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The MitoAging Project: Identification of single nucleotide polymorphisms (SNPs) in genes coding for mitochondrial proteins and their association with an increase in lifespan.

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RESUMEN

El envejecimiento está relacionado con una disminución en las funciones biológicas, en parte, debido a la acumulación de mutaciones en el ADN mitocondrial, las cuales causan problemas en la cadena de transporte de electrones y efectos negativos en procesos mitocondriales, como producción de ATP, funcionamiento celular y homeostasis. Sin embargo, algunas mutaciones, especialmente SNPs, juegan un rol importante en el mantenimiento de la estructura y función de la cadena de transporte de electrones, aumentando así la esperanza de vida. Por lo tanto, esta revisión sistemática tiene como objetivo identificar SNPs en genes que codifican proteínas mitocondriales y están asociados con un incremento en la esperanza de vida. Se encontró un total de 28 SNPs (mutaciones no sinónimas). Estos SNPs se reportaron en países asiáticos, tales como Japón, China, Turquía e India. La mutación C5178A en el gen ND2 se ha detectado en centenarios y semi-supercentenarios japoneses, nonagenarios turcos, al igual que, casos con FRDA y controles del norte de India. Esta mutación se ha relacionado con un efecto protector contra el daño oxidativo y algunas enfermedades, como el infarto de miocardio. Por otro lado, los polimorfismos G9055A (gen ATP6) y A10398G (gen ND3) se han asociado con un efecto protector contra la enfermedad de Parkinson. Por lo tanto, existen SNPs beneficiosos que podrían contribuir a envejecer con buena salud e incrementar la esperanza de vida.

Palabras clave: Mitocondria, ADN mitocondrial, SNP, proteína mitocondrial, longevidad, envejecimiento.

ABSTRACT

Aging is related to a decrease in biological functions, in part, due to the accumulation of mtDNA mutations, which cause problems in the electron transport chain and negative effects in mitochondrial processes such as ATP production, cellular functioning, and homeostasis. However, some mutations, especially SNPs, play an important role in the structure and function maintenance of the electron transport chain, thus increasing lifespan. Therefore, this systematic review aims to identify SNPs in genes coding for mitochondrial proteins and are associated with an increase in lifespan. A total of 28 SNPs (nonsynonymous mutations) were found. These SNPs were reported in Asiatic countries such as Japan, China, Turkish and India. The C5178A mutation in the ND2 gene has been detected in Japanese centenarians and semi-supercentenarians, Turkish nonagenarians, as well as FRDA cases and control from North India. This mutation has been related to a protective effect against oxidative damage and some diseases as myocardial infarction. On the other hand, G9055A (ATP6 gene) and A10398G (ND3 gene) polymorphisms have been associated with a protective effect against Parkinson's disease. Therefore, there are beneficial SNPs that could contribute to age in good health and increase lifespan.

Key words: Mitochondria, mitochondrial DNA, SNP, mitochondrial protein, longevity, aging.

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INTRODUCTION

Aging is a complex biological process related to a progressive functional decline in the performance of organisms' biological systems with time (Linnane et al., 1989; Kauppila et al., 2017). It is thought to be caused by accumulated damage that leads to the deterioration of cellular functions, tissue failure, organs dysfunction (Bratic & Larsson, 2013), and the probability of developing diseases such as diabetes, Alzheimer's and Parkinson's disease, cancer, atherosclerosis, among others (Belsky et al., 2015; Rea et al., 2018; Tanaka, 2002; Chocron et al., 2019). Therefore, beyond the fifty years of life there is an exponential increase associated with the development of different chronic conditions (Belsky et al., 2015). Furthermore, genetic, epigenetic, and environmental factors influence the aging process (Tanaka, 2002; Cevenini et al., 2010). Thus, the rate of aging, as it may be affected by endogenous and exogenous factors, could vary among different cells, tissues, organs, and even individuals, which explains why people of the same age may have diverse rates of aging (Tanaka, 2002; Capri et al., 2014).

Various theories have been proposed to explain the process of aging; however, one of the most recognized is the mitochondrial free radical theory of aging (MFRTA) (Bratic & Larsson, 2013). MFRTA states that the production and accumulation of reactive oxygen species (ROS) during mitochondrial oxidative phosphorylation (OXPHOS) conduct to the damage of several cellular components such as proteins, membrane lipids, and DNA, in particular, mitochondrial DNA (mtDNA) (Tanaka, 2002; Guney et al., 2014; Zhu et al., 2015). ROS is produced at level of Complex I due to the conversion of a large amount of oxygen that is used for energy production (Bratic & Larsson, 2013; Tanaka, 2002). The mtDNA is more susceptible to ROS damage owing to the lack of histones, the absence of genome

recombination, and its proximity to the electron transport chain (ETC) (Hollins et al. 2006; Guney et al., 2014; Zhu et al. 2015; Zinovkina, 2018).

The mitochondrial genome is a circular double-stranded molecule with a length of 16,569 base pairs, is maternally inherited, and encodes 2 ribosomal RNAs (12S rRNA and 16S rRNA), 22 transfer RNAs necessary for protein synthesis, and 13 proteins from over 1000 proteins related to mitochondrial function (Sharma & Sampath, 2019; Picard & McEwen, 2018; Tsang & Lemire, 2003; Zhu et al., 2015). These 13 proteins are part of the mitochondrial respiratory chain (MRC), which are: 7 subunits (ND1 – ND6, NDL4) from 45 polypeptides of Complex I (NADH: ubiquinone oxidoreductase), 1 subunit (cytochrome b) from 11 polypeptides of Complex III (cytochrome bc_1 complex), 3 subunits (COI – III) from 13 polypeptides of Complex IV (cytochrome *c* oxidase), and 2 subunits (ATP6 and 8) from approximately 16 polypeptides of Complex V or ATP synthase (Wallace, 2010; Picard & McEwen, 2018). The other mitochondrial proteins are encoded by nuclear genes and imported into the mitochondrion (Tsang & Lemire, 2003; Zhu et al., 2015; Guo et al., 2017).

Mitochondria are known as powerhouses of the cell because their main function is to provide energy to the cell through ATP production that is carried out by OXPHOS complexes (complexes I – IV and ATP synthase), which are located in the inner mitochondrial membrane (Sharma & Sampath, 2019; Baker et al., 2019; Bratic & Larsson, 2013). However, mitochondrial functions go further than energy-generated organelles. They are involved in diverse functions that are indispensable for cellular functioning such as cell signaling, control of cell cycle, cellular homeostasis, regulation of cell death (apoptosis), and dynamic modulation of respiratory capacity (Baker et al., 2019; McBride et al., 2006; Luo et al., 2018). Nevertheless, mitochondrial functions decline with age, including the activity of ETC complexes, being detected in different tissues such as muscle, heart, and liver (Chocron et al.,

2019; Bratic & Larsson 2013). Therefore, mitochondrial dysfunctions have been recognized as a hallmark of aging (Chocron et al., 2019).

Energy deficits are associated with the accumulation of mutations in mtDNA genes in somatic cells, possibly due to ROS production, contributing to aging and lead to the development of degenerative diseases, among others (Wallace, 2010; Tanaka et al., 1998). For instance, point mutations in mtDNA have a rate of approximately $6x10^{-8}$ mutations per bp per year, which has been observed to lead to age-associated functional decline of mitochondria and the individual (Zinovkina, 2018; Chocron et al., 2019). The investigation of mitochondrial mutations in somatic cells has shown the existence of some common mtDNA single nucleotide polymorphisms (SNPs) that could be affecting the complex interactions that exist between mitochondria and the nucleus, as well as the efficiency of OXPHOS (Dato et al., 2004; Ren et al., 2008). However, studies carried out in aged people suggest that some mutations, such as SNPs, could have a positive effect in the ETC and be possibly related to longevity (Guney et al., 2014).

Taking all of this evidence in account, it is crucial to find strategies to prevent our biological deterioration and live more time with good health. This systematic literature review (SLR) focuses on identifying single nucleotide polymorphisms (SNPs) on mitochondrial or nuclear genes that would be promoting changes in mitochondrial proteins related to an increase in lifespan and health. SLR also helps to 1) find the possible benefits that have been associated with the reported missense mutations (SNPs), 2) to identify study groups and populations where SNPs associated with longevity have been reported.

METHODOLOGY

Systematic literature review and data extraction

A systematic literature review was carried out to find information about SNPs in mitochondrial or nuclear genes that encode for mitochondrial proteins, which could be associated with an increase in lifespan. SCOPUS, PubMed, and EBSCO were selected as databases for published articles. The search terms used to retrieve information were the following (see Annex A for each database):

- *Organelle*: mitochondria, nuclear, mitochondrial DNA, mitochondrial protein, mitochondrial nuclear protein, mutation, snp.
- *Population*: human, *Homo sapiens*, mice, *Mus musculus*, rat, *Rattus*, *Caenorhabditis elegans*, zebrafish, *Danio rerio*, fruit fly, *Drosophila melanogaster*.
- *Characteristics 1*: polymorphism, mutation, variant, amino acid, protein, gene, mitochondrial DNA, mitochondrial nuclear DNA.
- *Characteristics 2*: longevity, aging, lifespan, lastingness.

In the SLR, we took in consideration SNPs found in animal models related to an increase in lifespan, most of them to mimic biological aging in humans, as well as study the consequences of this modification in longevity.

Articles found were reviewed using a screening protocol (see Annex B). First, the title and abstract of each article were evaluated. Only publications in English were included. Studies that did not discuss longevity, not mention any specific mutation, or the reported mutation causes decreased longevity were excluded. In the second screening, the complete article was read and assessed (see Figure 1 for the whole selection process of articles).

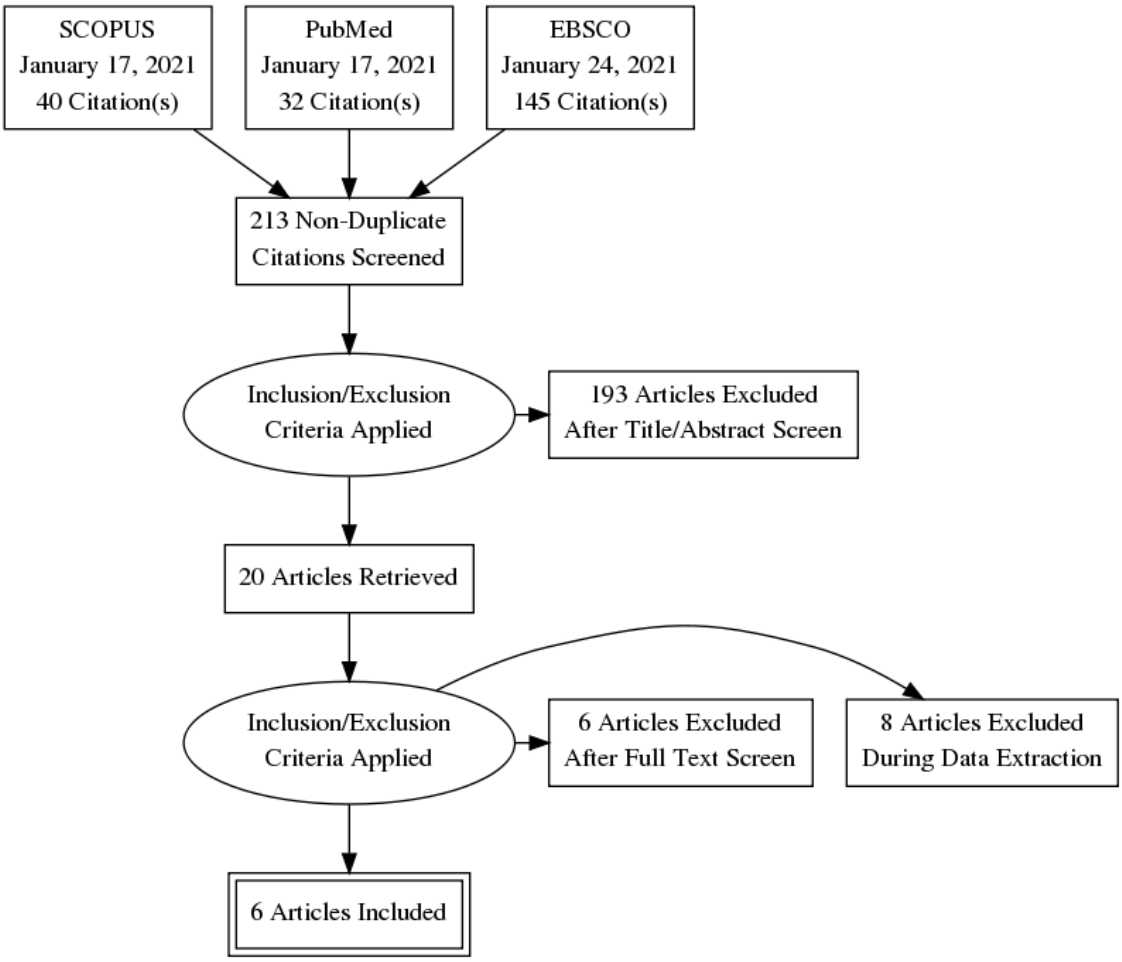

Figure 1. Selection process of publications according to PRISMA guidelines**.** The research was carried out in January of this year. 213 were compiled from the data bases after discarding 4 duplicated articles. 20 articles were retrieved after title/abstract screening and criteria applied. From these 20 articles, 6 were excluded after full text screen and 8 during data extraction, being classified as "Related article" (n = 3), "Negative association" (n = 1), and "Doesn´t mention a specific mutation" (n = 10) according to screening protocol (Annex B). Thus, 6 articles were included in this systematic review.

Later, relevant data were extracted from the potential publications. Supplementary information from each article was also taken into consideration for analysis. The search was focused on SNPs with nonsynonymous substitutions (missense mutations) because they change the amino acid sequence of the protein (Chu & Wei, 2019; Choudhuri, 2014). Thus, these variations in encoded proteins could be related to an increase in lifespan.

RESULTS

Systematic literature review

Six articles were included in this systematic review based on the methodology used. 28 SNPs were found in different mitochondrial genes that encode for mitochondrial proteins, specifically proteins from the mitochondrial respiratory chain: ND1-ND6 subunits from Complex I (Guney et al., 2014; Tanaka et al., 2000; Tanaka, 2002; Singh et al., 2015; Ren et al., 2008; Bilal et al., 2008), cytochrome b (cyt b) subunit from Complex III (Bilal et al., 2008), as well as ATP6 and ATP8 subunits from Complex V or ATP synthase (Ren et al., 2008; Singh et al., 2015). Results are summarized in Table 1, including gene, SNPs location, and protein mutation. Moreover, these variations of mitochondrial proteins were located in humans from Turkey, Japan, China and India (see Table 1).

Table 1. SNPs (missense mutations) found in different mitochondrial genes

Gene	***SNP location***	***Protein mutation***	***Species***	***Nationality***	***Source***
ND1	T3335C	I10T	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND1	T3356A	M17K	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND1	T4216C	Y304H	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND2	T4639C	I57T	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND2	C4640A	I57M	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND2	A4732G	N88S	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND2	A4917G	N150D	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND2	C5178A	L237M	*Homo sapiens*	Japanese, Turkish, North Indian	(Tanaka et al., 2000; Tanaka, 2002; Bilal et al., 2008; Guney et al., 2014; Singh et al., 2015)
ND2	G5262A	A265T	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND2	A5301G	I278V	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND2	A5322C	I285L	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND3	T10084C	I9T	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND3	A10398G	T114A	*Homo sapiens*	Japanese, Chinese, Turkish	(Tanaka et al., 2000; Tanaka, 2002; Bilal et al., 2008; Ren et al., 2008; Guney et al., 2014)
ND4L	C10654T	A62V	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND4	G11969A	A404T	*Homo sapiens*	Turkish	(Guney et al., 2014)

ND4	A12026G	I423V	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND4	T12083G	S442A	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND5	T12338C	M1T	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND5	A12397G	T21A	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND5	C13547T	T404M	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND5	G13759A	A475T	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND5	A13780G	A482V	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND5	A13966G	T544A	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND6	C14167G	E7D	*Homo sapiens*	Turkish	(Guney et al., 2014)
ND6	T14318C	N57S	*Homo sapiens*	Turkish	(Guney et al., 2014)
CYTB	T14979C	I78T	*Homo sapiens*	Japanese	(Bilal et al., 2008)
ATP6	G9055A	A177T	*Homo sapiens*	-	(Ren et al., 2008)*
ATP8	C8414T	L17F	*Homo sapiens*	Japanese, North Indian	(Tanaka et al., 2000; Tanaka, 2002; Bilal et al., 2008; Singh et al., 2015)

* The mutation in ATP6 was mentioned in this article but it was not reported in the study group.

Study groups and populations with SNPs related to longevity.

The ND2 gene presented the largest number of missense mutations (8) from the 28 SNPs found in *Homo sapiens* (see Figure 2). Among these 8 mutations, the C5178A was reported in Turkish nonagenarians (Guney et al., 2014), Japanese centenarians (Tanaka et al., 2000; Tanaka, 2002) as well as semi-supercentenarians (Bilal et al., 2008), and North Indian individuals [control group and Friedreich's ataxia (FRDA) patients] (Singh et al., 2015) (see Figure 3). This mutation causes a replacement from leucine to methionine at amino acid 237 of NADH dehydrogenase subunit 2 (ND2). Moreover, the mutation A10398G reported in the ND3 gene leads to a threonine to alanine substitution at amino acid 114 of NADH dehydrogenase subunit 3 (ND3). This variation was found in Turkish nonagenarians (Guney et al., 2014), Uygur Chinese individuals with an age range of 90 – 100 years (Ren et al., 2008), and Japanese centenarians (Tanaka et al., 2000; Tanaka, 2002) as well as semi-supercentenarians (Bilal et al., 2008) (see Figure 3). On the other hand, the mutation C8414T detected in the ATP8 gene was reported in both Japanese centenarians (Tanaka et al., 2000; Tanaka, 2002) and semi-supercentenarians (Bilal et al., 2008), as well as North Indian

individuals [control group and Friedreich's ataxia (FRDA) patients] (Singh et al., 2015) (see Figure 3). C8414T leads to a replacement from a leucine to phenylalanine at amino acid 17 of ATP synthase protein 8.

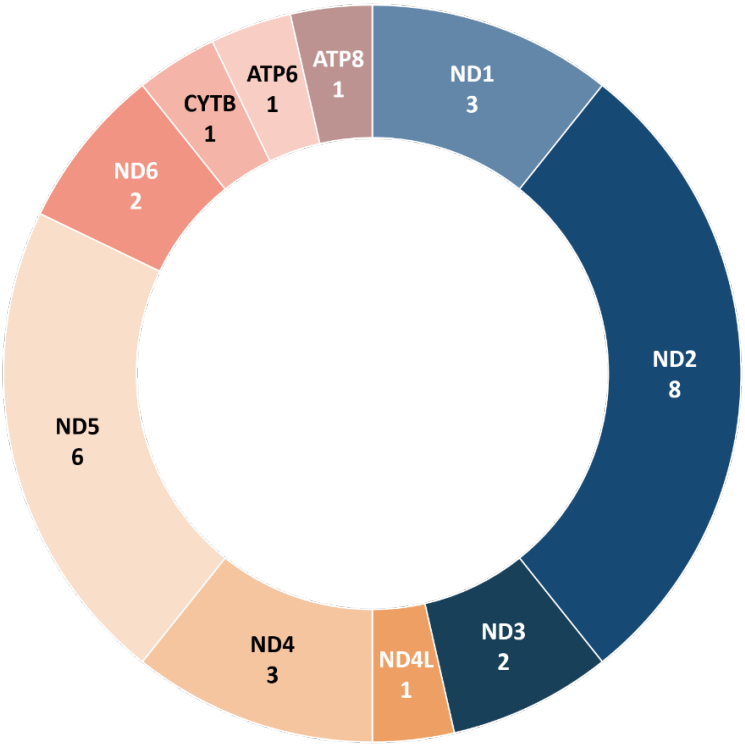

Figure 2. Mutations (SNPs) in mitochondrial genes found in *Homo sapiens***.** The number of SNPs (missense mutations) is below the gene name in each section. The width of section represents the percentage of missense mutations reported for each gene: ND1, 11% (3); ND2, 29 % (8); ND3, 7% (2); ND4L, 4% (1); ND4, 11% (3); ND5, 21% (6); ND6, 7% (2); CYTB, 4% (1%); ATP6, 4% (1); and ATP8, 4% (1).

Figure 3 shows 5 study groups that were identified from the SLR, which were: 11 Japanese centenarians (Tanaka et al., 2000; Tanaka, 2002), 112 Japanese (96 females and 16 males) with an age range of 105 – 115 years (Bilal et al., 2008), 98 Chinese from the Uygur region with a range of 90 – 100 years (Ren et al., 2008), 25 Turkish nonagenarians (13 females and 12 males) (Guney et al., 2014), and 30 FRDA cases and 62 healthy controls from North India (Singh et al., 2015).

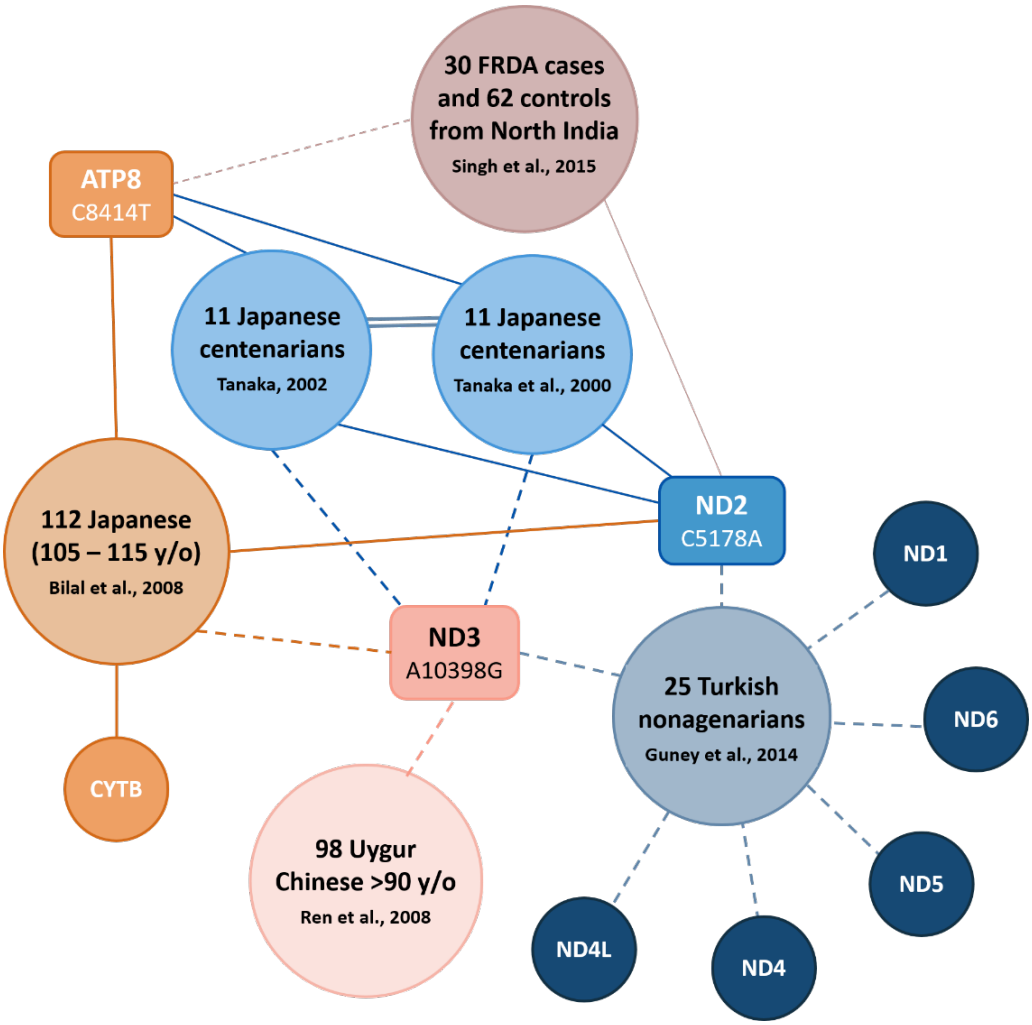

Figure 3. Study groups per mitochondrial gene with reported mutations in *Homo sapiens*. SNPs in mitochondrial genes shared by different study groups are represented by diamonds. Solid lines represent a significant relation between the reported missense mutations and the analyzed population; meanwhile, broken lines represent a non-significant relation. The parallel lines show that are the same study group.

The C5178A mutation in the ND2 gene had significant relation between 3 of 5 study groups (Figure 3): Japanese centenarians group (Tanaka et al., 2000; Tanaka, 2002), Japanese 105 – 115 years old group (Bilal et al., 2008), and among FRDA cases from North India (Singh et al., 2015). The C8414T mutation in the ATP8 gene had a significant relation between the Japanese centenarians group (Tanaka et al., 2000; Tanaka, 2002), and the Japanese 105 – 115 years old group (Bilal et al., 2008) (Figure 3). However, the A10398G mutation in the ND3 gene had a non-significant relation between any of the study groups (Figure 3).

DISCUSSION

The six articles retrieved for the SLR are investigations carried out in populations from Turkey (Guney et al., 2014), Japan (Tanaka et al., 2000; Tanaka, 2002; Bilal et al., 2008), China (Ren et al., 2008), and India (Singh et al., 2015). This suggests that most studies of SNPs in mitochondrial genes associated with an increase in lifespan have been focused on Asian countries. Therefore, investigations in the other continents need to be done in order to known if the reported mutations in this SLR could be present in other human populations and have a strong relation to longevity, or there are other SNPs in genes coding for mitochondrial proteins that could be associated with an increase of lifespan. Moreover, a limitation of this study is that the methodology used to do the SLR just compiled articles that have the defined search terms in either title, abstract, or/and keywords (Annex B). Hence, articles that have the search terms of interest in the introduction or/and theoretical framework will not appear in the identification of articles from databases despite the fact of reporting SNPs related to longevity in mitochondrial proteins.

Taking in consideration the possible positive effects that have been associated with the reported SNPs in the SLR, the C5178A mutation that leads to L273M replacement in the ND2 subunit (Table 1) might have a protective effect against oxidative damage (Tanaka et al., 2000; Tanaka, 2002). A possible reason is that L273M replacement causes the exposure of this methionine to the Complex I surface (Tanaka, 2002). Tanaka et al. (2000) and Tanaka (2002) cite Levine et al. that mentions the important role that methionine residues play as an antioxidant defense mechanism (Levine et al., 1996, 1999). Hence, this methionine at amino acid 273 in ND2 subunit might act as an oxidant scavenger (Tanaka et al., 2000; Tanaka, 2002). Furthermore, this mutation has been related to a low prevalence of myocardial infarction in Japanese individuals (Tanaka, 2002), a protective antiatherogenic effect in diabetics (Tanaka

et al., 2000; Tanaka, 2002), and a low triglyceride concentration in post-menopausal women (Tanaka, 2002). Singh et al., (2015) reported that the frequency of the C5178A mutation (3 of 30 cases) and other two mutations were statically higher in FRDA cases; however, the limited sample size of FRDA patients explains why there is not a significant correlation of this nonsynonymous variation with the age at onset of FRDA in their study. Therefore, the C5178A genotype might help to prevent the development of some diseases, but further studies are necessary to explain the biological mechanisms involved in these beneficial health effects.

Other SNPs that have been associated with a positive effect in health are G9055A in the ATP6 gene and A10398G in the ND3 gene (Table 1), even though this last mutation was not significant in any of the 4 study groups where it was reported (Figure 3). Ren et al. (2008) mentioned two studies where both mutations have been strongly related to a protective effect against Parkinson's disease (PD), especially in in women (van der Walt et al., 2003; Otaegui et al., 2004). Furthermore, van der Walt et al. (2003) carried out the research in PD patients of European ancestry and white subjects as control; whereas in the research from Otaegui et al. (2004), the sample was represented by Spanish population. Therefore, studies in other populations, where the A10398G genotype has been detected (Japanese, Chinese, and Turkish), are necessary to elucidate the protective effect against PD.

On the other hand, although the nonsynonymous SNPs detected in subunits (ND1 – ND6, NDL4) of the Complex I was not significant in Turkish nonagenarians (Table 1), Guney et al. (2014) suggest that these SNPs result in the alteration of the functionality of this complex, and therefore they might be related to an increase in lifespan.

Complex I, also known as NADH dehydrogenase, is one of the enzymes located in the inner mitochondrial membrane that is part of the respiratory chain (RC), having a fundamental role in energy conversion because electron entry to the rest of respiratory chain complexes

depends on it (Tanaka et al., 2000; Caballero et al., 2015; Guo et al., 2017). Moreover, during ATP synthesis through proton-motive force generation, complex I provides about 40% of the proton flux (Efremov & Sazanov, 2011).

Hence, the reported SNPs in the Complex I (e.g., C5178A, A10398G) might cause a better interaction between subunits (ND1 – ND6, ND4L), and therefore a structure and function maintenance of the electron transport chain, preventing its damage, controlling the ROS production, and increasing lifespan.

However, more research is needed to elucidate the protective effects. Future studies will need to explain the biological mechanisms and the pathways that the reported SNPs are involved. Moreover, aging and longevity are complex biological processes that are influenced by genetic and environmental factors. Therefore, an increase in lifespan might be related to a combination of mtDNA alleles and not just a single allele (Ren et al., 2008).

CONCLUSION

In conclusion, the existence of beneficial SNPs in genes coding for mitochondrial proteins (ND1 – ND6, ND4L, Cyt b, ATP6, and ATP8) in long-live people could be related to a correct functioning of complexes that are part of the electron transport chain, especially Complex I, and hence, ROS levels could maintain under control. Some SNPs (e.g., C5178A, A10398G, G9055A) have been associated with a protective effect against oxidative damage, myocardial infarction, atherosclerosis, and Parkinson's disease. Thus, these SNPs could contribute to age in good health and live longer.

The described SNPs in this systematic literature review were reported in Asiatic populations. Therefore, more studies are required to know if these SNPs are present in

populations from other regions and have the same protective effect, or if there are other SNPs in genes that encode mitochondrial proteins and are related to an increase in lifespan and help to age in good health. Moreover, the knowledge of signaling and metabolic pathways, where reported SNPs are involved, is necessary because this will help 1) to understand how SNPs are related to longevity and 2) to explain why they have a protective effect or reduce the risk of developing some diseases.

BIBLIOGRAPHY

Belsky, D. W., Caspi, A., Houts, R., Cohen, H. J., Corcoran, D. L., Danese, A., Harrington, H., Israel, S., Levine, M. E., Schaefer, J. D., Sugden, K., Williams, B., Yashin, A. I., Poulton, R., & Moffitt, T. E. (2015). Quantification of biological aging in young adults. Proceedings of the National Academy of Sciences of the United States of America, 112(30), E4104-10. https://doi.org/10.1073/pnas.1506264112

Bratic, A., & Larsson, N.-G. (2013). The role of mitochondria in aging. The Journal of Clinical Investigation.

Caballero, S., Duchêne, S., Garavito, M. F., Slikas, B., & Baker, C. S. (2015). Initial evidence for adaptive selection on the NADH subunit two of freshwater dolphins by analyses of mitochondrial genomes. Plos One, 10(5), e0123543. https://doi.org/10.1371/journal.pone.0123543

Capri, M., Santoro, A., Garagnani, P., Bacalini, M. G., Pirazzini, C., Olivieri, F., Procopio, A., Salvioli, S., & Franceschi, C. (2014). Genes of human longevity: an endless quest? Current Vascular Pharmacology, 12(5), 707–717. https://doi.org/10.2174/15701611116661312191103O1

Cevenini, E., Bellavista, E., Tieri, P., Castellani, G., Lescai, F., Francesconi, M., Mishto, M., Santoro, A., Valensin, S., Salvioli, S., Capri, M., Zaikin, A., Monti, D., de Magalhães, J. P., & Franceschi, C. (2010). Systems biology and longevity: an emerging approach to identify innovative anti-aging targets and strategies. Current Pharmaceutical Design, 16(7), 802–813. https://doi.org/10.2174/138161210790883660

Chocron, E. S., Munkácsy, E., & Pickering, A. M. (2019). Cause or casualty: The role of mitochondrial DNA in aging and age-associated disease. Biochimica et Biophysica Acta. Molecular Basis of Disease, 1865(2), 285–297. https://doi.org/10.1016/j.bbadis.2018.09.035

Choudhuri, S. (2014). Fundamentals of molecular evolution. In Bioinformatics for Beginners (pp. 27–53). Elsevier. https://doi.org/10.1016/B978-0-12-410471-6.00002-5

Chu, D., & Wei, L. (2019). Nonsynonymous, synonymous and nonsense mutations in human cancer-related genes undergo stronger purifying selections than expectation. BMC Cancer, 19(1), 359. https://doi.org/10.1186/s12885-019-5572-x

Dato, S., Passarino, G., Rose, G., Altomare, K., Bellizzi, D., Mari, V., Feraco, E., Franceschi, C., & De Benedictis, G. (2004). Association of the mitochondrial DNA haplogroup J with longevity is population specific. European Journal of Human Genetics, 12(12), 1080–1082. https://doi.org/10.1038/sj.ejhg.5201278

Efremov, R. G., & Sazanov, L. A. (2011). Structure of the membrane domain of respiratory complex I. Nature, 476(7361), 414–420. https://doi.org/10.1038/nature10330

Gingrich, J. R., Pelkey, K. A., Fam, S. R., Huang, Y., Petralia, R. S., Wenthold, R. J., & Salter, M. W. (2004). Unique domain anchoring of Src to synaptic NMDA receptors

via the mitochondrial protein NADH dehydrogenase subunit 2. Proceedings of the National Academy of Sciences of the United States of America, 101(16), 6237–6242. https://doi.org/10.1073/pnas.0401413101

Guney, O., Ak, H., Atay, S., Ozkaya, A. B., & Aydin, H. H. (2014). Mitochondrial DNA polymorphisms associated with longevity in the Turkish population. Mitochondrion, 17, 7–13. https://doi.org/10.1016/j.mito.2014.04.013

Guo, R., Zong, S., Wu, M., Gu, J., & Yang, M. (2017). Architecture of human mitochondrial respiratory megacomplex I2III2IV2. Cell, 170(6), 1247-1257.e12. https://doi.org/10.1016/j.cell.2017.07.050

Hollins, D. L., Suliman, H. B., Piantadosi, C. A., & Carraway, M. S. (2006). Glutathione regulates susceptibility to oxidant-induced mitochondrial DNA damage in human lymphocytes. Free Radical Biology & Medicine, 40(7), 1220–1226. https://doi.org/10.1016/j.freeradbiomed.2005.11.011

Kauppila, T. E. S., Kauppila, J. H. K., & Larsson, N.-G. (2017). Mammalian mitochondria and aging: an update. Cell Metabolism, 25(1), 57–71. https://doi.org/10.1016/j.cmet.2016.09.017

Levine, R. L., Mosoni, L., Berlett, B. S., & Stadtman, E. R. (1996). Methionine residues as endogenous antioxidants in proteins. *Proceedings of the National Academy of Sciences*, *93*(26), 15036–15040. https://doi.org/10.1073/pnas.93.26.15036

Levine, R. L., Berlett, B. S., Moskovitz, J., Mosoni, L., & Stadtman, E. R. (1999). Methionine residues may protect proteins from critical oxidative damage. *Mechanisms of Ageing and Development*, *107*(3), 323–332. https://doi.org/10.1016/s0047-6374(98)00152-3

Linnane, A. W., Marzuki, S., Ozawa, T., & Tanaka, M. (1989). Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. The Lancet, 1(8639), 642–645. https://doi.org/10.1016/s0140-6736(89)92145-4

Mousavian, Z., Khodabandeh, M., Sharifi-Zarchi, A., Nadafian, A., & Mahmoudi, A. (2020). StrongestPath: a Cytoscape application for protein-protein interaction analysis. BioRxiv. https://doi.org/10.1101/2020.11.12.380600

Otaegui, D., Paisán, C., Sáenz, A., Martí, I., Ribate, M., Martí-Massó, J. F., Pérez-Tur, J., & López de Munain, A. (2004). Mitochondrial polymporphisms in Parkinson's Disease. *Neuroscience Letters*, *370*(2–3), 171–174. https://doi.org/10.1016/j.neulet.2004.08.012

Picard, M., & McEwen, B. S. (2018). Psychological stress and mitochondria: A systematic review. Psychosomatic Medicine, 80(2), 141–153. https://doi.org/10.1097/PSY.0000000000000545

Rea, I. M., Gibson, D. S., McGilligan, V., McNerlan, S. E., Alexander, H. D., & Ross, O. A. (2018). Age and Age-Related Diseases: Role of Inflammation Triggers and

Cytokines. Frontiers in Immunology, 9, 586. https://doi.org/10.3389/fimmu.2018.00586

Sharma, P., & Sampath, H. (2019). Mitochondrial DNA integrity: role in health and disease. Cells, 8(2). https://doi.org/10.3390/cells8020100

Tanaka, M., Gong, J. S., Zhang, J., Yoneda, M., & Yagi, K. (1998). Mitochondrial genotype associated with longevity. *The Lancet*, *351*(9097), 185–186. https://doi.org/10.1016/s0140-6736(05)78211-8

Tanaka, M, Gong, J., Zhang, J., Yamada, Y., Borgeld, H. J., & Yagi, K. (2000). Mitochondrial genotype associated with longevity and its inhibitory effect on mutagenesis. Mechanisms of Ageing and Development, 116(2–3), 65–76. https://doi.org/10.1016/s0047-6374(00)00149-4

Tanaka, Masashi. (2002). Mitochondrial genotypes and cytochrome b variants associated with longevity or Parkinson's disease. Journal of Neurology, 249 Suppl 2, II11-8. https://doi.org/10.1007/s00415-002-1203-5

Tsang, W. Y., & Lemire, B. D. (2003). The role of mitochondria in the life of the nematode, Caenorhabditis elegans. Biochimica et Biophysica Acta, 1638(2), 91–105. https://doi.org/10.1016/s0925-4439(03)00079-6

van der Walt, J. M., Nicodemus, K. K., Martin, E. R., Scott, W. K., Nance, M. A., Watts, R. L., Hubble, J. P., Haines, J. L., Koller, W. C., Lyons, K., Pahwa, R., Stern, M. B., Colcher, A., Hiner, B. C., Jankovic, J., Ondo, W. G., Allen, F. H., Goetz, C. G., Small, G. W., … Vance, J. M. (2003). Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. *American Journal of Human Genetics*, *72*(4), 804–811. https://doi.org/10.1086/373937

Wallace, D. C. (2010). Mitochondrial DNA mutations in disease and aging. Environmental and Molecular Mutagenesis, 51(5), 440–450. https://doi.org/10.1002/em.20586

Zhu, Z., Lu, Q., Zeng, F., Wang, J., & Huang, S. (2015). Compatibility between mitochondrial and nuclear genomes correlates with the quantitative trait of lifespan in Caenorhabditis elegans. Scientific Reports, 5, 17303. https://doi.org/10.1038/srep17303

Zinovkina, L. A. (2018). Mechanisms of mitochondrial DNA repair in mammals. Biochemistry. Biokhimiia, 83(3), 233–249. https://doi.org/10.1134/S0006297918030045

ANNEXES

Annex A: Search terms for each database

Table 2. Search terms for SCOPUS

Items	***Search terms***
Organelle	((TITLE-ABS-KEY (mitochondri*)) AND ((TITLE-ABS-KEY (nuclear)) OR (TITLE-ABS-KEY (mtDNA)) OR (TITLE-ABS-KEY (mitochondrial DNA)) OR (TITLE-ABS-KEY (mitochondrial protein)) OR (TITLE-ABS-KEY (mitochondrial nuclear protein)) OR (TITLE-ABS-KEY (mitochondri* AND nucl*)))) AND (TITLE-ABS-KEY (mutation*)) AND (TITLE-ABS-KEY (snp*))
Population	((TITLE-ABS-KEY (human*)) OR (TITLE-ABS-KEY (homo sapiens)) OR (TITLE-ABS-KEY (h. sapiens)) OR (TITLE-ABS-KEY (person)) OR (TITLE-ABS-KEY (child*)) OR (TITLE-ABS-KEY (elder*)) OR (TITLE-ABS-KEY (people)) OR (TITLE-ABS-KEY (mice)) OR (TITLE-ABS-KEY (mus musculus)) OR (TITLE-ABS-KEY (m. musculus)) OR (TITLE-ABS-KEY (mouse)) OR (TITLE-ABS-KEY (rat*)) OR (TITLE-ABS-KEY (rattus)) OR (TITLE-ABS-KEY (caenorhabditis elegans)) OR (TITLE-ABS-KEY (c. elegans)) OR (TITLE-ABS-KEY (zebrafish)) OR (TITLE-ABS-KEY (danio rerio)) OR (TITLE-ABS-KEY (d. rerio)) OR (TITLE-ABS-KEY (drosophila melanogaster)) OR (TITLE-ABS-KEY (d. melanogaster)) OR (TITLE-ABS-KEY (fruit fly)))
Characteristics 1	((TITLE-ABS-KEY (mut*)) OR (TITLE-ABS-KEY (proteom*)) OR (TITLE-ABS-KEY (transcript*)) OR (TITLE-ABS-KEY (polymorphism*)) OR (TITLE-ABS-KEY (aminoacid*)) OR (TITLE-ABS-KEY (peptid*)) OR (TITLE-ABS-KEY (variation*)) OR (TITLE-ABS-KEY (protein*)) OR (TITLE-ABS-KEY (gene*)) OR (TITLE-ABS-KEY (missense*)) OR (TITLE-ABS-KEY (variant*)) OR (TITLE-ABS-KEY (error*)) OR (TITLE-ABS-KEY (allele*)) OR (TITLE-ABS-KEY (mitochondrial DNA)) OR (TITLE-ABS-KEY (mitochondrial nuclear DNA*)))
Characteristics 2	((TITLE-ABS-KEY (longevity)) OR (TITLE-ABS-KEY (aging)) OR (TITLE-ABS-KEY (youth)) OR (TITLE-ABS-KEY (ageing)) OR (TITLE-ABS-KEY (lifespan)) OR (TITLE-ABS-KEY (lifespan)) OR (TITLE-ABS-KEY (life time)) OR (TITLE-ABS-KEY (old age)) OR (TITLE-ABS-KEY (lastingness)))

TOTAL	((TITLE-ABS-KEY (mitochondri*)) AND ((TITLE-ABS-KEY (nuclear)) OR (TITLE-ABS-KEY (mtDNA)) OR (TITLE-ABS-KEY (mitochondrial DNA)) OR (TITLE-ABS-KEY (mitochondrial protein)) OR (TITLE-ABS-KEY (mitochondrial nuclear protein)) OR (TITLE-ABS-KEY (mitochondri* AND nucl*)))) AND (TITLE-ABS-KEY (mutation*)) AND (TITLE-ABS-KEY (snp*)) AND ((TITLE-ABS-KEY (human*)) OR (TITLE-ABS-KEY (homo sapiens)) OR (TITLE-ABS-KEY (h. sapiens)) OR (TITLE-ABS-KEY (person)) OR (TITLE-ABS-KEY (child*)) OR (TITLE-ABS-KEY (elder*)) OR (TITLE-ABS-KEY (people)) OR (TITLE-ABS-KEY (mice)) OR (TITLE-ABS-KEY (mus musculus)) OR (TITLE-ABS-KEY (m. musculus)) OR (TITLE-ABS-KEY (mouse)) OR (TITLE-ABS-KEY (rat*)) OR (TITLE-ABS-KEY (rattus)) OR (TITLE-ABS-KEY (caenorhabditis elegans)) OR (TITLE-ABS-KEY (c. elegans)) OR (TITLE-ABS-KEY (zebrafish)) OR (TITLE-ABS-KEY (danio rerio)) OR (TITLE-ABS-KEY (d. rerio)) OR (TITLE-ABS-KEY (drosophila melanogaster)) OR (TITLE-ABS-KEY (d. melanogaster)) OR (TITLE-ABS-KEY (fruit fly))) AND ((TITLE-ABS-KEY (mut*)) OR (TITLE-ABS-KEY (proteom*)) OR (TITLE-ABS-KEY (transcript*)) OR (TITLE-ABS-KEY (polymorphism*)) OR (TITLE-ABS-KEY (aminoacid*)) OR (TITLE-ABS-KEY (peptid*)) OR (TITLE-ABS-KEY (variation*)) OR (TITLE-ABS-KEY (protein*)) OR (TITLE-ABS-KEY (gene*)) OR (TITLE-ABS-KEY (missense*)) OR (TITLE-ABS-KEY (variant*)) OR (TITLE-ABS-KEY (error*)) OR (TITLE-ABS-KEY (allele*)) OR (TITLE-ABS-KEY (mitochondrial DNA)) OR (TITLE-ABS-KEY (mitochondrial nuclear DNA*))) AND ((TITLE-ABS-KEY (longevity)) OR (TITLE-ABS-KEY (aging)) OR (TITLE-ABS-KEY (youth)) OR (TITLE-ABS-KEY (ageing)) OR (TITLE-ABS-KEY (lifespan)) OR (TITLE-ABS-KEY (lifespan)) OR (TITLE-ABS-KEY (life time)) OR (TITLE-ABS-KEY (old age)) OR (TITLE-ABS-KEY (lastingness)))

Table 3. Search terms for PubMed

Items	***Search terms***
Organelle	(([Title/Abstract](mitochondria)) AND (([Title/Abstract](nuclear)) OR ([Title/Abstract](mtDNA)) OR ([Title/Abstract](mitochondrial DNA)) OR ([Title/Abstract](mitochondrial protein)) OR ([Title/Abstract](mitochondrial nuclear protein)) OR ([Title/Abstract](mitochondria AND nucleus))) AND ([Title/Abstract](mutation)) AND ([Title/Abstract](snp OR snps))
Population	(([Title/Abstract](human OR humans)) OR ([Title/Abstract](homo sapiens)) OR ([Title/Abstract](h. sapiens)) OR ([Title/Abstract](person)) OR ([Title/Abstract](child OR children)) OR ([Title/Abstract](elder)) OR ([Title/Abstract](people)) OR ([Title/Abstract](mice)) OR ([Title/Abstract](mus musculus)) OR ([Title/Abstract](m. musculus)) OR ([Title/Abstract](mouse)) OR ([Title/Abstract](rat OR rats)) OR ([Title/Abstract](rattus)) OR ([Title/Abstract](caenorhabditis elegans)) OR ([Title/Abstract](c. elegans)) OR ([Title/Abstract](zebrafish)) OR ([Title/Abstract](danio rerio)) OR ([Title/Abstract](d. rerio)) OR

	([Title/Abstract](drosophila melanogaster)) OR ([Title/Abstract](d. melanogaster)) OR ([Title/Abstract](fruit fly)))
Characteristics 1	(([Title/Abstract](mutation)) OR ([Title/Abstract](proteom)) OR ([Title/Abstract](transcript)) OR ([Title/Abstract](polymorphism)) OR ([Title/Abstract](aminoacid)) OR ([Title/Abstract](peptid)) OR ([Title/Abstract](variation)) OR ([Title/Abstract](protein)) OR ([Title/Abstract](gene)) OR ([Title/Abstract](missense)) OR ([Title/Abstract](variant)) OR ([Title/Abstract](error)) OR ([Title/Abstract](allele OR alleles)) OR ([Title/Abstract](mitochondrial DNA)) OR ([Title/Abstract](mitochondrial nuclear DNA)))
Characteristics 2	(([Title/Abstract](longevity)) OR ([Title/Abstract](aging)) OR ([Title/Abstract](youth)) OR ([Title/Abstract](ageing)) OR ([Title/Abstract](lifespan)) OR ([Title/Abstract](lifespan)) OR ([Title/Abstract](life time)) OR ([Title/Abstract](old age)) OR ([Title/Abstract](lastingness)))
TOTAL	(([Title/Abstract](mitochondria)) AND (([Title/Abstract](nuclear)) OR ([Title/Abstract](mtDNA)) OR ([Title/Abstract](mitochondrial DNA)) OR ([Title/Abstract](mitochondrial protein)) OR ([Title/Abstract](mitochondrial nuclear protein)) OR ([Title/Abstract](mitochondria AND nucleus))) AND ([Title/Abstract](mutation)) AND ([Title/Abstract](snp OR snps)) AND (([Title/Abstract](human OR humans)) OR ([Title/Abstract](homo sapiens)) OR ([Title/Abstract](h. sapiens)) OR ([Title/Abstract](person)) OR ([Title/Abstract](child OR children)) OR ([Title/Abstract](elder)) OR ([Title/Abstract](people)) OR ([Title/Abstract](mice)) OR ([Title/Abstract](mus musculus)) OR ([Title/Abstract](m. musculus)) OR ([Title/Abstract](mouse)) OR ([Title/Abstract](rat OR rats)) OR ([Title/Abstract](rattus)) OR ([Title/Abstract](caenorhabditis elegans)) OR ([Title/Abstract](c. elegans)) OR ([Title/Abstract](zebrafish)) OR ([Title/Abstract](danio rerio)) OR ([Title/Abstract](d. rerio)) OR ([Title/Abstract](drosophila melanogaster)) OR ([Title/Abstract](d. melanogaster)) OR ([Title/Abstract](fruit fly))) AND (([Title/Abstract](mutation)) OR ([Title/Abstract](proteom)) OR ([Title/Abstract](transcript)) OR ([Title/Abstract](polymorphism)) OR ([Title/Abstract](aminoacid)) OR ([Title/Abstract](peptid)) OR ([Title/Abstract](variation)) OR ([Title/Abstract](protein)) OR ([Title/Abstract](gene)) OR ([Title/Abstract](missense)) OR ([Title/Abstract](variant)) OR ([Title/Abstract](error)) OR ([Title/Abstract](allele OR alleles)) OR ([Title/Abstract](mitochondrial DNA)) OR ([Title/Abstract](mitochondrial nuclear DNA))) AND (([Title/Abstract](longevity)) OR ([Title/Abstract](aging)) OR ([Title/Abstract](youth)) OR ([Title/Abstract](ageing)) OR ([Title/Abstract](lifespan)) OR ([Title/Abstract](lifespan)) OR ([Title/Abstract](life time)) OR ([Title/Abstract](old age)) OR ([Title/Abstract](lastingness)))

Table 4. Search terms for EBSCO

Items	***Search terms***
Organelle	(((mitochondria) AND (nuclear)) OR ((mtDNA) OR (mitochondrial DNA) OR (mitochondrial protein) OR (mitochondrial nuclear protein) OR (mitochondri AND nuclear)) AND (mutation) AND (snp))
Population	((human OR humans) OR (homo sapiens) OR (h. sapiens) OR (person) OR (child OR children) OR (people) OR (mice) OR (mus musculus) OR (m. musculus) OR (mouse) OR (rat) OR (rattus) OR (caenorhabditis elegans) OR (c. elegans) OR (zebrafish) OR (danio rerio) OR (d. rerio) OR (drosophila melanogaster) OR (d. melanogaster) OR (fruit fly))
Characteristics 1	((mut) OR (proteom) OR (transcript) OR (polymorphism) OR (aminoacid) OR (peptid) OR (variation) OR (protein) OR (gene) OR (missense) OR (variant) OR (error) OR (allele) OR (mitochondrial DNA) OR (mitochondrial nuclear DNA))
Characteristics 2	((longevity) OR (aging) OR (youth) OR (ageing) OR (lifespan) OR (life time) OR (old age) OR (lastingness))
TOTAL	(((mitochondria) AND (nuclear)) OR ((mtDNA) OR (mitochondrial DNA) OR (mitochondrial protein) OR (mitochondrial nuclear protein) OR (mitochondri AND nuclear)) AND (mutation) AND (snp)) AND ((human OR humans) OR (homo sapiens) OR (h. sapiens) OR (person) OR (child OR children) OR (people) OR (mice) OR (mus musculus) OR (m. musculus) OR (mouse) OR (rat) OR (rattus) OR (caenorhabditis elegans) OR (c. elegans) OR (zebrafish) OR (danio rerio) OR (d. rerio) OR (drosophila melanogaster) OR (d. melanogaster) OR (fruit fly)) AND ((mut) OR (proteom) OR (transcript) OR (polymorphism) OR (aminoacid) OR (peptid) OR (variation) OR (protein) OR (gene) OR (missense) OR (variant) OR (error) OR (allele) OR (mitochondrial DNA) OR (mitochondrial nuclear DNA)) AND ((longevity) OR (aging) OR (youth) OR (ageing) OR (lifespan) OR (life time) OR (old age) OR (lastingness))

Annex B: Screening protocol

Category	*Exclusion criteria*
0. Null entries, duplicates, not in the language of interest, abstract is reported elsewhere and not in the time period of interest	01 - Null entries 02 - Duplicates 03 - Language of interest 04 - Abstract that is reported elsewhere
1. Nature of study	05 - Related article 06 - Not linked to longevity
2. Study population	07 - Yeast or plant model 08 - Prokaryotes 09 - Other mammal model 10 - Other non-mammal model
3. Outcome	11 - No functional link 12 - Inaccessible 13 - Negative association 14 - Doesn't mention a specific mutation
4. Potential	15 - Mitochondrial DNA mutations (mtDNA coding) 16 - Mitochondrial Nuclear mutations (DNA coding) 17 - Yeast model (human gene) 18 - Prokaryote (human gene) 19 - Protective of degenerative diseases 20 - Mitochondrial and Nuclear (mtDNA and DNA coding or non-coding) 21 - Mitochondrial (mtDNA non-coding) 22 - Protein analysis