Mitochondrial genetics and osteoarthritis

Ignacio Rego-Perez¹, Mercedes Fernandez-Moreno¹, Angel Soto-Hermida¹, Carlos Fernandez-Lopez¹, Natividad Oreiro¹, Francisco J. Blanco ¹,²,³

¹Rheumatology Division, Genomic Laboratory INIBIC-Hospital Universitario A Coruna, As Xubias 84, 15006-A Coruna Spain, ²CIBER-BBN- Instituto de Salud Carlos III-Madrid-Spain, ³Departamento de Medicina, Universidad de Santiago de Compostela, Santiago de Compostela, Spain

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1. ABSTRACT

The genetic contribution is one of the most notable factors that play a main role in the risk of OA. Despite the genetics of this disease is complex and the finding of risk-related genes has been very challenging, evidence for genetic predisposition has been reported. Besides, in the last years recent evidences indicate that the mitochondrion is implicated in OA. In this context, the mtDNA haplogroups, defined as individual groups characterized by the presence of a particular set of single nucleotide polymorphisms (SNPs) in the mtDNA sequence, emerged as new genetic variants involved in this pathology. Moreover, it has been described that mtDNA damage not only accumulates in OA chondrocytes, but also that OA chondrocytes have limited mtDNA repair capacity. In this review we will focus on the influence of mitochondrial genetics and the mtDNA haplogroups in the prevalence, severity and progression of the OA disease, as well as their incidence on many OA-related features, such as serum levels of OA-related molecular markers, Nitric Oxide production or telomere length.

2. INTRODUCTION

Osteoarthritis (OA) is the most common joint disease related to aging and is characterized by late-onset degeneration of articular cartilage (1). OA is a disease that affects more than 10% of the population after the age of 45 years, and its prevalence increases up to 40% of people older than 70 years of age (2). Since the life expectancy in the developed world increases, it is estimated that the number of OA cases will double in the next three decades. OA is also the main cause of permanent work incapacitation and one of the most common reasons for visiting primary care physicians and, however, there is not fundamental treatment. Among the factors that play a role in the risk of OA, gender, age, body mass index (BMI) and genetic contribution are the most notable (1, 3). Moreover, evidences described in this review support the idea that mitochondrial genetics is also a key factor in the complex aetiology of OA.

The genetics of OA is complex, as it does not usually follows the typical pattern of mendelian inheritance
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and is probably associated with multiple gene interactions; however, evidence for genetic predisposition to this pathology was reported. Stecher and collaborators demonstrated that the presence of Heberden nodules in OA fingers of the hand were three times more likely to occur in twins compared to the general population (4). In addition, several classical twin studies have been performed in OA, concluding that the influence of the genetic factors is 60% in hip OA and 39% in knee OA (3). Even radiographic severity and OA progression seem to be influenced, at least in part, by genetics (5, 6). Nowadays, thanks to both recent advances of high-throughput single nucleotide polymorphism (SNP) genotyping technology and the development of the HapMap project, it has been possible to test a large number of genetic markers across the genome by means of the Genome Wide Association Studies (GWAS). In the case of OA has proven very challenging to find risk-related genes, mainly due to i) the heterogeneity within clinical subsets of the disease and between different ethnic groups, ii) the influence of many loci each with a small effect and iii) the lack of analysis of rare large-effect mutations (3). However, interesting associations have been found, such as the rs143383 SNP in the growth differentiation factor 5 (GDF-5) gene with knee OA (7, 8).

Despite the glycolytic nature of articular chondrocytes, by which ATP production takes place mainly in the cytoplasm by the oxidative reactions of glycolysis, in the last years evidence indicates that the mitochondrion is implicated in OA (9). A significant decrease of complexes II and III of the mitochondrial respiratory chain (MRC) in OA chondrocytes has been described (10) and, in addition, the inhibition of complexes III and V of the MRC causes an increased inflammatory response potentially related to the production of reactive oxygen species (ROS) (11); besides, the apoptotic mitochondrial pathway is one of the major cellular pathways for apoptosis of OA chondrocytes (12) and even mitochondrial free radical production compromises chondrocyte function (13, 14) causing mitochondrial DNA (mtDNA) damage and reduced mtDNA capacity for repair (15, 16). In this context, the mtDNA haplogroups emerge as new genetic variants involved in the OA disease (17, 18). Briefly, these mitochondrial variants are defined as individual groups characterized by the presence of a particular set of SNPs, in the mtDNA sequence, that were accumulated sequentially along radiating maternal lineages (19). Among individuals of Caucasian ancestry, 95% of the population belongs to one of the following haplogroups: H, I, J, T, U, K, V, HV*, W or X. There is evidence about the importance of mtDNA haplogroups for energy production, in fact they show differences in their Oxidative Phosphorylation System (OXPHOS) coupling efficiency (20, 21) and, therefore, an increasing number of studies showing associations between some of the mtDNA haplogroups and multifactorial diseases have been carried out (22-24).

Taking into account this background, in this review we discuss some of the evidences implicating mitochondrial genetics in the risk of OA, as well as the causes of its involvement in degenerative diseases.

3. THE mtDNA HAPLOGROUPS

As stated above, the mtDNA haplogroups are related groups of mtDNAs characterized by stable polymorphic sites in mtDNA coding and non-coding regions (Figure 1a and 1b) (19) that were shaped by natural selection as humans migrated north into colder climates (Figure 1c) (20, 21). Specifically, European mtDNA haplogroups would also be expected to have been influenced by cold selection because of the episodic periods of cold associated with the repeated continental glaciations (Figure 1c) (25). Today, an increasing number of studies showing associations between the mtDNA haplogroups and different pathologies have been carried out, either as protective or risk factors. In this sense, one haplogroup may be linked to susceptibility to energy deficiency diseases, but also be protective for degenerative diseases and aging (21, 26). The explanation for this question is related to the multifunctional nature of the mitochondrion, whereby the OXPHOS system oxidizes the carbohydrates and fats of our diet with the oxygen we breathe to generate energy in the form of adenosine triphosphate (ATP) and heat to maintain our body temperature by means of a mechanism called coupling efficiency. Tightly coupled OXPHOS would produce maximum ATP and minimum heat, whereas partially uncoupled OXPHOS would generate more heat and less ATP. In this context, a mtDNA mutation that reduces the coupling efficiency of OXPHOS would diminish the ATP production, therefore increasing the susceptibility to energy deficiency diseases (i.e: LHON); on the contrary, the same mutation would keep the mitochondrial electron transport chain (ETC) more oxidized, thereby reducing reactive oxygen species (ROS) production and apoptosis, being protective for oxidative stress related diseases (i.e: Alzheimer, Parkinson, OA?) and increased longevity (Figure 2).

3.1. mtDNA haplogroups and OA

The involvement of mtDNA in the development of OA remained unclear until recently. A few reports analyzed the presence of mtDNA with 4977-base pair (bp) deletion in knee OA cartilage, concluding that the accumulation of this deletion increases with age and may play a role in the development of knee OA (27, 28). However, no study had shown a possible association between mtDNA polymorphisms and OA. Recent studies carried out by our group showed the relationship between the mtDNA haplogroups and OA. The first work demonstrated their role in the prevalence and severity of 457 knee OA patients from Spain, compared with 262 healthy controls, by which people carrying mtDNA haplogroup J may be at lower risk of developing knee OA (Odds Ratio (OR) 0.460; 95% Confidence Interval (CI) 0.282 – 0.748; p=0.002), and those carrying this haplogroup and suffered from OA present a lower radiological joint damage, attending to the Kellgren and Lawrence (K/L) score (OR 0.351; 95% CI 0.156 – 0.787; p=0.012) (17). These findings were replicated later in another study in which the authors analyzed 550 hip OA patients and 505 healthy controls from Spain, and showed that mtDNA haplogroup J was also associated with a lower risk of hip OA (OR 0.661; 95% CI 0.440 – 0.993; p=0.045)
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Figure 1. (A) Scheme of the human mtDNA molecule showing some of the characteristic polymorphisms (SNPs) that (B) identify the most common European mtDNA haplogroups. (C) mtDNA migrations, that permitted humans to adapt to different geoclimatic areas, gave rise to different mtDNA haplogroups. Reproduced with permission from Mitomap (18). In the same study, a cohort of patients with Rheumatoid Arthritis was also analyzed but no association between this pathology and the mtDNA haplogroups was detected, concluding that the protective effect of the haplogroup J affects only an age-related and oxidative stress disease such as OA (18).

Otherwise, a retrospective study carried out by our group in a well characterized follow-up cohort of 281 OA patients, revealed that mtDNA haplogroups also influence the radiographic progression of OA, so that patients with the most common European mtDNA haplogroup H are more apt to require joint replacement surgery than non-H carriers (Hazard Ratio (HR) 1.441; 95% CI 1.002 – 2.073; p=0.049). In this study, OA progression was defined as any radiographic worsening of the hip or knee K/L score in a follow-up period not less than 36 months, and total knee or hip replacement was recorded and considered as an outcome. Besides, to control for possible confusion bias, a multiple Cox regression analysis that permitted to analyze the influence of mtDNA haplogroups on radiographic OA progression probability in the follow-up, after adjusting for other non-genetic risk factors, such as age, gender and BMI, was also performed (Fernández-Moreno in press 2012).

A possible explanation for these findings could be related to biochemical differences between mtDNA haplogroups J and H (Figure 2). The partially uncoupled OXPHOS system in carriers of haplogroup J leads to an increased oxidation of the MRC and, therefore, a lower ROS production and apoptosis (21, 26, 29), two key factors involved in the development of OA (12, 14). On the contrary, mtDNA haplogroup H has the strongest OXPHOS coupling efficiency and ATP production, probably because of its high level of conserved aminoacids (29), and it is also the highest oxygen uptake consumer (30). These features related to its high performance are accompanied by an augmented production of ROS, increasing cellular damage and apoptosis (21). Therefore, the elevated levels of ROS found in haplogroup H carriers may be the reason why OA patients with this haplogroup are more likely to have more severe OA progression that leads to joint replacement.

A recent work carried out by our group analyzed the influence of mtDNA haplogroups in a well characterized cohort of 453 OA patients and 280 healthy controls from the United Kingdom (UK). The results obtained showed that mtDNA haplogroup T is a protective factor against knee OA in the UK (OR 0.581; 95% CI 0.365 - 0.926; p=0.021) (Soto-Hermida in press 2012).
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This population/geographic specific association may be explained by the fact that these two mtDNA variants (J and T) could represent an advantage to each of the different environmental conditions in northern Spain and in the UK respectively, as described in other cases (31). In fact, both haplogroups J and T have been described as sister haplogroups that share a common root, the m.4216t>c polymorphism (Figure 1b) (21), which means that mtDNA haplogroup T is also characterized by a partially uncoupled OXPHOS system, being related to lower ATP production (22, 32), and therefore a reduced ROS production is also expected (20, 21, 26). Therefore, the conclusion of these findings is the partially uncoupled OXPHOS system, characteristic of these two haplogroups, as the main cause of the protective role of both mtDNA haplogroups J and T against OA in Spain and UK respectively.

3.2. mtDNA haplogroups and OA-related biomarkers

Molecular markers in OA have been developed in order to detect changes in OA with more reliability and sensitivity, preferably in an earlier stage of the disease (33). These markers are molecules released into biological fluids during the process of tissue biosynthesis and turnover, and can be easily detected by immunoassays. Several molecular markers of bone, cartilage and synovium have been described as useful for early identification of patients with OA (34). In this context, interesting associations between the mtDNA haplogroups and some OA-related biomarkers have been described. In a recent work comparing the serum levels of type II collagen OA biomarkers between 75 OA patients and 75 healthy controls with haplogroups H, J or U, the authors showed that OA patients with the mtDNA haplogroup J had significantly lower serum levels of cartilage degradation markers (Col2-1, Col2-1NO2, C2C) than carriers of the haplogroup H (p ≤ 0.01 in all cases) (35). Similarly, the same authors also analyzed the influence of mtDNA haplogroups on serum levels of proteolytic enzymes in the same cohort of patients described above, concluding that the influence of these mitochondrial variants on serum levels of metalloproteinase 3 (MMP-3) was clearly dependent on the diagnosis (p=0.027), whereas the influence on serum levels of MMP-13 is independent of diagnosis (p=0.035), so that carriers of haplogroup J showed the lowest serum levels (36). As a consequence of these two works, the authors suggest to use mtDNA haplogroups as complementary molecular markers when the above mentioned protein markers are used for diagnosis of OA.

Following this line of mtDNA haplogroups-modulated proteins, and taking into account the particular characteristics by which some of the haplogroups are related to lower ROS production (21, 26), the authors analyzed their possible involvement on serum levels of certain antioxidant proteins, such as Catalase and
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Manganese Superoxide dismutase (MnSOD). No relationships between mtDNA haplogroups and serum levels of MnSOD were found, but a slight influence of mtDNA haplogroup J on serum levels of catalase was detected, so that carriers of haplogroup J showed higher serum levels than non-J carriers (p=0.057) (37).

All the associations with serum levels of different OA-related (potential) molecular markers are probably due to the different metabolic characteristics of mtDNA haplogroups, by which some of them show a different performance of the OXPHOS system (22, 38). This different metabolic profile leads to a different behaviour of the mitochondria in terms of OA-related features such as energy production, glucose metabolism, oxygen consumption, apoptosis, ROS production or calcium accumulation.

3.3. mtDNA haplogroups and OA-related features

Mitochondria play an integral role in ATP production in cells and are involved in glucose metabolism, insulin secretion and regulation of apoptosis (39). Besides, MRC is one of the most important sites of ROS production; specifically, complex I and III have been suggested to be the major ROS source (14). All these mitochondrial-related features, together with alterations in OXPHOS, are characteristic of disease syndromes with neurological, muscular or metabolic manifestations. In addition, oxidative stress within mitochondria can lead to a vicious cycle in which damaged mitochondria produce increased amount of ROS, leading in turn to progressive augmentation in both mitochondrial damage and somatic mutations. This sequence is one of the proposed causes of aging and degenerative diseases, like OA.

These many lines of evidence suggest a “mitochondrial” hypothesis of disease, and population genetic analysis suggests that common mtDNA variants or haplogroups may be relevant to longevity (40), neurodegenerative diseases (41), and metabolic traits like BMI (42) or type 2 diabetes (43). In this regard, a significant association of the mtDNA haplogroup X with decreased BMI and body fat mass (BFM) was demonstrated (42), and our group found an statistical association of mtDNA haplogroup J with lower BMI in a large cohort of samples from the north of Spain (personal data); however, other authors showed no significant associations of mtDNA haplogroups with metabolic traits including BMI (44).

3.4. mtDNA haplogroups and longevity

The mitochondria have been continually implicated in the aging process (29). Mitochondrial ROS are the major source of oxidative damage within the cell and lie at the heart of one of the most prominent theories postulated, the free radical theory of aging (45-47). The ROS produced cause cumulative damage to cellular constituents, DNA, RNA, proteins and lipids, resulting in aging and eventual death.

Successful aging and longevity are the result of the interaction between a variety of genes, environmental conditions and lifestyles; however, the hypothesis that mtDNA haplogroups play a role in longevity has been widely investigated. Three independent studies showed an overrepresentation of the haplogroup J in healthy centenarians compared to young individuals from Northern Italy (48), Northern Ireland (49) and Finland (40). In Spain, an accumulation of the haplogroup J in elderly individuals from Pyrenees Mountains was also detected, concluding that this mtDNA inherited variant could present a phenotypic survival advantage to environmental conditions (31). Similarly, several studies demonstrated the influence of Asian mtDNA haplogroups with longevity in the Japanese population (50-52). However, other studies did not find correlations between mtDNA haplogroups and successful aging probably because, as for other genetic factors, the association of mtDNA inherited variants with longevity is population/geographic specific (31, 53).

The possible explanation for these findings would be the same than for the OA. The partially uncoupled OXPHOS system of haplogroup J (Figure 2) would lead to a lower ROS production, resulting in less damage to cellular constituents, DNA, RNA, proteins and lipids and therefore in successful aging.

3.5. mtDNA haplogroups, Nitric Oxide (NO) and telomere damage

A growing body of evidence suggests that overproduction of NO is involved in the pathogenesis of OA (14, 54). NO inhibits ATP production by competing with oxygen to bind to cytochrome c oxidase on the mitochondria, thereby inhibiting the electron transport chain and the generation of ATP (55, 56); however, the most destructive effects of NO in articular cartilage are related to the ability of NO to combine with superoxide anions (O2−) to generate peroxynitrite (ONOO−) (57, 58). This strong oxidant induces not only damage to DNA, proteins and lipids, resulting in a loss of extracellular matrix and cell death (54, 59, 60), but also directly injure the guanine repeats in the telomere DNA, indicating that oxidative stress directly leads to telomere erosion, regardless of cell division (61, 62).

In a recent study carried out by Fernández-Moreno and collaborators (63), mtDNA haplogroup J appeared to be associated with a longer telomere length in peripheral blood leukocytes (PBLs) from 114 J and 52 non-J samples (p=0.025), as well as with lower NO production and iNOS mRNA expression in chondrocytes (p=0.043), confirming that carriers of this haplogroup suffer from less oxidative stress. This is probably due to the more oxidized MRC in carriers of this haplogroup that leads to a decreased generation of superoxide anion (O2−) and, therefore, a lower ONOO− production and lower telomere damage. Besides, since mitochondria are involved in the NO production through the reduction of nitrite by cytochrome c oxidase and regulated by oxygen on multiple levels (64, 65), and mtDNA haplogroup J show a lower oxygen consumption (30), we speculate that this could be one of the reasons why carriers of this haplogroup also show lower NO production.
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4. mtDNA DAMAGE AND OA

As stated above, there is a growing body of evidence indicating that mtDNA damage, caused by ROS and/or reactive nitrogen species (RNS) is involved in cellular dysfunction and death, and could play a causal role in disorders linked to the excessive generation of ROS. ROS-induced mtDNA damage leads to mtDNA mutations which in turn can lead to the synthesis of functionally impaired respiratory chain subunits, causing MRC dysfunction and augmented ROS production. This vicious cycle increases over time, resulting in enhanced aging and degenerative diseases.

Recent studies showed not only that mtDNA damage accumulates in OA chondrocytes, strengthening the presence of mitochondrial oxidative stress in these cells during disease progression, but also that OA chondrocytes have limited mtDNA repair capacity, indicating that these cells are less able to recover from free radical-induced damage (15). Another striking finding is related to the main proinflammatory cytokines that play a pivotal role in the development of OA, Interleukin-1 (IL-1) and Tumor necrosis factor-alpha (TNF-alpha), and their incidence in the mtDNA damage; IL-1 and TNF-alpha, which regulate apoptosis differently in human chondrocytes (66), disturb normal mitochondrial function in these cells by decreasing energy production and mitochondrial respiration; however, mitochondria from OA chondrocytes are more susceptible to damage induced by proinflammatory cytokines than mitochondria from normal chondrocytes. Otherwise, protection of human chondrocytes with the repair enzyme hOGG1 rescues mtDNA integrity, decreases mitochondrial ROS production, preserves ATP levels and greatly diminishes apoptosis following IL-1 and TNF-alpha exposure. With this scenario, the authors concluded that mtDNA damage plays a pivotal role in the mitochondrial dysfunction and apoptosis induced by proinflammatory cytokines (16).

5. CONCLUSION

Is clearly demonstrated that mitochondrial functions are altered in OA chondrocytes, and recent findings revealed that mtDNA inherited variants, called haplogroups, play a main role in the mechanism and progression of OA. These mitochondrial polymorphisms are also involved in several OA-related features; specifically, the mtDNA haplogroup J is associated with decreased serum levels of cartilage degradation biomarkers, lower BMI, as well as with lower NO production and longer telomere length. On the contrary, the most energetically efficient haplogroup H is related to increased serum levels of cartilage degradation biomarkers and increased risk of joint replacement surgery. We conclude that the effect of the mtDNA haplogroups in the development of the OA disease is mainly related to the ROS production, thus mainly affecting those OA phenotypes ROS-related.

Otherwise, mitochondria is the main source of ROS and also RNS, and increased ROS production and NO in human chondrocytes leads to mtDNA damage, proteins and lipids, causing an increased mitochondrial dysfunction that ultimately leads to the loss of extracellular matrix and cell death. In summary, the influence of the mitochondria and mitochondrial genetics in the OA disease is well documented, however, in order to strength this role it will be necessary to demonstrate these findings in appropriate animal models and in larger cohort of patients.

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**Send correspondence to:** Francisco J. Blanco, INIBIC-Complejo Hospitalario Universitario A Coruna, 15006-A Coruna, Spain, Tel: 34 981 176399, Fax: 34 981 176398, E-mail: fblagar@sergas.es