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# Detection of mtDNA with 4977 bp deletion in blood cells and atherosclerotic lesions of patients with coronary artery disease

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**Abstract**

Recent evidence suggests that somatic mutations in nuclear and mitochondrial DNA accumulated during aging, may significantly contribute to the pathogenesis of chronic-degenerative illness such as coronary artery disease (CAD). Mitochondrial DNA with 4977 bp deletion mutation (mtDNA$^{4977}$) is a common type of mtDNA alteration in humans. However, little attempt has been made to detect the presence of mtDNA$^{4977}$ deletion in cells and tissues of cardiovascular patients.

This study investigated the presence of mtDNA$^{4977}$ in blood samples of 65 cardiovascular patients and 23 atherosclerotic plaques of human coronaries with severe atherosclerosis. Moreover, the presence of the deletion has been investigated in blood cells from 22 healthy age-matched subjects.

The detection of mtDNA$^{4977}$ has been performed by using a nested polymerase chain reaction (PCR) protocol and normalized to wild-type mtDNA.

A significant higher incidence of mtDNA$^{4977}$ was observed in CAD patients with respect to healthy subjects (26.2% versus 4.5%; $P = 0.03$). Furthermore, the relative amount of the deletion was significantly higher in the patients compared to the control group ($P = 0.02$). The mtDNA$^{4977}$ was detected in 17 of the 65 patients blood samples (26.2%) and deletion levels ranged from 0.18 to 0.46% of the total mtDNA (mean: $0.34 \pm 0.02$%). For what concerns atherosclerotic lesions, 5 patients (21.7%) showed the deletion ranging from 0.13 to 0.45% of the total mtDNA (mean: $0.35 \pm 0.06$%). In both samples from patients, the incidence and the relative amount of mtDNA$^{4977}$ was not significantly influenced by atherogenic risk factors and clinical parameters.

The obtained results may suggest that the increase of oxidative stress in cardiovascular disease may be responsible for the accumulation of mtDNA damage in coronary artery disease patients.


*Keywords:* mtDNA; Blood cells; Atherosclerotic lesions; Coronary artery disease

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## 1. Introduction

Genotoxic damage is a common phenomenon occurring in several chronic-degenerative illness including age-related pathologies such as cancer, cardiovascular diseases, and neurodegenerative diseases [1–4]. In particular, several recent studies have suggested that human atherosclerosis is a condition associated with somatic instability of nuclear DNA, underlining the similarity between atherosclerotic and carcinogenic processes [1,4–6]. On the other hand, it has become increasingly clear in recent years, that also aging-related accumulation of somatic damage to mitochondrial DNA (mtDNA) is a potential contributor to human diseases, such as cardiomyopathies or more common forms of cardiovascular disease [7–10]. This is particularly relevant in the pathogenesis of ischemic heart disease, a condition characterized by an increase of oxidative stress [7,11]. It is well known, in fact, that the mitochondrial genome is more susceptible to increased oxidative damage than nuclear DNA since it is not protected by histones and it only reveals limited capability for DNA repair [12].

Various types of somatic mtDNA mutations have been reported. A specific deletion of 4977 bp, called "common deletion" ($mtDNA^{4977}$), is detected at high frequency in various tissues from aging humans as well as in ischemic myocardium [13,14]. This deletion is generated between a pair of 13-bp direct repeats, located at nucleotide positions 8470–8482 and 13 447–13 459 of mtDNA, respectively [15]. Recently, a significant accumulation of $mtDNA^{4977}$ has been demonstrated in the atrial tissue of patients with clinical atrial fibrillation [16,17].

However, little attempt has been made to detect the presence of this deletion in both cells or tissues of patients with ischemic heart disease.

Moreover, recent findings have also pointed out that some cardiovascular risk factors may cause mtDNA damage in cardiovascular tissues in mouse models from hypercholesterolemia and second-hand smoke exposure [18].

To the best of our knowledge, only two studies have documented the presence of $mtDNA^{4977}$ in cardiac muscle from patients suffering from coronary atherosclerotic heart disease [19,20].

In the present study, we examined the presence of 4977 bp deletion of mtDNA in blood cells and atherosclerotic lesions from patients with proven angiographically coronary artery disease (CAD). In addition, we tested the hypothesis that the $mtDNA^{4977}$ may be correlated to the presence of common atherogenic risk factors.

## 2. Materials and methods

### 2.1. Study populations

We studied 65 unrelated adult patients consecutively admitted to the Clinical Cardiology Department of our institute (CNR, Institute of Clinical Physiology, G. Pasquinucci Hospital). All patients underwent coronary angiography. Coronary stenosis was considered to be significant if there was the presence of a luminal diameter narrowing of >50% in at least one epicardial coronary artery. The severity of CAD was expressed simply by the number of affected vessels (one-vessel, two-vessel or three-vessel disease). At the time of sampling, a complete history, including cardiovascular risk factors such as smoking habits, hypertension, diabetes, dyslipidemia, was collected for all subjects. Hypertension was defined as blood pressure >140/90 mmHg; patients using antihypertensive medication were also classified as hypertensive. Subjects with a history of diabetes, or those receiving any antidiabetic medication, were considered to be diabetic; subjects were deemed dyslipidemic when their total cholesterol concentration was $\geq$220 mg/dL or their triglyceride concentration was $\geq$200 mg/dL, or they were receiving lipid-lowering drugs. Smoking history was coded by grouping patients into those who were non-smokers and had never smoked, and those who were current smokers. Patients who underwent recent radiological procedures (<1 month) have been excluded from the present study. Informed consent was obtained from all the patients according to our ethical committee.

In addition, 23 atherosclerotic plaques of human coronaries with severe atherosclerosis were obtained from patients undergoing coronary endarterectomy. Tissues were frozen in liquid nitrogen and stored at −80 °C. The tunica media, mainly composed of smooth muscle cells, was used for total DNA extraction. The tissues were homogenized and underwent proteinase K digestion for 5 h at 55 °C, followed by two extractions with a phenol/water/chloroform mixture and two