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Relationship between catalase haplotype and arterial aging

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ABSTRACT

Background: Although many conventional factors have been associated with the development of arterial aging, cardiovascular diseases remain the first cause of death in old age. Therefore, identification of new risk factors may prove promising for monitoring this serious health problem. Oxidative stress and particularly catalase (*CAT*), an antioxidant enzyme, play an important role in endothelial cell pathophysiology, in shear stress response and ultimately in arterial aging.
Objective: Examine the relationships between *CAT* haplotypes and phenotypes of arterial aging (mean internal diameter, mean intima–media thickness of the common carotid arteries (CCA), presence of atheromatous plaques) in two French cohorts.
Methods and results: 564 middle-aged French individuals (mean age 53 ± 12 years) from two cohorts (ERA and STANISLAS cohorts) were included in the study. Blood pressure, CCA intima–media thickness, CCA internal diameter and number of atheromatous plaques were measured. Catalase rs769214 SNP genotyping was performed. We identified a *CAT* haplotype that influences arterial aging. Individuals carrying the *CAT2* haplotype had a higher mean internal diameter of CCA with aging and/or with an SBP ≥140 mmHg and were associated with a greater number of atheromatous plaques than *CAT1* haplotypes carriers. This *CAT2* haplotype appeared as an independent risk factor of arterial aging, similarly to previously identified factors such as age, systolic blood pressure, male, sex, tobacco use, hs-CRP, BMI and diabetes.
Conclusion: The present study highlights the roles of *CAT* haplotypes in arterial aging and underlines the beneficial impact of the *CAT1* haplotype on mean internal diameter of the CCA and atheromatous plaque number as well as on potential associated diseases.

1. Introduction

Arterial aging, characterized by arterio-atherosclerosis, involves a number of conventional risk factors [1]. Despite large scale epidemiological studies, nearly half the burden of atherosclerotic cardiovascular disease remains unexplained by these factors, although modification of risk factors (when possible) is associated with improved outcome [2].

Oxidative stress plays an important role in endothelial cell pathophysiology [3,4] as well as in various aspects of the vascular response to stretch or shear stress [5,6], and in vascular aging [7]. By converting hydrogen peroxide into water, catalase constitutes a primary antioxidant defense system and could protect cells from reactive oxygen species and its deleterious consequences on diseases [8]. In the same manner, age-associated atherosclerosis has been induced in catalase activity-deficient mice [7]. In contrast, overexpression of catalase in a murine model leads to a delayed onset of atherosclerosis [9,10], lower blood pressure (BP) [4] and an increase in life span [11].

Catalase (*CAT*) is a 33.14 Kb single copy gene located on chromosome 11p13. It encodes a 526-amino acid protein with 89% similarity with mice. Subsequent human studies have identified a haplotype of the *CAT* gene presenting different allelic frequencies according to ethnic origin [12]. This haplotype is characterized by

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three single-nucleotide polymorphisms (SNPs) [rs769214 (-844G/A), rs7943316 (-89A/T) and rs1049982 (-20T/C)] clustering an 824-bp region spanning the catalase promoter region. The rs769214A allele has been reported to create a PAX6 binding site on the *CAT* gene promoter that modifies *CAT* transcription [13].

We have previously reported that the allele A in the *CAT* gene promoter is associated with a defect in renutrition of an elderly malnourished population because of an insulin-resistant state [13,14]. In a longitudinal association study of a Japanese population, the same SNP was found to predict the risk of hypertension (HTN) progression [15]. Furthermore, an association was found between this SNP and essential HTN in Caucasian and Chinese populations, but not in African Americans [16,17]. In a larger genetic study targeting a Caucasian population, T allele carriers of the rs1049982 presented a lower risk of HTN as well as lower blood pressure values [18]. Recently, Wang et al. demonstrated that subjects bearing the [-844A, -89T, -20C] haplotype presented a higher susceptibility to essential HTN and a precocious onset for HTN when compared with those carrying the [-844G, -89A, -20T] haplotype of the *CAT* gene [19].

Our hypothesis was therefore that the *CAT* haplotype may be implicated in the pace of age-related arterial alterations.

1.1. Objective

Examine the relationships between *CAT* haplotypes and phenotypes of arterial aging (CCA internal diameter and intima–media thickness, presence of atheromatous plaques) in two cohorts of middle-aged French individuals (ERA and STANISLAS cohorts) respectively recruited in two French centres (Paris and Nancy) performing regular health check-ups.

2. Methods

2.1. Study population

In the present investigation, data obtained from 564 middle-aged French individuals are presented.

Among this population, 379 individuals were part of the ERA cohort: individuals participating in the ERA (Evolution de la Rigidité Artérielle) study were selected from a Parisian cohort that had a health check-up at the IPC (*Investigations Préventives et Cliniques*) centre, which is one of the medical centres of the French national health care system (Securité Sociale-CNAM). The details of this study have been presented previously [20]. In this cohort, 353 patients had been explored for CCA measurements and 378 for carotid plaque number. All individuals signed an informed consent form and the study protocol was approved by the institution's ethics committee (*Comité d'Ethique du Centre Hospitalier Universitaire de Cochi*n).

The remaining 185 individuals were part of the STANISLAS cohort: a family based study, of which members were free of chronic or acute disease and were selected at the Centre for Preventive Medicine of Vandoeuvre-lès-Nancy (east of France) [21]. Individuals included in this study had been explored for CCA measurements. The research protocol was approved by the "*Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale de Lorraine*" and each individual gave a written informed consent.

The mean age of the study population was 53 years with a mean age of 58 years for ERA and 42 years for STANISLAS ($p < 0.0001$) and included 30% and 50% women ($p < 0.03$) respectively.

2.2. Clinical and biological data

All individuals underwent complete medical examination including weight and height measurements. Blood samples were collected according to the manufacturer's recommendations for assays. Plasma fasting glucose, serum total cholesterol, HDL-cholesterol, triglyceride and CRP high-sensitive concentrations were measured using commercially available kits (Merck, Darmstadt, Germany) on an AU5021 apparatus (Olympus, Merck).

2.3. BP measurements

In the two cohorts, BP was measured with a manual sphygmomanometer under standardized conditions (room temperature between 19 °C and 21 °C and the individual in supine position). The values reported for systolic blood pressure (SBP) and diastolic blood pressure (DBP) are the means of three readings at each examination. Pulse pressure was calculated as the difference between DBP and SBP.

2.4. Common carotid artery intima–media thickness (IMT) and internal diameter measurements

Measurements of CCA internal diameter and IMT in ERA cohort were obtained by using an ultrasound imager (Aloka SSD-650), with a transducer frequency of 7.5 MHz. Acquisition, processing and storage of B-mode images were computer-assisted with specifically-designed software as previously described (M'ATHS, Metris, France) [22]. For IMT and internal diameter measurements, far and near walls of the right and left CCAs, 2–3 cm proximal to bifurcation, were imaged. For each side, at least two optimal longitudinal images were captured and stored for off-line analysis. The IMT was measured at a site free of any discrete plaques along a 1 cm-long segment of the far wall of the CCA and measured as the distance between the lumen–intima interface and the media–adventitia interface using an automated edge detection algorithm. A mean of 50 measurements were automatically performed on each image (two images per side) and on each side (left and right). The mean of the right and left CCA internal diameter and IMT measurements was used in the analysis.

CCA internal diameter and IMT measurements in the STANISLAS cohort were obtained by using a B-mode ultrasound imager (HITACHI EUB 565, lotec software system (Data Processing Co, Paris, France)). The right and left CCA were examined with a 7.5 MHz probe, according to a protocol already described [23]. Briefly, measurements were taken at 3 cm proximal to the CCA bifurcations in anteroposterior projection and on a segment of at least 1 cm of longitudinal length free from any atheromatous plaque. The average IMT obtained from the selected area represented the mean of at least 50 successive measurements. The CCA internal diameter was calculated automatically along the same length as the IMT by averaging the distance between near and far lumen–intima interfaces. For each subject, two IMT and internal diameter measurements were obtained on each arterial segment. The average of right and left measurements was used to calculate the IMT and the internal diameter.

Mean CCA IMT and internal diameter were respectively 0.73 ± 0.11 mm and 5.82 ± 0.69 mm for the ERA population and 0.62 ± 0.05 mm and 5.37 ± 0.67 mm for the STANISLAS cohort. These parameters were significantly different between the two cohorts ($p < 0.0001$ and $p < 0.005$, respectively). However, this difference disappeared after adjustment for age and gender.

2.5. Atheromatous plaque measurements in the ERA cohort

Measurements of atheromatous plaques in the ERA cohort involved scanning of the common carotid arteries, the carotid bifurcations, and the origin (first 2 cm) of the internal carotid arteries. At the time of the examination, the near and far walls of these