Atherosclerosis 276 (2018) 91–97

Contents lists available at ScienceDirect

Atherosclerosis

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

Prognostic value of mitochondrial DNA^{4977} deletion and mitochondrial DNA copy number in patients with stable coronary artery disease

Cecilia Vecoli*, Andrea Borghini, Silvia Pulignani, Antonella Mercuri, Stefano Turchi, Clara Carpeggiani, Eugenio Picano, Maria Grazia Andreassi**

CNR Institute of Clinical Physiology, Pisa, Italy

ARTICLE INFO

Article history:
Received 3 April 2018
Received in revised form
2 July 2018
Accepted 11 July 2018

Keywords:
Major adverse cardiac events
mtDNA^{4977} common deletion
Mitochondrial DNA copy number
Coronary artery disease

ABSTRACT

Background and aims: Mitochondrial DNA copy number (mtDNA-CN) depletion has been recently associated with an increased cardiovascular risk. However, the integrity of mtDNA is another key aspect of the energy metabolism and mitochondrial function. We investigated the prognostic role of peripheral blood common mitochondrial deletion (mtDNA^{4977}) and mtDNA-CN on long-term major adverse cardiac events (MACEs) and all-cause mortality in a cohort of patients with coronary artery disease (CAD).

Methods: Within the Italian GENOCOR (Genetic Mapping for Assessment of Cardiovascular Risk) cohort, we studied 515 patients (450 males, 65 ± 8 years) with known or suspected stable CAD. mtDNA^{4977} deletion and mtDNA-CN were assessed in peripheral blood using qRT-PCR.

Results: During a mean follow-up of 4.5 ± 1.1 years, 78 (15%) patients had MACEs (15 cardiac deaths, 17 nonfatal myocardial infarction and 46 coronary revascularizations) and 28 patients died for non-cardiac causes. Patients with high levels of mtDNA^{4977} deletion (>75th) had increased risk of MACEs (log rank = 7.2, p=0.007) and all-cause mortality (log rank = 5.7, p=0.01) compared with patients with low mtDNA^{4977} deletion (≤75th).

Multivariate Cox regression analysis showed that log mtDNA^{4977} was a significant predictor of MACEs (HR = 2.17; 95% CI, 1.31–3.59; p=0.003) and all-cause mortality (HR = 2.03; 95% CI: 1.13–3.65, p=0.02). Log mtDNA-CN was not significantly associated with MACEs or all-cause mortality. However, patients with high mtDNA^{4977} deletion (>75th) and low mtDNA-CN (<25th) had significantly increased risk for MACEs (HR: 3.73; 95% CI: 1.79–7.79; p=0.0005).

Conclusions: Mitochondria DNA damage was associated with an increased risk of MACEs and all-cause mortality in patients with stable CAD, confirming the critical role of mitochondrial dysfunction in atherosclerosis.

1. Introduction

Mitochondria play a central role in the metabolism of cardiac cells because of the high-energy demand of heart tissue. The mitochondrial DNA copy number (mtDNA-CN), representing the number of mitochondria per cell, as well as the number of mitochondrial genomes per mitochondrion, is an indirect biomarker of mitochondrial function, with less function mirrored by lower copy number associated with greater cardiovascular risk [1,2].

However, the integrity of mtDNA is another important key aspect of the energy metabolism and mitochondrial function [3].

Indeed, mtDNA mutations are associated with cardiac disorders, especially in hypertrophic-, dilated- or restricted cardiomyopathy [4,5]. Additionally, very recent experimental studies have clearly shown that mtDNA damage is present in atherosclerosis and promotes its progression [6,7]. mtDNA mutations probably promote atherogenesis through the impairment of mitochondrial function, resulting in increased reactive oxygen species (ROS) production, which in turn further damages mitochondria and promotes a vicious cycle of mitochondrial function decline and oxidative stress increase, leading to inflammation, apoptosis, and vascular cell senescence [8].

The 4977-bp mitochondrial deletion (mtDNA^{4977}), usually called

* Corresponding author. CNR-Institute of Clinical Physiology, Via Moruzzi 1, 56124, Pisa, Italy.
** Corresponding author. CNR-Institute of Clinical Physiology, Via Moruzzi 1, 56124, Pisa, Italy.
E-mail addresses: vecoli@ifc.cnr.it (C. Vecoli), andreassi@ifc.cnr.it (M.G. Andreassi).

https://doi.org/10.1016/j.atherosclerosis.2018.07.015
0021-9150/

the “common” deletion, is the first large scale deletion identified to cause human diseases [9]. This deletion occurs between two 13-bp direct repeats at positions 8470–8482 and 13447–13459 and results in complete or partial truncation of genes codifying for key subunits of respiratory chain complexes (two complex V subunits, one complex IV subunit, four complex I subunits and five intervening tRNAs), ultimately causing an increased amount of ROS [10]. The mtDNA^{4977} deletion accumulates physiologically during aging in many tissues and it has been suggested as a potential biomarker of mtDNA oxidative damage and mitochondrial dysfunction [11]. Mitochondrial copy number and mtDNA^{4977} deletion may be asymmetrically affected by physiologic or pathologic conditions, and in general, an increase of mtDNA-CN may be considered adaptive and (up to a point) beneficial [12], whereas an increase of mtDNA^{4977} deletion may be associated with various disease states [11].

To date, although undoubtedly relevant, only very few studies with a small number of patients have investigated the role of mtDNA^{4977} deletion in atherosclerotic cardiovascular disease [13–18]. The purpose of this study was to examine the prognostic value of peripheral blood mtDNA^{4977} deletion, either alone or in combination with mtDNA-CN, in predicting the risk of adverse cardiovascular outcomes in a relatively large cohort of patients with coronary artery disease (CAD).

2. Materials and methods

2.1. Study population

Within the Italian GENOCOR (Genetic Mapping for Assessment of Cardiovascular Risk) cohort, we studied 515 patients (450 males, 65 ± 8 years), who were admitted in our Institute with known or suspected stable CAD. Inclusion criteria included documentation of CAD defined as angiographically significant coronary stenosis in at least one vessel diseased (>50% lumen reduction). The severity of CAD was determined as the number of affected vessels (one-, two-, or three-vessel disease). At hospitalization, data were collected on smoking status, arterial hypertension (systolic blood pressure> 140 mmHg and/or diastolic pressure >90 mmHg or use of blood pressure–lowering drugs), hypercholesterolemia (plasma cholesterol >220 mg/dl or cholesterol-lowering drugs), obesity (body mass index>30 kg/m^2), diabetes (fasting plasma glucose > 125 mg/dl or glucose-lowering drugs) according to the guidelines [19–21], and coded in a dichotomized fashion. The left ventricular ejection fraction (LVEF) was obtained by echocardiography or left ventricular angiography. A history of previous coronary revascularization and myocardial infarction (MI) was confirmed by definitive clinical evidence in the medical record. At discharge, all patients received standard medical treatment on the basis of the current standards of care recommended by published guidelines. The study was approved by the local ethics committee (Comitato Etico Sperimentazione Farmaco - Azienda Ospedaliera Universitaria Pisana, Italy) and written informed consent was obtained from all patients. ClinicalTrials.gov Identifier is NCT01506999 (January 10, 2012).

2.2. Follow-up

After discharge, all patients were subject to a follow-up program that involved an annual telephone interview with patients or family members and validation of major advance cardiac events (MACEs) defined as coronary related death, nonfatal MI, and coronary revascularization. The cause of death was derived from medical records or death certificates provided by local health authorities. The definition of cardiac death required the documentation of either significant arrhythmias, cardiac arrest, or death attributable to congestive heart failure or MI in the absence of any other precipitating factor. The diagnosis of MI was based on the documentation of persistent electrocardiographic ST segment changes or new Q wave development, associated with cardiac specific biomarker increase. Patients were censored after the first cardiovascular adverse event during follow-up.

2.3. Analysis of amount of mtDNA-CN and mtDNA^{4977} deletion

Blood sample was collected from participants and DNA was extracted by using the QIAGEN BioRobot® EZ1 System. The levels of both mtDNA^{4977} deletion (nucleotides between 8.470 and 13.447 bp) and mtDNA-CN were determined by quantitative RT-PCR in DNA extracted from whole blood as previously described [22]. Briefly, NDI1 gene in undeleted region for the reference sequence of mtDNA as an internal control (mtNDI1), the remaining fragment after mtDNA^{4977} deletion and human β-globin gene of genomic DNA (gDNA) were amplified by PCR in both gDNA and mtDNA. The difference in the average threshold cycle (Ct) number values was used for the measurement of relative content. Specifically, ΔCT (Ct = Ct mtDNA^{4977}- Ct mtNDI1) as the difference between the Ct for the mtDNA^{4977} deletion and the Ct for the *NDI1* gene was used for calculate the levels of mtDNA^{4977} deletion respect to mtDNA-CN. The percentage of the mtDNA^{4977} deletion was calculated as $2^{-\Delta Ct}$ x 100%. In addition, ΔCt values were computed as the difference between the Ct for the β-globin gene and the Ct for the *NDI1* gene and used for the measure of mtDNA-CN relative to gDNA. mtDNA-CN was calculated by using ($2^{\Delta Ct}$) method (ΔCt = Ct mtNDI1-Ct gDNA). In order to assess qRT-PCR efficiency, a calibration curve (from 3.12 ng to 50 ng in 2–fold dilution) was included for each plate using pooled human DNA from ten healthy donors. The resulting data for the calibration curve are shown in Supplemental Table 1. Standard curve with linearity R [2]> 0.98 was accepted. For quality control, all samples were run in triplicate in a CFX 384 RT-PCR System (Bio-Rad, Hercules, CA, USA) in order to assess the intra-assay precision. To ensure consistency, samples showing a high variation (>10%) were rerun. The intra-assay coefficient of variation (CV) within triplicates were 0.7% for NDI1 gene, 0.8% for the mtDNA^{4977} deletion, and 0.4% for the β-globin gene. The inter-assay CVs for the mtDNA^{4977} and mtDNA-CN ratio were 6.4% and 3.8%, respectively.

2.4. Statistical analysis

Values are presented as mean ± standard deviation (SD), median with interquartile range (25th-75th percentile) in non-normal distribution or percent according to the nature of the data. Comparisons of normally distributed variables between groups were performed by using unpaired t-tests. Comparisons of non-normally distributed variables between groups were performed by using the Mann–Whitney *U* test as appropriate. The Spearman rank correlation was used to test the association between mtDNA^{4977} deletion, and other continuous parameters. The Kaplan-Meier survival curves and log-rank test were used to assess the effect of both high mtDNA^{4977} deletion (above the 75th percentile) and low DNA-CN (below the 25th percentile) on the survival time of patients. For the Cox proportional hazards regression models, $\log_{10}$-transformed mtDNA^{4977} and mtDNA-CN were fitted as continuous variables. The interaction between mtDNA^{4977} deletion and mtDNA-CN was tested by introducing an interaction term into the Cox regression models and the likelihood ratio test was used to assess for statistical significance. Age, gender, smoking, hypertension, hypercholesterolemia, diabetes,