. There may be a relationship between environments and ageing under which the organisms evolve a. In case of human there may be a primate lineage in getting longevity as shown by evolutionary theory. Weismann first proposed that the longevity depends on the way of evolution in the species or group in 1891. Later Weismann proposed another theory in organisms that separate reproductive cells (germ cells) from somatic cells use more energy in germ cells rather than in somatic cells. So ageing occurs in somatic cells followed by its deterioration (de Magalhaeus 2012). However, it has now been accepted that ageing is controlled by genetically regulated processes and some environmental factors.

AGEING GENETICS

Ageing is determined by Genetic, Environmental and the Lifestyle maintained by the people. It has been found that longevity depends on the life style maintained by the people as non-smokers, without any obesity, regularity and the capacity of coping well with stress. Another important point for increasing longevity in human is due to the advancement of biomedical research in preventing and treating infectious and age-related diseases. The direct consequence is a steady increase in the proportion of people living in the country where their health and life style are well maintained to delay ageing. It has been estimated that the percentage of people in UK over the age of 65 will rise to over 25% by the year 2050, compared to 14% in 2004 (de Magalhaes 2012, Smith 2004). Most normal function and physiological function start to decline with age as well as there is a loss of muscle and bone mass with a gradual reduction in height, weight and lower metabolic rate leading to susceptibility of many diseases. The most critical organ is the heart as it has no period of rest, so the major cause of death for older people particularly after 85 is heart disease or heart failure.

Thus ageing is the reason for causing some physiological changes leading to a functional decline and loss of equilibrium between different physiological systems and their capacity to respond to environmental challenges that is known as Homeostasis (de Magalhaes 2012). Thus this progressive deterioration of physiological function leads to mortality in old age. But there is a in loss of function and rate of morbidity between individuals of the same groups that may be due to some genetic difference Each organ of the human consists of many small organelles that are again formed by proteins programmed by genes. With the progress of ageing lot of changes occur in cells and different molecules including DNA. So the understanding of genetics and other non-genetic factors of ageing is necessary to delay ageing and age related disorders. If the identification of gene responsible for ageing is possible then anti-ageing therapies can be used to stop or delay ageing. Finally with the help of Gene transfer technology the manipulation of the process of human ageing may be done.

There are two theories of ageing. One is Stochaistic process meaning thereby that ageing (senescence) occurs randomly by genetic mutations which accumulate with time. Another theory is the non-stochaistic process meaning thereby that it is a programmed senescence where ageing is predetermined and is a function of the expression of structural genes (Stuart-Hamilton 2011). Structural genes code for the synthesis of specific polypeptides that is regulated by structured mRNA elements. Senescence or age related changes may also occur due to accumulation of free radicals and through gradual loss of immune system. The free radical has an unpaired electron in the outer ring of any molecule produced during aerobic respiration. The reactive oxygen produced in certain types of mitochondria during oxidative stress may also play an important role in ageing due to oxidative damage followed by senescence (Pankow and Solotoroff 2007). Free radicals are unstable molecules that are produced during aerobic respiration, mitochondrial electron transport and other physiological processes that cause cumulative damage. Naturally these free radicals in the human body are destroyed by protective enzyme systems. The accumulation of extra free radicals may cause aging, damage to different organs leading finally to death. The increase in damage due to free radicals are due to point and other deleterious mutations in the mitochondrial DNA (mt DNA) leading to altered mitochondrial respiration with the formation of many free oxygen radicals (Troen 2003). These free radicals have played an important role in regulating differential gene expression, cell replication, differentiation and apoptotic cell death (Troen 2003).

Many scientists believe that Life-span or longevity is under genetic control and genetic mutations can modify ageing. As people become older with the increase of age, point mutations are also increasing and the efficiencies of enzymes are decreasing in the metabolic reactions. In the senescent cells many defective proteins and altered DNA polymerase enzymes are found. In addition to this the occurrence of defects in DNA-repair enzymes may also lead to premature ageing syndrome in human (Gilbert 2000).

Genes for Ageing

The database of genes related to aging as per GenAge Database can be divided into genes related to longevity or age-related genes in model organisms (GEnData Base).

There are over 1800 genes altering lifespan in different model organisms like 838 in C. elegans, 883 in yeast, 170 in Drosophila and 126 in mouse . Some of the genes of model organisms are CG31196 gene from transcript CG31196-RA in *Drosophila melanogaster* (Anti longeivity, maximum life span change 49%), ADP/ATP Carrier in Yeast *Saccharomyces cerevisiae* (Anti longevity, maximum life span change 90%), AMP-activated kinase in *Caenorhabditis elegans* (Pro-longevity), etc.(Taken from Gen Database). With the progress of research in understanding the increase in longevity or lifespan of human, many genes have been identified. Some of these are APOE (Apolipoprotein E), SIRT1 (Sirtuin 1), DAF16 (producing FOXO1 transcription factor), FOXO3, SOD2(Superoxide dismutase), IGF1(Insulin like growth factor), CLOCK1 (mCLK1), AMPK (Adenosine monophosphate-activated protein kinase), klotho, CETP and GATA6 (GATA binding protein6). Thus genetic studies of human ageing are needed to understand the mechanism of longevity. Detailed studies of ageing genes particularly tissue-specific genes may help to predict which tissues of particular organs will deteriorate rapidly and which will decline gradually. This will help to identify the occurrence of age-related diseases in a specific organ to bring decline in longevity through death. Further this may also help in taking early intervention to bring healthy years of life (Wheeler and Kim 2011).

In this recent age, Whole Genome Sequencing studies are used to find out the genetic variability in every individual in coding and non-coding regions to identify genetic reasons for longevity. The female generally carries homozygosity with some common variants for their role in metabolism to promote longevity in GWAS (Genome Wide Association Studies) studies while male shows no such similar stretch of homozygosity resulting in increase of longevity in woman in certain population.

Some of the longevity genes are discussed below

Apoe

(Apolipoprotein E) : It is known as cholesterol carrier and has an important role in the transport of fats and cholesterol through blood, maintenance of blood-glucose level in the body, brain physiology, neuronal cell homeostasis and cardio vascular death (Nasser Bin-Jumah *et al* 2022).. This gene codes for a protein Apoprotein that binds with the lipid to form Lipoprotein. There are three alleles of the gene such as e2, e3 and e4 for APO E2, APO E3 and APO E4 proteins. About 50% of world population has e3 genotype. The allele e4 is responsible for causing Alzheimer and cardiovascular diseases. The shorter lifespan of about 4 years has been noted in human population having greater frequency of e4 allele. The gene APOE is located in the chromosome 19: q13.3 along with APOC 1, APOC 2 and APOC 4 (Abondio *et al* 2019). Some variations in the sequence of amino acids are found in these proteins leading to binding variability with corresponding molecules like LDL receptors(Low Density Lipoprotein), cell surface HSPGs (Heparin sulphate proteoglycans) receptor and ABCA1 (ATP binding cassette protein). It has been noted through Genome-wide association study (GWAS) that the allele APO e4 is missing in most of the centenarians. In a group of population (cohorts) of Japan and Italy the APO ϵ allele (APOE isoforms) has been found to be associated with an increased longevity (Serbezov *et al* 2018). The variability of different isoforms of APOE gene has been found to be associated with longevity and age related diseases in different human populations, Studies on DNA methylation variations in different tissues of human populations have helped to define the role of this gene in longevity (Abondio *et al* 2019). In the GWA studies of longevity, SNPs (Single Nucleotide Polymorphism) are found near the APOE locus. The variations in SNPs near the APO E locus including some rare variants have lot of significance in GWA studies of longevity. A GWAS meta analysis identified the TOMM40/ APO E/ APO C 1 locus that are associated with reaching ≥ 90 years of age (Nasser *et al* 2022). It has also been noted that variants of APO E and FOXO3A genes are associated with longevity. Detailed studies are needed to understand the role of different gene in longevity. The variant of APOC gene (5′-UTR APOC3) is found in the elderly people of Russian population. One variant in the promoter of the same gene was found in the Ashkenazi Jewish population which was associates with longevity (Atzmon *et al* 2006; Serbezov *et al* 2018). GWAS studies again show that there is an association between variant rs 16835198-G of the gene FNDC5 (which synthesizes a pro-hormone upregulated by muscular exercise) and APOE alleles in the Japanese individuals with extreme longevity (Abondio *et al* 2019).

Research on Candidate gene association with longevity is also important. Canidadte gene is referred to genes that are related to a particular character or trait like disease, longevity and others. This identified gene as a candidate is important for the detailed study of particular characters as life span extension in this case. Of the genes identified for longevity, the most important ones are different variants of APOE and FOXO3 and SIRTULINS (Btooks-Wilson 2013).

Sittulins

Sirtulin gene is commomly known as anti-ageing genes in Yeast, Drosophila , *Caenorhabditis elegans* and in Mammals. This gene family consists of seven members . There are mitochondrial (SIRT3, SIRT 4 and SIRT 5) , cytoplasmic (SIRT2) and nuclear (SIRT1, SIRT6, SIRT7). SIRT 1 to SIRT7 have an important role in regulating cell growth, metabolism, stress resistance, neural function and ageing. With the information gathered from research on Yeast that the overexpression of SIRT2 gene may extend the lifespan of at least by 70%, the importance of Sirtulin gene in longevity has got special emphasis (Grabowska *et al* 2017; Kaeberlein *et al* 1999). It has also been noted from the findings of results after Caloric restriction in diet on Yeast, Worm, Drosophila and Mouse that SIRT1 is one of the important factors in longevity extension. This result has been corroborated by using SIRT1 activating compounds Resveratrol in several organisms including yeast showing anti-ageing activity of SIRT1 (Duan 2013). The mechanism of SIRT1 on longevity may be due to deacetylation of histone and nonhistone substrates leading to gene silencing through interaction with DNA and decline of enzymatic activity. These deacetylation processes have caused diverse effects in metabolism, survival promotion and autophagy. Sirtulins are under class 3 histone deacetylases and its catalytic activity depends on NAD+ and is regulated by NAD+ level and NAD+/NADH ratio suggesting that Sirtulins might have evolved as sensors of energy and redox status on the cell (Grabowska *et al* 2017). In addition to deacylate histones, Sirtulins can also deacylate some transcription factors

and cytoplasmic proteins thus affecting catalytic activity, stability and binding to other proteins or chromatin. SIRT1 has also been found to suppress genes involved in fat storage, apoptosis and inflammation showing thereby its importance in longevity. Some fraction of SIRT2 can translocate to the nucleus for regulating the cell cycle. SIRT3, SIRT4 and SIRT5 are found mainly in the mitochondria whereas SIRT1, SIRT6 and SIRT7 are located in the nucleus. SIRT2 is found in the cytoplasm where it functions as a tubulin deacetylase and is giving nuclear export signal. Some abnormalities in energy metabolism and mitochondrial function are linked to ageing due to decline in physiological functions followed by DNA damage. It has been found that SIRT1is regulating most of the metabolic activities by controlling acetyation process, metabolic enzymes and transcriptional factors. Another longevity regulator PARP (Poly ADP-ribosyl polymerses is influencing the activity of SIRT1 by controlling metabolic activity and in assisting DNA repair mechanism (Canto and Auwerx 2011). Another important Sirtulin is SIRT7 that is also involved in different cellular processes and has an important role in health and ageing processes in human. This gene is located chromosome 17 in the region 17q 25.3 of 6238 base pairs. SIRT7 is also located in the nucleolus. SIRT7 has a close relationship in ageing and age-related processes (Lagunas-Rangel 2022). This gene produces protein of 400 amino acids having molecular weight pf 44.9 KDa and has been found to carry out many enzymatic activities using the co-substrate NAD+ and id regulated by the NAD+/NADH ratio (Grabowska *et al* 2017). Proteins produced by SIRT7 gene is called the protein SIRTUIN (anti-ageing proteins / longevity proteins) which is essential for the survival of living organisms. It has also been noted that the anti-ageing proteins Sirtuin reduces the reproduction activity to use resources on longevity. The reduction in calorie intake with regular physical activity has some effects on the production of the longevity protein, Sirtuin, leading to the increase of life expectancy. This protein has other important functions like the maintenance of Cardiac health, sleep-wake cycle etc which help to regulate different metabolic syndrome, diabetes, risk of cancer, neuro-degenerative diseases and other age related diseases. For this reason Sirtulin is an important longevity gene. It has been noted that a chemical, Resveratrol can activate the gene Sirtulin to prolong longevity. The protein produced from the gene SIRT2 has been found to co-ordinate metabolic activities to maintain physiological homeostasis in human. Physiological homeostasis is known as the tendency of the body to maintain some important physiological parameters like blood glucose level, blood pressure, blood salinity, body temperature to keep a stable equilibrium of the internal environment.

It has already been known that mitochondria is the power house of cell by producing ATP as well as the source of generating Reactive oxygen species (ROS) and the initiation of Apoptosis signalling pathway. When the mitochondrial proteins are deacylated by NAD+ dependent mitochondrial deacytylases, the function of mitochondrial proteins including proteins of SIRT3, SIRT4 and SIRT5 genes are altered. The role of SIRT3 in ageing is important as it has the ability to suppress ROS which is one of the important factors in the process of ageing (Duan 2013). Another role of SIRT3 gene has been found in the prevention of Age-related hearing loss (ARHL) , a very common disease of elderly people indicating thereby that SIRT3 may be the critical modulators of longevity or lifespan (Duan 2013).

daf 16/ FOXO: The daf16 is another longevity gene producing protein DAF2 is found in many organisms like nematode (C. elegans), mice, Drosophila and human. It encodes the protein FOX O (Forkhead box protein). This protein FOXO is necessary in the Insulin/IGF1 signalling pathway which has an important role in longevity, lipogenesis, dauer formation and stress responses. In Caenorhabditis elegans DAF 16 is the only forkhead box transcription factors class O (FOXO) homolog that acts as a signal to make changes in many genes in ageing, development, stress, metabolism and immunity (Zecic and Braeckman 2020). In the research of Ageing, *Caenorhabditis elegans* is being used as a model organism due to its short lifespan, fully known gene sequence and other available data. Another advantage of this microscopic round worm is that it attains within two to three weeks. Further it has been noted that one single gene mutation in DAF2 showed two times increase in longevity in C. elegans (Kenyon *et al* 1993). Originally DAF16 has been identified during studies of dauer formation in the larva of the worm. Under adverse condition the larva goes to pause their normal development and metamorphose in dauer state to survive harsh condition. When the environment becomes normal, then the larva starts normal development leading to the idea that ageing is mainly controlled by genes in addition to physiological processes and other environmental factors. If the longevity is controlled by single gene as shown in C. elegans, then the idea has come that either ageing can be slowed down or it can be reversible. It has been noted that the gene daf16 is found to be functional unless it forms a complex with a transcription factor DAF16/FOXO. This transcription factor integrates signals from different pathways particularly the Insulin/IGF1 signalling to regulate ageing and longevity. Most of the work on ageing and longevity has been done in the model organism, C. elegans to understand the molecular mechanism of signalling pathway, microarray analysis to know the expression of different genes in the ageing process (Murphy *et al* 2003). There are other signalling pathways like TOR (Target Of Rapamycin) signalling, AMPK (AMP-activated protein kinase) pathway, JNK (c-Jun N-terminal kinases) pathway and germline signalling that are involved in ageing and longevity (Sun *et al* 2017). Of these signalling processes, the Insulin/IGF1 is the most important.

There is another gene daf2 where the protein (DAF2) encoded by this gene is like the receptor protein in human and functions as insulin receptor (Kimura *et al* 1997). In addition to ageing, this gene daf2 regulates stress resistance, metabolism and development. The molecular mechanism and function of daf2 have been studied in details in C. elegans showing that daf2 regulates many other genes leading to control many physiological processes at different stages of life using transcription factors produced by daf 16. Scientists have an idea on various genes producing proteins may have an effect on longevity in acting as antioxidants, regulating metabolism and exerting an antibacterial effect (Gems and McElwee 2003).

GATA 6

This gene encoding GATA-binding protein 6 regulates ageing of human mesenchymal stem/ stromal cells. It is a member of the zinc finger transcription factors that generally has a role in the regulation of cellular differentiation and organogenesis in vertebrate development. It has been noted that induced pluripotent stem cells have a role in slowing down and reversing the ageing process.

If the dedifferentiated or mature somatic cells are converted to Pluripotent state then somatic cells will lost its identity of dedifferentiation that is called reprogramming of cells to differentiate for generating any somatic cell type or embryonic stem cell like state or re-establishment of self renewal capabilities (Simpson *et al* 2021). The induction of pluripotent cells is due to the overexpression of four transcription factors (Oct 3 /4, Sox2, KLf4 and c-Myc) that is called Yamanaka Factors or OSKM factors (Takahasi and Yamanaka 2006; Simpson *et al* 2021). It has been found that GATA 6 binding protein and FOXP1 regulate cellular reprogramming or induced changes of mesenchymal stem cells/stromal cells (epigenetic rejuvenation) to reduce biological age or age-related activities. Again ageing process is associated with a variety of molecular hallmarks like telomere reduction, genetic instability, genetic or DNA damage, mitochondrial dysfunction, transcriptotomic and epigenetic alterations etc. The effect of all these factors in ageing may throw a light in reversing the process of ageing and rejuvenation of phenotypic effect (Gill *et al* 2022). In one experiment of reprogramming fibroblasts for 10-17 days, it has been found under the light microscope that the cells were transformed from mesenchymal to epithelial state followed by a colony structure indicating thereby that morphological reversion is possible after reprogramming (Gill *et al* 2022). This reprogramming is also connected with reactivation of the expression of telomerase responsible for maintaining telomere length which has an important role in ageing.

With age changes are found in impaired responses in skeletal muscle, thinning of skin epithelium and hypercellularity of bone marrow in vertebrate cells of mouse. After rejuvenation, the restoration of youthful changes in the tissues and functions have been noted like thickness of the skin epithelium, reduction of hyercellularity in bone marrow and enhancement of muscle regeneration (Rando and Chang 2012). Fig.1 may be added from this paper. This rejuvenation of an old cell to young ones without any change in differentiated cells is called epigenetic rejuvenation. The important molecular changes in ageing is the accumulation of damaged macromolecules like DNA, Protein and Lipids leading to cellular dysfunction and alterations of gene expression (Rando and Chang 2012; Seviour and Lin 2010). With the increase of age, accumulation of mutations leads to undergo apoptosis, malignant transformation and senescence. In addition to genetic factor, the epigenetic factors like changes in DNA methylation, histone modification, chromatin remodelling may have an important role in ageing. Metabolic changes in several pathways like AMPK (AMP- Activated Protein Kinase) pathway, Insulin like signalling pathway and Oxidative stress, Calorie restriction etc.

Some Factors controlling Ageing

Insulin-like signalling Pathway

This is the most important pathway in the ageing process. It depends on the Insulin-like growth factor 1 (IGF1) binding to IGF1 receptor that activates the cellular phosphoinositol-3-kinase which finally activates the kinases and serum and glucocorticoid-inducible kinase to regulate growth processes in the cell (Bozzini and Falcone 2017). This signalling activity has an important role in long lived animals. One of the interesting facts is that mutation in IGF1 gene is associated with prolonged life.

This IGF1 gene also activates FOXO gene . There are four FOXO genes that regulate ageing such as FOXO1, FOXO3, FOXO4nd FOXO6. Of these genes FOXO3 has been associated with multiple candidate gene studies in diverse groups of German, Italian and Chinese populations of 95 years and more (Bozzini and Falcone 2017). The influence of gene FOXO3 in longevity may be due to oxidative stress, insulin sensitivity and cell cycle progression. It has been found that the mutation in Insulin-like growth factor (IGF1) receptor of C. elegans makes the lifespan to double as compared to its wild type showing thereby that there is a link between Insulin signalling and longevity. Again this receptor of C. elegans has a similarity with the receptor of mammalian IGF 1 factor. Again genetic studies of long-lived humans showed that there is a mutation in IGF 1 receptor or FOXO transcription factor in longer life span and better health of human population (Meiliana *et al* 2022). It has been noted the importance of the somatotrophic axis in co-ordinating growth, metabolism and lifespan with the secretion of Insulin /IGF1 receptor or other factors of growth hormone showing that neuroendocrine modulation is vital for energy homeostasis and ageing (Meliana *et al* 2022). IGF1 pathway is related to many functions associated with metabolic regulation and energy management. It has been noted that the presence of genetic variants of gene controlling IGF 1 pathway in centerarians reduces the efficiency of IGF-1 R (insulin Like Growth Factor 1 Receptor) to control many age related diseases. It has been noted in C. elegans (nematode) that loss of function mutation in daf2 gene , that encodes Insulin like growth factor (IGF 1) receptor, increases longevity than its wild type showing thereby that this pathway may affect ageing (Bareja *et al* 2019). Again this pathway also regulates metabolism and production of free radicals ($-O_2$ and H_2 O_2) and these free radicals control senescence and ageing through neurons in the nervous system.

AMPK Pathway

This pathway controls the regulation of metabolism, cell survival and growth, cell death and others that play the most important part in regulating ageing and life span. The activation of AMPK may lead to delay ageing and i the increase of longevity. AMPK pathway promotes ATP producing and the inhibition of ATP consuming pathways in tissues and thus acting as a regulator of cellular energy homeostasis. It has been noted that the extension of longevity is linked with signalling pathways that are controlled by AMPK. The overexpression of AAK-2 (AMP activated Kinase 1)/AMPK and its activation by some drug like Metformin and some hormones may extend the life span. The AMPK activation can also be increased by Dietary Restriction or Calorie Restriction and Exercise. It has also been noted that AMPK stimulates energy production from glucose and fatty acids during stress and inhibits energy consumption for protein, cholesterol and glycogen synthesis and thus showing 11the role of AMPK in regulating the ageing processes by reducing the metabolic rate (Salminen and Kaarniranta 2012). In addition the organisation of different signalling pathways to an integrated network has also some effect on the activity of AMPK.

Telomere Shortening: The eukaryotic chromosome terminates in a special region having multiple repeats (30-70) of species specific short sequences such as TTAGGG (tandem repeats) in humans and TTTAGGG in plants (*Arabidiopsis thaliana*).

Tandem repeat is a sequence of two or more bases that is repeated several times on a chromosome. Although these sequences are somewhat variable in different species , the basic repeat unit in all species has the pattern 5′ T_{1-4} A_{o-4} G_{1-8} - 3′ . Telomeres have either two strands of DNA covalently linked with a subterminal nick or have a single stranded 3′ end i.e. , the G- rich strand extends by 12-16 nucleotides beyond the C- rich strand. The protruding strand is single stranded. Telomere sequences are added by a special enzyme called Telomere terminal transferase or Telomerase which is a ribonucleoprotein. This unique structure of telomere performs three important functions like i) it prevents exonuclease from degrading the ends of linear DNA molecule; ii) it resists the fusion of ends with other DNA molecule and iii) it facilitates replication of the ends of DNA molecule without loss of terminal (Roy and De 2011). Telomeres will shorten by several repeats (tandem) after each cell division unless maintained by telomerase. After each cell division the enzyme telomerase adds telomeric repeat sequences as replicative DNA polymerase could not replicate telomeric repeats. These tandem repeats at the end of chromosome act as a cap and protect the damage of DNA for maintaining genomic stability. But sometimes the telomerase gets silenced. In such condition, when telomeres become too short after several divisions the silenced telomerase cannot add repeats and so cell divisions will stop. This condition is called Telomere Attrition. This telomeric attrition leads to telomere syndrome (telomeropathies) and age-related diseases followed by loss of longevity. Telomeropathies may cause diseases like Werner syndrome and Hutchinson-Gilford Progeria causing premature aging and increased disease risk). In addition to this, telomeric attrition may also cause bone marrow failure, hair loss, emphysema (shortness of breath), liver cirrhosis, osteoporosis and pulmonary fibrosis (Lidzbarsky *et al* 2018).

It has been found that shortening of telomeres may be used as a biomarker of ageing. The telomere length of Leukocyte has been correlated with measures of health and ability in elderly individuals. Leucocyte telomere length was found to correlate with physical ability in Danish twins aged 77 years and inversely with disability on American seniors. Centenarians of Ashkenazi Jews (Diaspora population along the Rhine in Western Germany and Northern France; now spread to different countries) and their offspring showed longer telomeres for their age than controls. Variation in genes involved in telomere maintenance has also been associated with longevity (Brooks-Wilson 3013). Again the telomere is bounded by a multiprotein complex (Shelterin) that prevents DNA repair proteins to come near the telomere. So, telomerase becomes active to add repeats to the telomere after its shortening. Otherwise it leads to senescence, apoptosis (cell death) and chromosome fusion . It has also been observed that telomere shortening also takes place during ageing and ageing van be reverted by telomerase activation (Lopez-Otin *et al* 2013).

Role of Free Radicals in Ageing

Free radicals (mainly - O_2, H_2 O_2 and –OH) are produced as intermediates in many metabolic processes and function in regulating some processes like glucose metabolism, cell growth and proliferation. But free radicals my also cause some deleterious effects mainly by causing damage in DNA, Protein and Lipid leading to the effect on ageing. It is also known that mitochondria is an important source of free radicals and thus mitochondrial damage is also related with ageing along with neuronal network systems. When free radicals help in the production of hormonal signals from neurons, there will be no ageing and the stage of youth will continue. But if the free radicals interfere the neuron signalling pathway and hormonal signals from neurons are not produced, the degeneration of neurons or neural cell death occurs leading to ageing. Thus the maintenance of neural signalling pathway remains in a normal condition by regulated production of free radicals in humans showing the importance of free radicals in ageing (Funch and Ruvkun 2001). The production of free radicals is related with the metabolic rate because free radicals are created by metabolic processes. So slower metabolism (slow metabolic rate) can produce less free radicals resulting into the extension of lifespan or longevity. This has been found in animals as larger animals (say Elephants) live longer than smaller animals (Rats) due to their slow metabolic rates (Grabski *et al* 2020).

Dietary influences Calorie Restriction on Longevity

It has been noted that dietary restriction or calorie restriction can extend life span to human and other organisms provided there is no basic nutritional deficiency. It also protects against the deterioration of biological functions as well as by reducing the risk factor in Diabetes, Cardio-Vascular diseases and Cancer. In recent days, there is a progressive rise globally in food intake in almost all populations resulting in increase of obesity and overweight followed by several diseases like diabetes, cardiovascular and other diseases resulting in the loss of longevity. Then gerontologists are thinking about the importance of Calorie restriction (20 – 40% reduction) without making any nutritional deficiency (taking sufficient vitamins, minerals and essential nutrients) to increase the life span in human. There is a report that in fifteenth century, an Italian Luigi Cornaro was eating voraciously till his middle age showing his ill health. With the advice of his physician he started to eat less leading to good health and lived to 102 years (Everitt *et al* 2010). Calorie restriction studies on rats were first done by McCay (1935) showing thereby that 40% reduction in Calories prolonged the lifespan of rat in the laboratory. In twentieth century, the longevity in human has brrn increased due to proper medical treatment as the cardiovascular diseases were decreased with the use of drugs to lower blood pressure and cholesterol including artery bypass surgery and use of defibrillators. The lower rate of cigarette smoking has reduced death from lung cancer. All these factors have helped to increase living percentage of old persons including centenarians which have been doubling every 10 years in developed countries (McCormack 2000, Vaupel and Kistowski 2005, Everitt *et al* 2010). There is evidence that the maximum lifespan has increased in Sweden from 101 years in 1860s to 108 years in 1990 and one French woman Jeanne Calment (longest lived woman) died at the age of 122years and 164 days (Coles 2004, Everitt *et al* 2010).

In the twenty first century the reduction of rate of mortality is due to overweight and obesity due to increase of food intake, fatty junk food and lack of physical exercise. The reason for the increase of diabetes and cardio-vascular diseases is the overweight and obesity which are reducing the longevity. Data from Framingham study dhow that the overweight at the age 40 with BMI of 25- 30) reduces longevity by 3 years and if there is obesity then it will be shortened by 7 years. Framingham study is a long term research report to identify the risk factors of cardiovascular disease.

It is a cohort study among a group of individuals to determine the natural history of certain diseases to identify the risk factors of the diseases particularly for heart disease. It has been noted that 25% Calorie restriction of the individual of age less than 50 along with increasing physical exercise showed a 10% fall in body weight, fat mass and fasting insulin level leading to the reduction of life-threatening diseases like diabetes, hypertension, myocardial infarcation, stroke and cancers (Everitt *et al* 2010). The risk factors of diabetes and coronary heart diseases have also been identified in obese children. Overweight can also increase the dementia and Alzheimer`s disease. The effect of Calorie Restriction on anti-ageing process may be due to the reduction of oxidative stress and inflammatory responses as well as delaying age-related diseases. So, the lifestyle for healthy and long life should be by decreasing food intake and by eating low calorie foods, whole grain foods, fruits, vegetables, fish, lean meat, egg along with regular physical exercise.

In the study of calorie restriction the diet maintained by the population of Okinawa of Japan is very important as long life expectancy has been reported within Japan. Their traditional diet is low in calorie but with high in nutrient value containing phytonutrients (flavonoids and antioxidants) suggesting the role of calorie restriction in their increased longevity. The traditional dietary pattern of Okinawa population is : i) Low calorie intake ii) High consumption of vegetables iii) High consumption of legume (mostly soy) iv) Low consumption of dairy products v) Low consumption of meat and meat products vi) Moderate consumption of fish products vii) Low consumption of Refined starches and low overall Glycaemic load viii) Low consumption of saturated fat and high consumption of omega-3 fat ix) moderate alcohol consumption (Rosenbaum *et al* 2010). Of the three diets like Mefiterranean, DASH (Dietary Approaches to Stop Hypertension) diet and Okinawa diet , the latter is lowest in calories and saturated fat. People of Okinawa is known as the naturally calorie restricted population. The higher longevity in Okinawa population may be due to their traditional calorie restricted diet from their early age. In addition there may be some genetic factors responsible for their longevity as they are living in a small island with a restricted gene flow. However calorie restricted diet and lifestyle from their early age resulted in positive energy balance to maintain longevity. Popular foods used by the population of Okinawa is Sweet potatoes (Pulp,skin and leaves), bitter melon, turmeric, ginger, peppers, mugwort (Artemisia vulgaris) and Carotenoid rich marine foods to scavenge free radicals (Meliana *et al* 2015).

It has been found that when Okinawan families are living in Brazil, they changed their lifestyle to Western one changing their diet and physical activity resulting into overweight, obesity and loss of longevity of about 17 years. The changes of Calorie restriction in diet may change the hormonal status of the body like Insulin like Growth Factor-1(IGS-1), thyroid hormones, oxidative stress, inflammation, mitochondrial function etc which have relations with ageing processes. Comparative data from normal and controlled conditions showed that Calorie restriction with adequate nutrition has beneficial effects in human on longevity and gives protection against obesity, cardiovascular disease, hypertension, body weight, cholesterol and cancer. These data were obtained from Calorie Restriction Society (CRS) members. The beneficial effect of Calorie restriction in food depends on the limited amount of macronutrients like Proteins, Carbohydrates and fats. The limited consumption of certain amino acids like Methionine and Tryptophane can also increase longevity (Al-Regaiey 2016). Another chemical, Resveratrol, has been found in Grapes and in Red wine which has the potentiality to increase lifespan by preventing many age-related diseases (Meiliana *et al* 2015). Actually Calorie restriction activates SIR2 proteins encoded bt Sirtulin genes which increases longevity through overexpression of Sirtulin 2 gene. From the survey of different data, it can be said that longevity in Okinawa population is not due to their genetic factors alone but Calorie restricted diet has played an important part by reducing many age-related diseases as these diets are healthier with high plant food consumption, lower salt intake, lower glycaemic load and healthier fat intake.

Role of DNA and Histone modifications in Ageing

It has been found that ageing does not depend only on genes or on the changes in the sequence of DNA but more on the alterations in the expression of genes. This type of study is known as Epigenetics and alterations found in gene expression or gene function is known as Epigenetic Alterations. Ageing is also due to the accumulation of macromolecular damage including DNA damage. DNA damage is occurring continuously by endogenous and environmental factors. Cells have their own DNA damage repair mechanism by triggering signals to transform damaged cells to senescence or apoptosis to stop replications of damaged DNA. This DNA damage process has an indirect effect on aging as it may lead to mitochondrial and metabolic disorder, altered proteostasis and inflammation. However, it has also been noted that epigenetic alterations like histone modifications, altered gene expressions due to DNA methylation, environmental factors like toxic chemicals altering gene expressions have played an important role in ageing. DNA Methylation data may be accepted as biomarkers of ageing as DNA methylation pattern has some relations with ageing or longevity. For example, DNA in leukocytes has been found to be hypomethylated with age while specific CpG sites in gene promoters are hypermethylated. Generally CG dinucleotides within promoters tend to be protected from methylation in normal development. Hypomethylation occurs from loss of methylation at normally heavily methylated repeat elements. There is a link between DNA methylation and methylation in promoter because if the prompter is methylated the expression of genes will be ceased (silenced) leading to ageing and many diseases even cancer. So DNA methylation has a role in histone modification, chromosome structure and activity of promoters leading to gene silencing. Normally the function of DNA methylation is to attach the methyl group to the specific places of DNA to block some proteins that are not needed i.e. silencing of that gene. But with the rise of ageing hypermethylated condition occurs in the genes and promoters to create loss of function of many genes. There are many examples of specific genes in the innate immunity pathway that are dysregulated by aging or by methylation (hypo vs hyper methylation). This differential methylation has been found in 1859 genes in rheumatoid arthritis. The analysis of whole blood gene expression identified 1497 genes that were differentially expressed with age. Gene expression profiles van be used to calculate transcriptomic age of individuals (Morris *et al* 2019). Of the different factors in ageing , another important factor in regulating ageing is the transcriptome changes through chromatin remodelling.Histone has played an important role in chromatin remodelling or chromatin

reorganization. Chromatin is a nucleosome polymer having DNA and histone protein. The process of chromatin remodelling helps to hide or expose regions of DNA for transcription through histone methylation. Histone has another imporyant character that it undergoes Post translational modifications for modulating chromatin structure in order to activate or silencing gene expression. Caloric restriction in diets and other environmental changes affect histone post translational modifications and cellular phenotypes including ageing through signalling pathways. Many researches are still going on to show a direct link between diet, lifestyle and longevity through histone modification-associated mechanisms (Molina-Serrano *et al* 2019).

WAYS TO REVERSE AGEING

As the people become older they develop loss of function at the cellular level and in different vital organs leading to many serious diseases like cardiovascular, neurological, metabolic disorders and cancer along with painful death around the world. This increases the expenses on medicines and health so much that puts pressure on the economy of the family. As many factors in regulating ageing processes have already been discussed, it will be wise to discuss the way to maintain longer youthful period. Further, if the ageing process can be slowed down or can be reversed to youth then the older people will be stronger, healthy, happier, productive and less prone to many age-related diseases leading to less pressure on the economy of country, person concerned and health services. With the advancement of stem cell and nuclear transfer technology it has been shown that young cells and even the clones of an animal can be regenerated from either an old differentiated cell or from stem cells by the process of rejuvenation or reprogramming. Somatic cell nuclear transfer technology has shown that age-related changes are reversible as evidenced by the formation of cloned animals (Dolly the sheep) by transferring the nucleus from differentiated somatic cells to an enucleated unfertilized egg cell to form an embryo that is genetically identical to the donor cell leading to develop an idea that youthful period can be brought back to the aged man as exemplified in Mahabharata.

Reprogramming through epigenetic rejuvenation has shown that cellular phenotypes of aged cells can be transformed to stem cell like state or younger state (Simpson *et al* 2021). It has been noted that cellular reprogramming may turn a cell to a pluripotent state where there is a potentiality to generate any type of somatic cell that is from dedifferentiation (mature cell) state to young cell. Takahasi and Yamanaka (2006) showed that overxpression of four transcription factors (Oct3/4, Sox2, Klf4 and c-Myc) known as Yamanaka. Factors convert the mature somatic cells to the puripotent state showing thereby that cellular identity is controlled by epigenetic changes rather than alterations or mutations in the genomic DNA. For this important research Yamanaka gas got Nobel Prize in Medicine in 2012.This part of the genome is known as Epigenome. Although genes are identical in all cells of the individual still the expression of genes may be different that is due to some epigenetic factor that is mainly the environment. Another factor in altering gene expression or ageing is the Yamanaka factors which have played an important role in longevity research.

Although the reverse of Ageing is now possible in worms (nematode C.elegans) and mice but it has not been successful in human as the system here is very complex. Still research is going on in several laboratories of the world to reverse aging in human using Yamanaka factors (OSMK genes) and ageing genes. Different techniques like Gene transfer technology of transferring OSK genes to somatic cells of older people and CRISPR based gene editing are used to inactivate senescence or ageing for bringing youthful state in human. Besides study of genes in ageing, the study of pathways and some important factors related with ageing is needed to prevent many age-related diseases. This objective of the ageing research is not only to extend lifespan but also to enjoy the healthy years of old age. Sinclair`s group of Harvard Medical School showed that when elderly mice was injected with AAVs as a vector (Adeno Associated Virus) carrying OSK genes could reverse loss of vision in elderly mice. Again the analysis of muscles, kidneys and retinas showed some reversed epigenetic changes in mice.

These important observations may open a new vistas of controlling ageing so that one day will come when we can drive an animal`s age forward and backward at our will. In near future Reverse Ageing Technology or Cell reprogramming technique may also be able to show same effect of forwarding and reversing of age in human.

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