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Letter to the editor

Reduction of leukocyte mitochondrial DNA copy number in knee osteoarthritis

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Knee osteoarthritis (OA) is a degenerative joint disorder characterized by articular cartilage deterioration, osteophyte formation, and subchondral bone sclerosis.(1) The knee is the most significant site of primary OA involvement. The etiology and pathogenesis of OA remain poorly understood; however, they have been associated with several physiological factors. Mitochondria produce energy by synthesizing adenosine triphosphate (ATP) to drive normal cellular physiological functions. As mitochondrial DNA (mtDNA) lacks the protection of introns, as well as histones, it mutates faster, and with a higher frequency than that seen in nuclear DNA.(2) Moreover, a greater copy content of mtDNA is one mechanism through which dysfunctional mtDNA is repaired.(3) mtDNA content could be a possible biomarker associated with oxidative stress and inflammation. The underlying mechanisms mediating the mitochondrial dysfunction in OA have been suggested as increased chondrocyte oxidative stress and apoptosis, decreased chondrocyte biosynthesis, upregulated chondrocyte inflammation and matrix catabolism, and accelerated cartilage matrix calcification.(4) However, data on association of mtDNA content in primary knee OA are sparse. This study aimed to investigate the relative mtDNA copy number in peripheral blood leukocytes (PBLs) in knee OA patients compared with healthy controls and to determine possible association between mtDNA copy number and radiographic severity of knee OA.

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This case-control study included 215 knee OA patients (mean age 64.32 ± 7.22 years), diagnosed following the criteria of the American College of Rheumatology, and age-matched 218 healthy volunteers (mean age 64.05 ± 6.08 years). This research has been approved by the Institution Review Board (IRB) of the Faculty of Medicine, Chulalongkorn University. A total of 5 ml of peripheral blood was drawn from each participant, and transferred into coded sodium citrate anticoagulant tubes. Following this, genomic DNA was extracted using illustra blood genomic Prep Mini Spin kit (GE Healthcare, Buckinghamshire, UK). The genomic DNA was aliquoted and stored at "80°C analysis. Relative mtDNA copy number was determined using quantitative real-time polymerase chain reaction (PCR) as described previously.(5) DNA samples were amplified in 10 μl reactions using a StepOnePlus Real Time PCR system (Applied Biosystems, Foster City, CA, USA). The primer sequences for mitochondrial NADH dehydrogenase 1 (ND1) gene and nuclear human globulin (HGB) gene were as follows: ND1 F 5'-CCCTAAAACCCGCCACATCT-3' and R 5'-GAGCGATGGTGAGAGCTAAGGT-3'; HGB F 5'-GTGCACCTGACTCCTGAGGAGA-3' and R 5'-CCTTGATACCAACCTGCCCAG-3', respectively. Both reactions conducted contained 5 μl QPCR Green Master Mixes (2x) (Biotech Rabbit, Germany), 2 μl DNA template (1.56 ng/ml) and 0.2 μl forward and reverse primers (10 μM). The thermal cycling profile for both ND1 and HGB gene started with 95°C incubation for 30 s by 1 cycle, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 50 s with signal acquisition. All amplification specificity was regulated by melt curve analysis. To calculate the real time-PCR results was in the term of threshold cycle (C_T) values. The relative mtDNA copy number was

estimated by $\Delta\Delta C_T$ method through using fold induction $2^{-\Delta\Delta CT}$ equation. [6]

The relative mtDNA copy number was significantly lower in knee OA patients than in healthy controls ($P < 0.0001$) (Figure 1). The mtDNA copy number was also shown to diminish with age and was inversely associated with age in healthy controls ($r = -0.38$, $P < 0.001$) (Figure 2). Interestingly, the mtDNA content of OA patients was directly correlated with age ($r = 0.15$, $P = 0.027$) (Figure 2). There was no correlation between mtDNA copy number and the severity of knee OA.

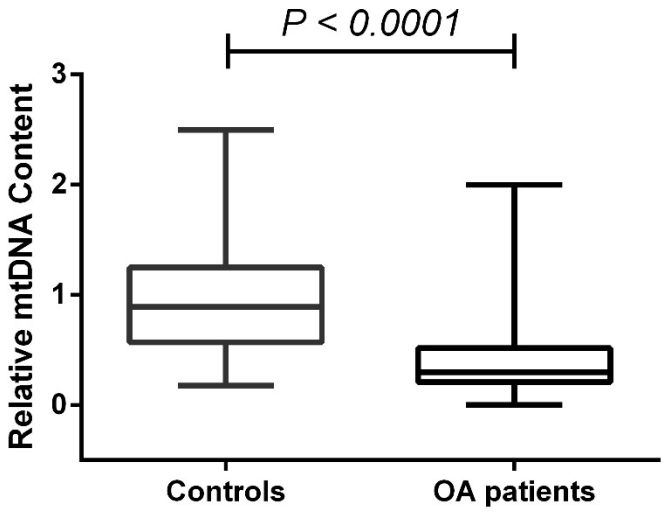

Figure 1. Analysis of leukocyte relative mtDNA copy number in controls and OA patients. The calculated mean ± standard deviation of mtDNA copy number was 0.963 ± 0.489 in control group and 0.422 ± 0.335 in OA group.

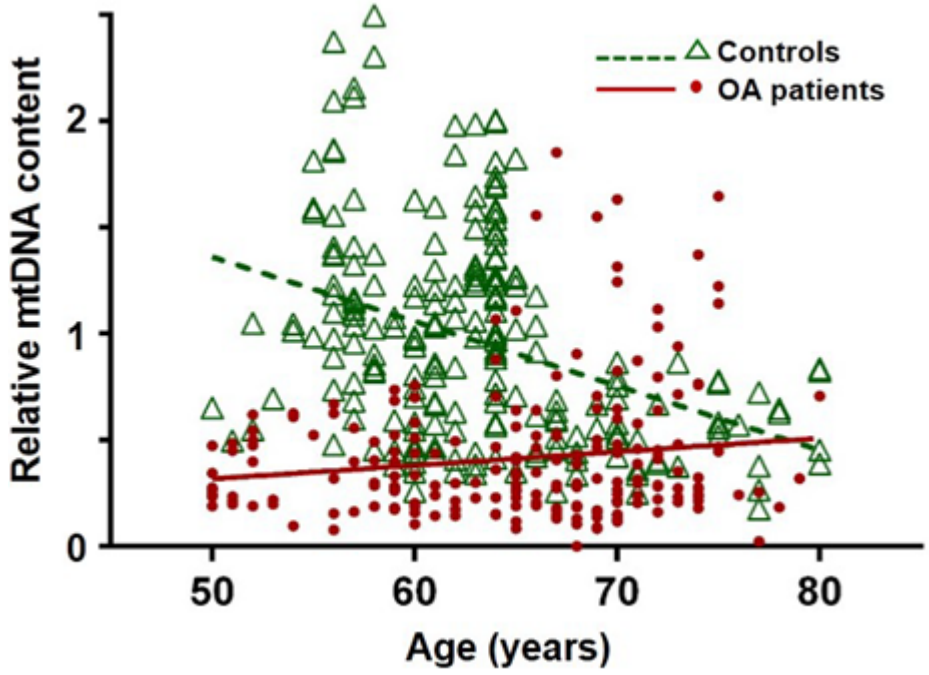

Figure 2. Plot of leukocyte relative mtDNA copy number of controls and OA patients against their ages. Relative mtDNA copy number decreased with age in control group ($r = -0.38$, $P < 0.001$). Relative mtDNA copy number was correlated positively with age in OA group ($r = 0.15$, $P = 0.027$).

The present findings demonstrate that knee OA was associated with reduced mtDNA copy number in PBLs. Gene expression, differentiation and migration of normal cells were influenced by mtDNA function which in turn was affected by copy number and integrality of mtDNA, regulated by multiple factors. [7] Mitochondria dysfunction of peripheral leucocytes could be associated with OA development through the increase of reactive oxygen species (ROS) and apoptosis.[8, 9] Moreover, previous study found leukocyte mtDNA decreased in various diseases including metabolic syndrome, cancer, as well as neurodegenerative diseases. [10] In recent years, previous study showed that mtDNA copy number was increased in knee OA patients compared with controls but mtDNA copy number was not different among various subgroups in OA patients. [11] The explanation of these conflicting findings remain obscure but could be attributed to differences in clinical setting, disease advancement, populations, ethnic groups, or assays applied.

Although mtDNA copy number of PBLs in OA patients was lower than that in controls, mtDNA copy number of OA patients tends to increase with age. However, in healthy controls, we observed that mtDNA copy number decreased with age, which was in agreement with the study in general population reported by Knez J, *et al*. [12] Free radicals may result in accumulative oxidative damage to mtDNA and a decline in mtDNA copy number with age in controls. Conversely, age-related increase in mtDNA copy number in knee OA might represent a compensatory mechanism for mitochondria damage from oxidative stress. Despite underlying mechanisms that account for mtDNA content change, their functions in OA and other diseases are still unknown. We demonstrate that mtDNA copy number changes in leukocytes, and might contribute to the pathological process of age-related OA.

It is worth to point out that this study is limited to PBLs, which are a mixture of various cell populations. Besides, mtDNA copy number in different cell types from synovium and cartilage of knee OA was not determined in this study. Additional evaluation of mtDNA copy number in these various cell populations and their correlation with aging could provide a better understanding on how mtDNA copy number alters during aging in controls and knee OA patients.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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