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Relationship Between the Dynamics of Telomere Loss in Peripheral Blood Leukocytes From Knee Osteoarthritis Patients and Mitochondrial DNA Haplogroups

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ABSTRACT. ***Objective.*** To evaluate the evolution of telomere length from peripheral blood leukocytes (PBLs) in subjects from the Osteoarthritis Initiative (OAI) cohort in relation to the incidence of osteoarthritis (OA), and to explore its possible interactive influence with the mitochondrial DNA (mtDNA) haplogroup.

Methods. Dynamics of telomere sequence loss were quantified in PBLs from initially healthy individuals (without symptoms or radiological signs), 78 carrying the mtDNA cluster HV, and 47 with cluster JT, from the OAI, during a 72-month follow-up period. The incidence of knee OA during this period (n = 39) was radiographically established when Kellgren-Lawrence (KL) score increased from < 2 at recruitment, to ≥ 2 at the end of 72 months of follow-up. Multivariate analysis using binary logistic regression was performed to assess PBL telomere loss and mtDNA haplogroups as associated risk factors of incidence of knee OA.

Results. Carriers of cluster HV showed knee OA incidence twice that of the JT carriers (n = 30 vs 9). The rate of PBL telomere loss was higher in cluster HV carriers and in individuals with incident knee OA. Multivariate analysis showed that the dynamics of PBL telomere shortening can be a consistent risk marker of knee OA incidence. Subjects with nonincident knee OA showed a slower telomere loss than those with incident knee OA; the difference was more significant in carriers of cluster JT than in HV.

Conclusion. An increased rate of telomere loss in PBLs may reflect a systemic accelerated senescence phenotype that could be potentiated by the mitochondrial function, increasing the susceptibility of developing knee OA.

Key Indexing Terms: biomarkers, cohort studies, genetic studies, osteoarthritis, polymorphism

Telomeres are essential elements for maintaining genome integrity since they protect the chromosome ends from exonucleolitic degradation and end-to-end fusion.[1] Yet, they progressively shorten with consecutive cell divisions. When a telomere becomes critically shortened and chromosome capping cannot be properly exerted, cell proliferation is inhibited, and it may enter into apoptosis. This phenomenon is known as replicative senescence and limits cell longevity in most somatic cells.[1,2]

Because of their relationship with cellular turnover, the quantification of the telomere DNA sequences from peripheral blood leukocytes (PBLs) has been found to be a potential marker of biological age and a predictor of longevity.[3] Telomere size from PBLs strongly correlates with that from other tissues from the same individual; thus, its determination may report information about telomere status in less accessible tissues.[4,5] Telomere size determined in PBLs has been revealed to be a general associated risk factor for age-related chronic diseases, such as some types of cancer, type 2 diabetes mellitus, dementia, and cardiovascular disease.[2,6,7]

Osteoarthrithis (OA) is a common age-related chronic disease defined by confined progressive destruction of articular cartilage, leading to pain and work incapacitation.[8] Due to chondrocyte dysfunction, the extracellular matrix is degraded, progressing to subchondral bone remodeling, osteophyte formation, local inflammation, degeneration of ligaments, and loss of normal joint function. Chondrocytes release proinflammatory cytokines that contribute to inflammation and promote apoptosis, aggravating the progression of the disease.[9] OA chondrocytes

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The authors declare no conflicts of interest relevant to this article.

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show telomeres shorter than those from healthy individuals; this may be related to accelerated articular senescence and could contribute to the incidence and progression of OA.[10]

Mitochondrial genetics also has a significant influence in the pathogenesis of OA. Several studies established associations between specific mitochondrial DNA (mtDNA) haplogroups and longevity, particularly with disorders such as hypertension, diabetes, obesity, neurodegenerative diseases, and OA.[11] In White populations, mtDNA variants belonging to the mtDNA haplogroup cluster JT have been associated with a reduced risk of prevalence of knee and hip OA and lower rates of incidence and progression of knee OA, in comparison to subjects with the most common haplogroup H.[12,13,14] Interestingly, individuals carrying the mtDNA haplogroup J exhibit a PBL telomere length longer than non-J carriers.[15]

Previous evaluation of subjects from the cohort study belonging to the Osteoarthritis Initiative (OAI) from the United States indicated that PBL telomere size is shorter in patients with concurrent knee OA and in patients with hand OA.[16,17]

The aforementioned association was found to be even stronger regarding future incidence of hand OA.[17] In the abovementioned studies, all were cross-sectional and PBL telomere size was determined only at the time of recruitment of the patients with OA and controls. However, telomeres decrease progressively with time, with varying rates among individuals. Here we evaluated for the first time, to our knowledge, the evolution of PBL telomere length in subjects from the OAI cohort in relation to the incidence of OA and explore its possible interactive influence with the mtDNA haplogroups.

METHODS

Subjects. The study included individuals from the OAI, from a subsample of controls at recruitment (i.e., without symptoms or radiological signs; n = 125, 45 males and 80 females), and carriers of mtDNA cluster HV (n = 78) or cluster JT (n = 47). The subsample was restricted to White subjects with an age range of 45–77 years (mean 57.6 yrs). Subjects carrying these mtDNA clusters were selected because they constitute the majority of the clusters in the White population and are the most studied and relevant in terms of the incidence and/or progression of OA. Of these subjects, BMI was 26.57 ± 4.36 and 19.2% were hypertensive. The Western Ontario McMaster Universities Osteoarthritis Index (WOMAC), a clinical measure of disease activity, was also recorded from the OAI database.

Evaluation of knee OA. Individuals were assessed through the use of radiologic knee images, according to the Kellgren-Lawrence (KL) score, ranging from 0 to 4. The interreader agreement between 2 readings was good (κ = 0.70–0.80).[18] The presence of radiographic knee OA was defined when an individual had a maximum KL grade (max-KL) ≥ 2 considering both knees. Incident knee OA was defined when max-KL grade increased from < 2 at recruitment to ≥ 2 at the end of 72 months of follow-up. Baseline and 72 months KL data were obtained from the OAI database.

Evaluation of telomere sequences. DNA from PBLs was extracted from blood samples obtained at recruitment and after a 72-month follow-up. Coded DNA samples were processed by personnel blinded to the status of the subjects. The average telomere amount in PBLs was measured with a standard validated quantitative PCR (qPCR)-based assay as described.[10] This method measures the average ratio of telomere repeat copy number to a single gene (*36B4*) copy number (T/S ratio) in each sample and may be considered an indirect measure of mean telomere length. DNA samples from baseline and after 72 months from the same individual were simultaneously processed in the same PCR plate to avoid possible interassay variability. The relative percentage of telomere loss in each individual was defined as [(T/S ratio at recruitment – T/S ratio after 72 months) / T/S ratio at recruitment] × 100.

mtDNA haplogroups genotyping. A combination of the single-base extension assay with the PCR-restriction fragment length polymorphism technique was employed to obtain the different single-nucleotide polymorphisms (SNPs) that characterize the mtDNA haplogroups, as previously described.[19]

Statistical analysis. Data were analyzed using SPSS software (IBM Corp.). Since the percentage of PBL telomere loss was not normally distributed, as ascertained by the Kolmogorov-Smirnov test, nonparametric Mann-Whitney *U* test was performed for homogeneity testing. A Pearson chi-square test was used to determine statistical differences between percentages of incidence. Multivariate analysis using binary logistic regression was performed to assess PBL telomere loss, mtDNA haplogroups, age, sex, hypertension, and WOMAC, as associated risk factors of incidence of knee OA. The linearity of relative PBL telomere loss and log odds was confirmed by linear regression (R^2 = 0.974). Significance was defined as $P < 0.05$.

Ethics. The OAI study was approved by the institutional review boards at each OAI clinical site and the coordinating center (University of California, San Francisco), and informed consent was obtained from the participants. The study was also approved by the Local Ethics Committee (Comité de Ética de la Investigación de A Coruña-Ferrol) with registry code 2018/129.

RESULTS

The study was performed in a group of 78 individuals carrying mtDNA cluster HV and 47 carrying cluster JT, all of them without radiological signs of knee OA at recruitment. The percentage of radiologic OA incidence and the relative percentage of telomere loss in PBLs were evaluated after a follow-up period of 72 months. From the 125 subjects, 39 developed knee OA (i.e., incident).

Regarding mtDNA polymorphisms, individuals with incident knee OA were increased in carriers of cluster HV (38.46%), 2 times higher in comparison with carriers of cluster JT (19.15%; P = 0.02). Moreover, the relative percentage of telomere loss in PBLs was significantly lower in subjects carrying cluster JT than in those carrying cluster HV ($P < 0.001$; Figure 1A). The relative PBL telomere shortening was also lower in nonincident subjects than in incident individuals ($P < 0.001$; Figure 1B, Table 1).

In the multivariate analysis of the joint role of both variables (mtDNA cluster and relative PBL telomere shortening), using binary logistic regression, the relative PBL telomere loss remained significantly associated with incidence as a risk marker (OR 1.06, 95% CI: 1.02–1.10, P = 0.001); therefore, higher PBL shortening rates were associated with a higher risk of knee OA incidence. The OR is per percentage unit of telomere loss. Otherwise, the mtDNA cluster lost statistical significance (OR 0.70, 95% CI 0.26–1.80, P = 0.45; data not shown).

When the analysis was stratified by the cluster, the relative percentage of PBL telomere shortening was significantly higher in the individuals with incident knee OA than in the nonincident knee OA individuals carrying the JT cluster (P = 0.003; OR 1.13, 95% CI 1.04–1.22, P = 0.005; Figure 1C, Table 1). In carriers of the HV cluster, the differences in PBL telomere shortening rates between nonincident and incident individuals were smaller and extremely close to the limit of statistical significance (P = 0.08, OR 1.04, 95% CI 0.99–1.08; Figure 1C, Table 1).

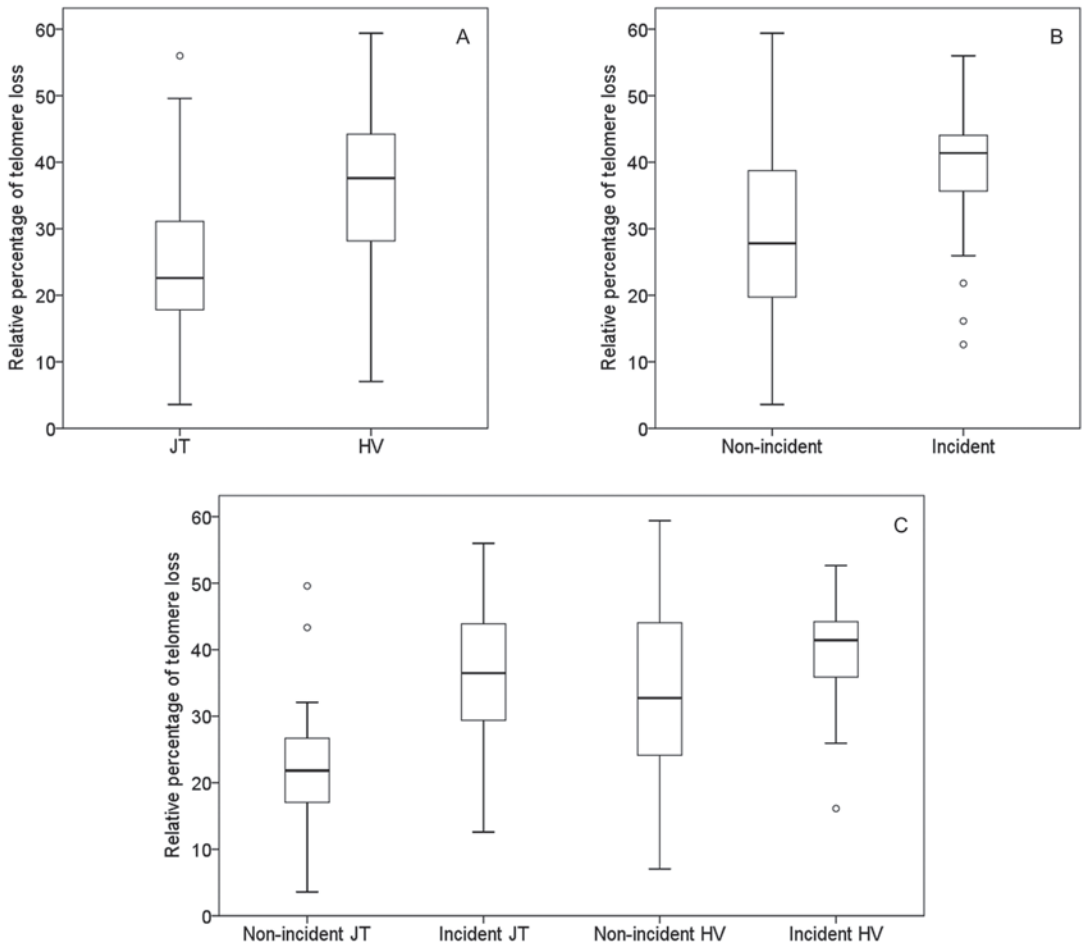

Figure 1. Relative percentage of telomere loss in peripheral blood leukocytes from (A) subjects carrying the mtDNA clusters JT and HV; (B) subjects with nonincident and incident knee OA; and (C) subjects with nonincident and incident knee OA carrying the mtDNA clusters JT and HV. OA: osteoarthritis.

Table 1. Statistical values of the relative percentage of telomere loss in peripheral blood leukocytes and analysis of significant differences in subjects with incident or nonincident knee OA, as well as individuals with mtDNA cluster HV or JT.

			n	Q1	Q2	Q3	*P**
Subjects	Nonincident		86	19.39	27.79	39.31	< 0.001
	Incident		39	35.47	41.36	44.22	
mtDNA cluster	HV	Nonincident	48	23.91	32.72	44.41	0.08
		Incident	30	35.88	41.43	44.27	
	JT	Nonincident	38	16.82	21.83	27.05	0.003
		Incident	9	25.60	36.49	44.91	

Quartiles (Q1: lower, Q2: median, Q3: upper) are indicated. * Mann-Whitney *U* test. mtDNA: mitochondrial DNA; OA: osteoarthritis.

When age, sex, BMI, WOMAC, and hypertension were included in the multivariate analysis, using binary logistic regression, the relative PBL telomere loss remained significantly associated with incidence (OR 1.05, 95% CI 1.01–1.09, $P = 0.02$), together with BMI (OR 1.26, 95% CI 1.12–1.42, $P < 0.01$) and WOMAC (OR 1.25, 95% CI 1.04–1.50, $P < 0.02$). Age, sex, and hypertension were not significant.

DISCUSSION

In previous reports, reduced PBL telomere length determined at a single timepoint was associated with prevalence of knee OA[16] and with prevalence and incidence of hand OA.[17] Since the telomeres evolve over time, the determination of longitudinal changes of PBL telomeres must provide a more stringent testing. This study confirms the strong association between the dynamics

of PBL telomere shortening with the risk of knee OA incidence. Moreover, mitochondrial function may have a relevant role since the difference in PBL telomere decay between incident and nonincident subjects was more significant in carriers of mtDNA cluster JT than in HV. This fact is due to the much lower percentage of PBL telomere decay in nonincident subjects with cluster JT than with HV.

The polymorphisms (SNPs) that characterize haplogroup cluster JT may decrease the efficiency of mitochondrial coupling, reducing both adenosine triphosphate and reactive oxygen species (ROS) production, as well as lowering the expression of genes related to the inflammatory response, complement, and apoptosis.[12,20]

Senescent cells accumulate with age and in age-related diseases; they are also associated with loss of tissue function. One main factor associated with the development of knee OA must be the progression of biological aging of the joint cartilage. Replicative senescence at the cellular level is triggered when telomeres are excessively shortened, so this mechanism would be more plausible in high-turnover tissues. Nevertheless, chondrocytes do not exhibit a high proliferation rate and their telomeres are higher than those from PBLs in the same subject.[10] Importantly, telomere DNA damage can occur independently of length, and this has been shown to contribute to the senescent phenotype. Telomeres may act as sensors of intrinsic and extrinsic stresses, and they maintain genomic stability by limiting replication of cells that have accumulated significant genomic damage.[21] The higher ROS generation in chondrocytes carrying mtDNA cluster HV may damage telomeric DNA, favoring the telomere length-independent senescence, unlike in the less inflammatory and oxidative phenotype from carriers of cluster JT.

The decay of telomeres in PBLs provides information about how this systemic aging process is evolving, and subsequently how the articular senescence status is progressing. Knee OA is mainly developed in the group of subjects with a higher telomere loss over time (i.e., with an accelerated biological aging). The influence of the mtDNA cluster HV or JT on the risk of knee OA incidence seems to be derived from the differential damaging oxidative activity in the body. However, this harmful effect is seen in PBL telomere shortening, as previously mentioned. PBL telomere loss includes the presumed information of the risk of incidence of knee OA provided by the mtDNA cluster, which could explain its loss of significance in the multivariate analysis. Further, the rate of PBL telomere shortening may also provide information concerning other additional risk factors of incidence, independent of mtDNA haplogroup.

The main limitation of this study is that the results are not generalizable to non-White populations. It would be interesting to replicate this in racially diverse samples. In addition, the sample size of the individuals with incident knee OA is not very high. It is a very difficult task to obtain healthy subjects, with 6 years of follow-up, to evaluate the incidence or nonincidence of knee OA. Despite these issues, our results are remarkably consistent with previous epidemiologic studies in this field.

The slower telomere decay in PBL is associated with a lower risk of incidence of knee OA over time. Moreover, this slow telomere shortening is more significant in nonincident subjects carrying the mtDNA cluster JT than in those with cluster HV. The influence of the mtDNA haplogroup as a risk factor of incidence may be incorporated in the dynamics of PBL telomere shortening.

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