Experimental Gerontology 83 (2016) 56–62

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

TNFA gene variants related to the inflammatory status and its association with cellular aging: From the CORDIOPREV study

Oriol Alberto Rangel-Zúñiga [a,b,1], Andreea Corina [a,b,1], Beatriz Lucena-Porras [a,b], Cristina Cruz-Teno [a,b], Francisco Gómez-Delgado [a,b], Rosa Jiménez-Lucena [a,b], Juan Francisco Alcalá-Díaz [a,b], Carmen Haro-Mariscal [a,b], Elena María Yubero-Serrano [a,b], Javier Delgado-Lista [a,b], Javier López-Moreno [a,b], Fernando Rodríguez-Cantalejo [c], Antonio Camargo [a,b], Francisco José Tinahones [b,f], José María Ordovás [d,e], José López-Miranda [a,b,1], Pablo Pérez-Martínez [a,b,*,1]

[a] Lipids and Atherosclerosis Unit, IMIBIC/Reina Sofia University Hospital/University of Córdoba, Córdoba, Spain
[b] CIBER Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain
[c] Biochemical Laboratory, Reina Sofia University Hospital, Córdoba, Spain
[d] Nutrition and Genomics Laboratory, J.M.-US Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Boston, MA, USA
[e] IMDEA-Food and CNIC, Madrid, Spain
[f] Biomedical Research Laboratory, Endocrinology Department, Hospital Virgen de la Victoria, Malaga, Spain

ARTICLE INFO

Article history:
Received 8 April 2016
Received in revised form 26 July 2016
Accepted 27 July 2016
Available online 29 July 2016

Keywords:
TNFA genetic variants
Inflammation
Oxidative stress
Cellular aging
Telomere length
CORDIOPREV study

ABSTRACT

Background: Several single nucleotide polymorphisms have been proposed as potential predictors of the development of age-related diseases.
Objective: To explore whether Tumor Necrosis Factor Alpha (*TNFA*) gene variants were associated with inflammatory status, thus facilitating the rate of telomere shortening and its relation to cellular aging in a population with established cardiovascular disease from the CORDIOPREV study (NCT00924937).
Materials and methods: SNPs (rs1800629 and rs1799964) located at the *TNFA* gene were genotyped by OpenArray platform in 840 subjects with established cardiovascular disease. Relative telomere length was determined by real time PCR and plasma levels of C-reactive protein by ELISA. In a subgroup of 90 subjects, the gene expression profiles of *TNFA*, *IKKβ*, *p47phox*, *p40phox*, *p22phox* and *gp91phox* were determined by qRT-PCR.
Results: GG subjects for the SNP rs1800629 at the *TNFA* gene showed shorter relative telomere length and higher plasma levels of hs-CRP than A-allele subjects ($p < 0.05$). Consistent with these findings, the expression of pro-inflammatory (*TNFA*) and pro-oxidant (*p47phox* and the *gp91phox*) genes was higher in GG subjects than A allele subjects ($p < 0.05$).
Conclusion: Subjects carrying the GG genotype for the SNP rs1800629 at the *TNFA* gene show a greater activation of the proinflammatory status than A-allele carriers, which is related to ROS formation. These ROS could induce DNA damage especially in the telomeric sequence, by decreasing the telomere length and inducing cellular aging. This effect may also increase the risk of the development of age-related diseases.
© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Aging is associated with cellular senescence, which is characterized by irreversible cell cycle arrest and dramatic changes in cell morphology and functionality (Colavitti and Finkel, 2005; Collado et al., 2007; Correia-Melo et al., 2014). One of the main contributors to cell cycle arrest is mediated by autocrine signaling involving the secretion of growth factors, inflammatory and immune-modulatory cytokines and chemokines (Coppe et al., 2008). Among the proinflammatory cytokines, overexpression of Tumor Necrosis Factor Alpha (*TNFA*) has been implicated in a number of pathological conditions related to chronic inflammation and aging. In particular, TNFA induces prolonged growth arrest, decreased telomerase activity and telomeric disruptions (shortening, losses and fusions) (Beyne-Rauzy et al., 2004). Moreover, chronic inflammation exerts its effects through mechanisms that include excessive production of free radicals and depletion of antioxidants, thus leading to excess oxidative stress. The latter has been implicated in the pathogenesis of several age-related diseases such as diabetes and cardiovascular disease (CVD) (Ceriello and Motz, 2004; Durackova, 2010). Reactive oxygen species (ROS) are likely to be involved in both the induction and stabilization of cellular senescence and numerous reports point to links between oxidative damage and the aging process (Hamilton et al., 2001). ROS is also associated with a

* Corresponding author at: IMIBIC/Reina Sofia University Hospital/University of Cordoba, Av. Menendez Pidal s/n, 14004 Cordoba, Spain.
E-mail address: pablopermar@yahoo.es (P. Pérez-Martínez).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.exger.2016.07.015
0531-5565/© 2016 Elsevier Inc. All rights reserved.

gradual loss of DNA at the ends of chromosomes and eventual telomere dysfunction (Erusalimsky and Kurz, 2005) that contributes to cellular senescence (Khan et al., 2012). The shortening rate can be accelerated by several factors, including age (Armanios, 2013), phenotype (Masi et al., 2014), oxidative stress (von Zglinicki, 2000) and endothelial dysfunction (Gonzalez-Guardia et al., n.d.). For instance, when cells are exposed to high levels of oxidative stress, the amount of telomere attrition per cell division significantly increases (Jena, 2012). Its study is therefore crucial for understanding mechanisms associated with the development of age-related diseases (Armanios, 2013).

Both aging and age-related diseases have a significant genetic component (Johnson et al., 2015; Richardson and Schadt, 2014), and several studies have demonstrated associations between single nucleotide polymorphism (SNPs) and aging-associated diseases including CVD (Angelakopoulou et al., n.d.; Corsetti et al., n.d.; Vasto et al., 2007). Some of these studies have pointed out the significant relationship between the *TNFA* locus and CVD (Cui et al., 2012; Mellick, 2007), in particular, it has been demonstrated that two SNPs transition substitution-type rs1800629, NM_000594.3:c, −488A > G, also known as −308G > A, and rs1799964, NM_000595.3:c, −838C > T, located in the *TNFA* gene, are associated with CVD development (Chu et al., 2012; Hernandez-Diaz et al., 2015; Rodriguez-Rodriguez et al., 2011; Sandoval-Pinto et al., 2015). Moreover, the development and progression of CVD is related to cellular senescence, and proinflammatory cytokines may be the nexus for both processes. However, the mechanisms are still unclear and genetic tools may be the key to unravelling the mechanisms driving the aging process and its inter-individual differences observed in the population.

Based on this concept and the existing evidence, we explored whether genetic variants at the *TNFA* gene were associated with the inflammatory status, thus mediating telomere length and its relation to cellular aging in a population with established CVD.

2. Materials and methods

2.1. Population

The current work was conducted within the framework of the CORDIOPREV study. The CORDIOPREV study is an ongoing prospective, randomized, opened, controlled trial including 1002 patients with coronary heart disease (CHD), who had their last coronary event more than six months before enrolment. Patients were randomized in two different dietary models (Mediterranean and low-fat) over a period of five years, in addition to conventional treatment for CHD. Patients were recruited from November 2009 to February 2012, mostly at the Reina Sofia University Hospital (Cordoba, Spain), but patients from other hospital centres from the Cordoba and Jaen provinces were also included. The subjects mostly received treatment with four types of medications: antihypertensives, beta blockers, antiaggregants and hypolipemiant drugs (see Supplementary Table 1). In summary, patients were between 20 and 75 years old, with established CHD without clinical events in the last six months, had no other severe diseases or a life expectancy of less than five years, and were considered able to follow a long-term dietary intervention. Details of the trial design were provided in the Clinicaltrials.gov (NTC00924937) (https://clinicaltrials.gov/ct2/show/NCT00924937?term=%20CORDIOPREV&rank=1). This trial was designed and conducted by the authors and the protocol and all amendments were approved by the local ethics committees, all of which followed the Helsinki Declaration and good clinical practices. The current analyses were conducted on those subjects for whom we had complete information about all the variables included (i.e. clinical, anthropometric, biomarkers, genetic and telomere length). 98.9% of the subjects included in this study were white-Caucasian and 96.4% were unrelated individuals; 697 were men (83%) and 143 women (17%) (n = 840).

2.2. Laboratory measurements

Blood samples were collected from the participants after a 12-h overnight fast at the beginning of the study and once a year during the follow-up period. In the present work, we have used the information about biochemical parameters obtained at the beginning of the study (basal time. Year 0; time 0). Samples were collected in EDTA tubes (final concentration of 0.1% EDTA) and plasma was separated from the red cells by centrifugation at 1500 × *g* for 15 min at 4 °C and immediately frozen at −80 °C. The biochemical measurements were performed at the Reina Sofia University Hospital by personal who were unaware of the interventions. Lipid variables were assessed with a DDPPII Hitachi modular analyzer (Roche, Basel, CH) using specific reagents (Boehringer-Mannheim, Ingelheim am Rhein, DE). Plasma triglycerides (TG) and cholesterol concentrations were assayed by enzymatic procedures. High density lipoproteins (HDL-C) were measured following precipitation of a plasma aliquot with dextran sulphate-Mg^{2+}. Low density lipoprotein (LDL-C) concentration was calculated by the Friedewald equation, using the following formula: LDLc = CT − (HDL + TG / 5). Glucose measurements were performed by the hexokinase method. The hs-C-Reactive Protein (hs-CRP) was determined by high-sensitivity ELISA (BioCheck, Inc., Foster City, CA, USA).

2.3. DNA isolation from blood samples

Blood samples for DNA isolation were collected as described above and blood cells were obtained from buffy coat fraction. DNA isolation was carried out through the *salting-out* method (Miller et al., 1988) using 10 mL of Montreal-Baltimore buffer (0.32 M sucrose, 0.1 mM Tris HCl pH 7.5, 0.025 mM $MgCl_2$, 1% Triton X-100) and mixing and centrifuging to separate the nuclear fraction. Then, the nucleic pellet was homogenized with 3 mL of nuclei lysis buffer (10 mM Tris-HCl pH 8.2, 2 mM EDTA, 0.4 M NaCl) and 10% SDS and proteinase K. The DNA was precipitated with 6 M NaCl and washed with 100% ethanol. Finally, the genomic DNA was extracted and resuspended in 500 μL of 1 X TE buffer.

2.4. Genotyping

SNPs rs1800629 and rs1799964 at the *TNFA* gene were genotyped using the TaqMan assays, C___7514879_10 and C___7514871_10 provided by Life Technologies (*Data base: https://www.lifetechnologies.com/es/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays.html*). We performed genotyping on the OpenArray™ SNP Genotyping System (Life Technologies, Carlsbad, CA, USA) using microscope slide-sized plates and the Openarray Accufill autoloader (Life Technologies, Carlsbad, CA, USA), following the manufacturer's instructions. Four to six plates were thermally cycled simultaneously using a flat-block Geneamp PCR System 9700 thermal cycler (Life Technologies, Carlsbad, CA, USA) and subsequently read on the OpenArray™ NT Imager as an endpoint assay. The Hardy–Weinberg equilibrium (HWE) was determined using the X^2 test with 1 degree of freedom. Genotypes were called using the TaqMan Genotyper software V 1.3 (Life Technologies, Carlsbad, CA, USA), which also provided information regarding the minor allelic and genotypic frequencies. To compare our allelic frequencies, the database 1000GENOMES (http://www.1000genomes.org/1000-genomes-browsers) was used, selecting 1000GENOMES:phase_3:IBS (Iberian populations in Spain) as the reference population. Haploview v4.2 (Barrett et al., 2005) was used to estimate the Linkage Disequilibrium (LD) level among *TNFA* SNPs by analyzing the D′ and r^2 values. In addition, we analyzed genetic frequencies for SNPs rs1800629 and rs1799964 according to gender.

2.5. Quantitative PCR analysis of telomere length

Relative Telomere Length (RTL) was determined at the beginning of the study, in the basal time, using the Cawthon method by qPCR

Download English Version:

https://daneshyari.com/en/article/1906091

Download Persian Version:

https://daneshyari.com/article/1906091

Daneshyari.com