

Mitochondrial DNA haplogroups and ageing mechanisms in osteoarthritis

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MITOCHONDRIAL DNA AND AGEING

Osteoarthritis (OA) is the most common form of arthritis affecting more than 12% of people over the age of 60.¹ Although late-onset articular cartilage degeneration is common and age is one of the most important risk factors for the disease, the relationship between old age and OA is not fully understood.² In the past it was believed that the link with age was due to 'wear and tear' of articular cartilage by continuous mechanical stress; we now know, however, that OA involves an active response to injury comprising remodelling of articular cartilage and subchondral bone, in addition to synovial inflammation and damage to other joint structures such as ligaments and menisci.³

Biological ageing is a complex process and it is now widely accepted that ageing starts with molecular damage, leading to cell, tissue and, ultimately, organ dysfunction.⁴ Extensive evidence from animal models and in vitro studies indicates that mitochondria contribute to specific aspects of the ageing process, including cellular senescence, chronic inflammation and the age-dependent decline in stem cell activity.⁵

Perhaps the best known and most longstanding hypothesis to explain ageing is the free radical theory that proposes a central role for the mitochondrion as the principal source of intracellular reactive oxygen species (ROS) leading to mitochondrial DNA (mtDNA) mutations.^{4, 5} Somatic (acquired) mtDNA mutations and their association with the decline in mitochondrial function during ageing are well described, but these observations do not necessarily imply a causal relationship between mitochondrial dysfunction and human ageing. The maternally inherited mtDNA sequences encode the key proteins involved in energy production, although

the relevance of high sequence variability of mtDNA had been considered of little functional relevance. Latorre-Pellicer and coauthors showed recently that transferring mtDNA from a mouse strain to the nuclear DNA (nDNA) background of another strain results in huge differences in insulin signalling, obesity and longevity throughout the life of the mouse.⁶ The two mtDNA sequences differ in genetic variants that confer 12 amino acid substitutions and 12 changes in RNA molecules involved in mitochondrial protein synthesis; this level of variation is enough to result in striking differences in the ROS generation, insulin signalling, obesity and cell-senescence-related parameters such as telomere shortening and mitochondrial dysfunction. Showing the direct relevance of mtDNA in human ageing and in age-related diseases, such as OA, is a big challenge and one which is, at least in part, addressed in this issue.⁷

mtDNA in OA

Over the past 10 years, the group led by Francisco Blanco and Ignacio Rego-Perez has shown that differences in mtDNA haplogroups correspond to variations in the prevalence and progression of cartilage loss in large joint OA.⁸ In a series of studies from Spanish OA cases and controls, the evidence has accumulated for an association between OA prevalence and the J haplogroup^{9, 10} (table 1). However, two studies in samples from the UK have failed to find an association with the J haplotype,^{9–11} whereas evidence of association of the T haplotype with lower disease risk was found in a small UK cohort⁹ (table 1).

The mtDNA haplogroups J and T share the same phylogenetic origin and a set of common uncoupling mitochondrial polymorphisms.¹² These uncoupling polymorphisms confer different metabolic characteristics compared with other mitochondrial lineages, particularly the most common and highly efficient mtDNA haplogroup H.¹³

The jury is still out regarding the role of mtDNA T and J haplogroups with regard to genetic susceptibility in populations with large joint OA, particularly when compared with the evidence accumulated for nuclear genetic variants

identified from genome wide association studies (GWAS) or otherwise.¹⁴ To date, eight variants associated with knee OA have been reported with significance of $p < 1 \times 10^{-7}$ and 11 variants with hip OA in Caucasians. At least three other variants have been reported at high significance levels in Asians (see ref. 14 for details).

On the other hand, with the exception of variants mapping to *GDF5* and *FTO* genes, the mechanisms underlying the risk conferred by variants linked to knee OA are yet to be unveiled.¹⁴ Importantly, as of today, very few efforts have been made to identify genetic risk factors contributing to risk of progression or incidence of disease.

The mtDNA haplotypes T, J and the JT cluster, on the other hand, are significantly associated in populations from the USA, the Netherlands and Spain with radiographic incidence and progression of the disease^{7, 15} (table 1). Fernandez-Moreno and coauthors report that the mtDNA haplogroup J, the same haplogroup associated with lower OA prevalence, lower disease progression and lower cartilage loss, is also associated with a significantly lower risk of incident knee OA in a population of 3124 individuals from two prospective cohorts from the Netherlands and the USA.⁷

FUNCTIONAL ANALYSIS OF MTDNA VARIANTS

From previous studies, it is known that the low OA risk haplogroup J is associated with lower serum levels of markers of collagen type-II degradation and of matrix metalloproteinases, but all of these studies failed to address the key question arising from this large body of evidence: 'What is the functional role of these mtDNA haplogroups?'

To answer this question, Fernandez-Moreno *et al*⁷ used cytoplasmic hybrid (cybrid) cell lines. Cybrids incorporate mitochondria from human subjects and perpetuate the mtDNA-encoded components while maintaining the nuclear background of different cybrid lines as constant.¹⁶ Thus, this technique allows investigators to assess the influence of mtDNA variation on cell function. To investigate the role of mtDNA haplogroups, they also created cybrids using osteosarcoma cell lines with the same nuclear background, one of them harbouring the haplogroup J (which protects against OA) and another harbouring the haplogroup H (linked to higher risk of OA).

The cybrids carrying the haplogroup H produced higher ATP levels than those

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Table 1 Selected associations between T, J and TJ cluster mitochondrial DNA and OA prevalence, progression and incidence of OA

Study	Origin	Haplogroup	Trait studied	Total N	Effect (95% CI) p value	Reference
Case-control	Spain	J	OA prevalence	2557 OA, 1339 controls	OR=0.57 (0.46 to 0.71) p<0.00001	10
Case-control	UK	J	OA prevalence	7846 OA, 5402 controls	OR=1.19 (0.72 to 1.95) ns	10
Case-control	UK	T	OA prevalence	453 OA, 280 controls	OR=0.57 (0.35 to 0.94) p<0.027	9
CHECK cohort	The Netherlands	T	OA progression	431 OA	HR=0.645 (0.419 to 0.978) p<0.05	15
OAI	USA	T	OA progression	891 OA	HR=0.50 (0.28 to 0.88) p<0.05	24
Spanish OA cohort	Spain	T	OA progression	281 OA	HR=0.69 (0.38 to 1.28) ns	25
Meta analysis		T	OA progression	1603 OA	HR=0.61 (0.45 to 0.82) p=0.001	15
CHECK cohort	The Netherlands	JT	OA progression	431 OA	HR=0.71 (0.50 to 0.96) p<0.05	15
OAI	USA	JT	OA progression	891 OA	HR=0.81 (0.59 to 1.11) ns	15
Spanish OA cohort	Spain	JT	OA progression	281 OA	HR=0.80 (0.50 to 1.26) ns	15
Meta analysis		JT	OA progression	1603 OA	HR=0.77 (0.62 to 0.94) p=0.009	15
CHECK cohort	The Netherlands	J	OA incidence	635	HR=0.73 (0.47 to 1.00) p<0.05	7
OAI	USA	J	OA incidence	2579	HR=0.68 (0.47 to 0.97) p<0.05	7
Meta analysis		J	OA incidence	3214	HR=0.70 (0.54 to 0.91) p=0.006	7

OA, osteoarthritis; OAI, osteoarthritis initiative.

with the haplogroup J, but this higher energetic efficiency was accompanied by higher production of ROS and the proportion of cells that survived in the presence of hydrogen peroxide was almost half the number of cybrids with haplogroup J. In chondrocytes during OA, oxidative stress may act together with inflammatory and/or mechanical stress to accentuate catabolic processes by increasing the levels of ROS relative to antioxidants.^{17 18} The increased levels of ROS also contribute to the senescence secretory phenotype, in which the age-related decline in the responses of chondrocytes to anabolic growth factors are related to increased oxidative stress.^{19 20} The depletion of antioxidants promotes mitochondrial dysfunction in chondrocytes,²¹ which in turn can amplify the stress responses through increased production of nitric oxide and ROS and activation of NF-κB signalling.^{21–23}

In the presence of staurosporine, which induces cell apoptosis, the cybrids with the haplogroup H had over 50% more apoptotic cells than the cybrids with the low OA risk haplogroup J.⁷ These data, therefore, prove the functional relevance of mtDNA variation linked to risk of OA on cell function and survival and is in agreement with recent work by the same group showing that OA cartilage exhibits signs of early molecular ageing compared with healthy age-matched cartilage.²

CLINICAL RELEVANCE

The data accumulated on the role of mtDNA on cell function and on OA risk have potential clinical implications. On the one hand, it may allow investigators in

the future to define an 'age-related OA' genetic type (haplogroup H) versus one which is protected from the effects of ageing. This group with lower incidence and progression can be excluded from clinical studies that require rapidly progressing OA populations. At the same time, haplogroup J carriers are not fully protected from OA; therefore, studying risk factors in this haplogroup can help identify a group of individuals where other molecular mechanisms linked to OA, for example, those derived from bone changes or from inflammation, may be stronger predictors for progression. These data also raise the important question of the contribution of interactions between nDNA and mtDNA haplogroups, which have yet to be investigated. Finally, OA is a disease that occurs together with cardiometabolic comorbidities which are known to be influenced by mitochondrial dysfunction. Haplogroup H carriers may therefore be the group of OA sufferers at higher risk of metabolic syndrome and cardiovascular disease and with the most chance to benefit from regenerative therapies targeting early cartilage damage or, at more advanced stages, early joint replacement.

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