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mtDNA haplogroups and osteoarthritis in different geographic populations

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ABSTRACT

Objective: To compare the frequency distribution of the mtDNA haplogroups in OA patients and healthy controls between the United Kingdom (UK) and Spain.
Methods: We used the single base extension (SBE) assay to obtain the European mtDNA haplogroups in 1471 OA patients and 406 healthy controls from Spain, and 453 OA patients and 280 healthy controls from the UK. Some differential haplogroup J-related single nucleotide polymorphisms (SNPs) between both populations were analyzed. The whole data was analyzed with SPSS software (v.18) following appropriate approaches that included chi-square contingency tables and logistic regression models adjusting by gender and age.
Results: The haplogroup J appeared underrepresented in OA patients from Spain when compared with healthy controls (OR = 0.636; 95% CI: 0.444–0.911; p = 0.013). Individuals from the UK carrying the haplogroup T showed a decreased risk of OA (OR = 0.574; 95% CI: 0.350–0.939; p = 0.027). The comparison of the frequency distribution of the haplogroup J between the UK and Spain showed a decreased presence of this haplogroup in healthy controls from the UK when compared with healthy controls from Spain that is in borderline of the statistical significance (p = 0.06). The analysis of some haplogroup J-related SNPs in OA patients and healthy controls from Spain and the UK showed that the SNP m.3394t>c appeared underrepresented in the UK cohort (p = 0.038).
Conclusions: The proposed mitochondrial uncoupling mechanism derived from the mtDNA haplogroups J and T could be behind their protective role against OA. The different association found in Spain and the UK could reflect the adaptation of the mtDNA haplogroups to different climatic patterns. The genetic composition of the haplogroup J between the UK and Spain seems to be slightly different, being the m.3394t>c SNP one of the differentially expressed haplogroup J-related polymorphisms.

1. Introduction

Osteoarthritis (OA), the most common form of joint disease and cause of musculoskeletal disability in elderly people, is a disease affecting articular cartilage, bone and soft tissue leading to joint destruction and severe impairment of mobility (Felson and Zhang, 1998). It is also the main cause of work incapacity and one of the most common reasons for visiting primary physicians. OA is a multifactorial disease, influenced by both environmental and genetic risk factors (Southam et al., 2011; Valdes and Spector, 2011).

Despite the glycolytic nature of articular chondrocytes, a growing body of evidence suggests that mitochondria are involved in the pathogenesis of OA (Blanco et al., 2011; Terkeltaub et al., 2002). A significant decrease in complex II and III activity in OA chondrocytes compared with normal chondrocytes has been demonstrated (Maneiro et al., 2003), the apoptotic mitochondrial pathway is implicated as one of the major cellular pathways of apoptosis in OA chondrocytes (Kim and Blanco, 2007), the inhibition of complexes III and V of the mitochondrial respiratory chain (MRC) causes an increased inflammatory response potentially relevant to the production of reactive oxygen species (ROS) (Cillero-Pastor et al., 2008), and mitochondrial-free radical production compromises chondrocyte function (Blanco et al., 2004; Henrotin and Kurz, 2007) causing mtDNA damage and reduced mtDNA capacity for repair (Grishko et al., 2009).

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A new mitochondria-related genetic association has been described in OA, the mtDNA haplogroups. Briefly, each mtDNA haplogroup is defined as an individual group characterized by the presence of a particular set of single nucleotide polymorphisms (SNPs) in the mtDNA sequence (Torroni et al., 1996). Recent findings showed that mtDNA haplogroup J is a protective factor for knee (Rego-Perez et al., 2008) and hip OA (Rego et al., 2010) in Spanish populations, and also modulates the serum levels of type II collagen OA-related molecular biomarkers (Rego-Pérez et al., 2010) and metalloproteinases (Rego-Perez et al., 2011), as well as the nitric oxide production by chondrocytes and telomere length in peripheral blood leukocytes (Fernández-Moreno et al., 2011). The proposed mechanism relies on the different performance of the oxidative phosphorylation system (OXPHOS) among the mtDNA haplogroups, even making that some of these mtDNA haplogroups are clearly biochemically different among them (Martínez-Redondo et al., 2010; Wallace, 1999).

The presumable cause of this behavior is the role that selection has played in shaping regional mtDNA variation, being the climate one of the selective influences, so ancient mtDNA variants permitted humans to adapt to colder climates (Mishmar et al., 2003; Ruiz-Pesini et al., 2004; Wallace et al., 2003). According to this theory, the cold selection that took place during the episodic periods of cold associated with the repeated continental glaciations (Ambrose, 1998) influenced the coupling efficiency of the electron transport chain (ETC) in European mtDNAs. This resulted in a proportionately more heat production (uncoupled efficiency) to be advantageous in cold climates (Brand, 2000), at the expense of a lower ATP production, thereby reducing ROS production and apoptosis (Ruiz-Pesini et al., 2004). While this sequence could represent a risk factor for diseases caused by ATP deficiency (i.e., LHON, multiple sclerosis), it would be protective for degenerative diseases caused by oxidative stress (i.e., Parkinson, aging-associated diseases, OA?).

With all this background, and taking into account that most of the European mtDNA haplogroups were introduced into Europe from the Near East around the time of the Last Glacial Maximum (Richards et al., 1998) and the Franco-Cantabria area was considered one of the main refugia for human populations during the height of the last Ice Age, an era of great climatic uncertainty (Pala et al., 2012), the aim of this work is to analyze the frequency distribution of the European mtDNA haplogroups in two OA cohorts of populations from different geographic locations and different ancestral climatic patterns. To perform this study, we analyzed a well characterized cohort of knee OA patients and healthy controls from the UK, and compared this frequency with that of the well characterized cohort of knee and hip OA patients and healthy controls from the north of Spain.

2. Materials and methods

2.1. Patients and controls

The Spanish cohort analyzed in this study is an updated and larger cohort used in previous studies, and consisted of 1471 unrelated North Spanish patients (994 females and 477 males) from Hospital Universitario A Coruña, diagnosed as having primary knee and/or hip OA following the American College of Rheumatology (ACR) criteria (Altman et al., 1986). The radiological stage of the disease was a Kellgren and Lawrence (1957) grade II or more in all cases, with over 73% being grade IV. The average age of the cases was 69 years with an age range of 42–98 years. The donors who met the inclusion criteria for healthy controls assessed by anamnesis, clinical examination and radiographic studies included 406 individuals (244 females and 162 males), of which 200 visited the hospital for ailments unrelated to OA, and the remaining 206 consisted of macroscopically healthy knees obtained from cadavers as well as healthy hips that suffered joint fracture. The average age for the healthy controls was 66 years, with an age range of 42–95 years. In all cases, informed consent and the agreement of the ethical committee from Galician Health Administration were obtained.

The cohort from the UK has been previously described in detail (Southam et al., 2007). The cases were ascertained through the Nuffield Orthopaedic Centre in Oxford (n = 453; 275 females and 178 males). They had undergone total joint replacement of the knee for primary OA. The cases were also ascertained using the criteria of signs and symptoms of OA sufficiently severe to require joint replacement surgery. The radiological stage of the disease was a Kellgren and Lawrence (1957) grade II or more in all cases, with over 90% being grade III or IV. The average age of the cases was 73 years with an age range of 53–89 years. The control group comprised 280 individuals (96 females and 184 males) with no signs or symptoms of arthritis or joint disease. The average age of the controls at recruitment was 71 years, with an age range of 51–88 years. Similarly to the cohort from Spain, ethical approval for the study was obtained from appropriate ethics committees.

Both cohorts from Spain and the UK consisted of individuals of Caucasian origin exclusively.

2.2. mtDNA haplogroup genotyping

The mtDNA haplogroups were assigned in total isolated DNA by a well described method (Rego-Perez et al., 2008). Briefly, a multiplex PCR was performed to amplify 6 mtDNA fragments that contain each of the informative SNPs that characterize the most common European mtDNA haplogroups (H — including V and HV*, U — including the UK subtypes, J and T). The resulting PCR fragments were further purified and analyzed by single base extension (SBE) assay and the informative SNPs were visualized after loading the purified SBE product into an ABI 3130 XL genetic analyzer (Applied Biosystems). The less common haplogroups (W, I, X and other non-European variants) were assessed by means of PCR-RFLP according to the hierarchical scheme described elsewhere (Macaulay et al., 1999). All the primers and conditions used for the mtDNA haplogroup assignment are available in Rego-Perez et al. (2008).

The genotyping of the SNPs m.14798t>c (sub-haplogroup J1c), m.15257g>a (sub-haplogroup J2a) and m.3394t>c was performed by both SBE assay (m.14798t>c and m.15257g>a) and direct sequencing of the PCR product (m.3394t>c). The primers for the amplification of the fragment containing the SNP m.3394t>c were: 3192F (5′-CTT AGT ATT ATA CCC ACA CCC A-3′) and 3560R (5′-AGT AGA AGA GCG ATG GTG AG-3′), the primer 3192F being subsequently used for the sequencing of the PCR product.

The primers and conditions used for the assignment of the SNPs m.14798t>c and m.15257g>a are available in Rego-Perez et al. (2008).

2.3. Statistical analyses

Statistical analyses were performed using SPSS software, release 18 (Chicago, USA). As a first approach, the frequencies of the most common haplogroups between cases and controls for each of the two populations were compared using the chi-square test from contingency tables. Odds ratios (ORs) and their 95% confidence interval (CI) were also calculated to assess the odds of carrying each mtDNA haplogroup in OA cases compared to controls. To carry out this analysis, we followed a previously described approach, comparing each haplogroup with all other haplogroups pooled into one group (Rego-Perez et al., 2008). Similarly, the chi-square test from contingency tables was also used to compare the frequency of the most common mtDNA haplogroups and candidate differential SNPs between the two populations analyzed. For all cases, the Bonferroni correction for multiple comparisons was applied and consequently, p-values were multiplied by the number of outcomes tested in each case. As an additional statistical approach, logistic regression analyses adjusting for confounder variables age and gender were also performed to assess the incidence of the mtDNA haplogroups in both populations.