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Association of human mitochondrial DNA variants with plasma LDL levels

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Abstract

Increasing evidence supports the relationship between mitochondrial DNA variability and differences in energy metabolism, which may have pathophenotypic consequences. MtDNA pathological mutation has been also described to be associated with hypercholesterolemia. The target of this work consisted in studying the possible existence of an association between the mitochondrial DNA variability and plasma cholesterol levels. For this, two populations of 61 sedentary and 83 sportsmen were used to estimate the association of the lipidemic levels with the mitochondrial DNA variant harboured by them. Triglycerides, HDL-c, LDL-c and cholesterol/HDL-c were essayed, and mitochondrial DNA polymorphisms were assessed by HVR I sequencing and PCR/RFLP analysis. Major Caucasian mtDNA clades (HV, JT, U and IWX) did not associate with lipidemic levels in the sedentary population. However, in the case of a more disciplined population in term of nutritional habits and life style as sportsmen are, a significantly higher and lower level of LDL-c was associated with HV and JT clade, respectively. This observation could have relevant significance for metabolic distress diseases affecting plasma cholesterol levels.

Keywords: Mitochondrial DNA; LDL; HDL; Cholesterol; Haplogroups; Variability

1. Introduction

It is well known the frequent appearance of nucleotide polymorphisms in human mitochondrial DNA (mtDNA). These single nucleotide polymorphisms (SNPs) are present in coding or non-coding (hypervariable I and II) region and define a large human mitochondrial variability. These SNPs are phylogenetically linked defining major genetic lineages (clades) each one integrating different haplogroups (Wallace, 2005; Macaulay et al., 1999; Finnila et al., 2001). This mitochondrial variability can be assessed by high resolution analysis of the whole mitochondrial genome using Restriction Fragment Length Polymorphisms (RFLPs), or sequencing the hypervariable control region I (HVR I). Thus, in Europe, nine major haplogroups have been identified: H, I, J, T, U (including subhaplogroup K), V, W and X, and these haplogroups have been further subsumed within four mtDNA clades: HV, U, TJ and WIX. (Torroni et al., 2000; Richards et al., 2000).

The first association between the mitochondrial genetic background and a disease, was in the Leber's Hereditary Optic Neuropathy (LHON) (for review see Wong, 2007). Mitochondrial haplogroup J was overrepresented in LHON patients and it was postulated that this haplogroup increased the penetrance of the pathological mutation. Since then, mtDNA haplogroups have been associated with decreased sperm motility (Ruiz-Pesini et al., 2000; Montiel-Sosa

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et al., 2005), with aging-related diseases, such as Parkinson (Van der Walt et al., 2003; Ghezzi et al., 2005; Pyle et al., 2005) and Alzheimer disease (Chagnon et al., 1999; van der Walt et al., 2004), with metabolic syndrome, type 2 diabetes, myocardial infarction and atherothrombotic cerebral infarction (Tanaka et al., 2000; Fuku et al., 2007; Nishigaki et al., 2007a, 2007b), as other diseases and phenotypes.

The mitochondrial variability has been proposed as responsible for individual changes in electron transport chain activity and coupling (Ruiz-Pesini et al., 2004). The main function of the mitochondria is to produce ATP by OXPHOS, and while the uncoupling of OXPHOS generates heat, it concomitantly reduces the production of ATP due to proton leak (Kadenbach, 2003). Uncoupling of OXPHOS also lowers the production of reactive species ROS, which are its obligatory by-products.

On the other hand, some studies have emphasized the role that mitochondria could play in the vascular changes occurring in atherogenesis (Ballinger et al., 2002). In fact, mitochondria are both important sources and targets for ROS (Esposito et al., 1999; Wallace, 1992), and there are evidences that risk factors for coronary artery diseases are associated with increased levels of ROS (Alexander, 1998; Ballinger et al., 2002). Mitochondria also contribute to signal transduction pathways, and their function in cells with relatively low energy demands, such as the endothelium, have received growing attention (Puddu et al., 2005; Ramachandran et al., 2002).

The first genetic report on the association of a mtDNA polymorphism with serum lipid levels was described in the Japanese population (Kokaze et al., 2001). After adjustments for age and body mass index, the high-density lipoprotein cholesterol concentration in males carrying an mtDNA 5178A was higher than that in males carrying mtDNA 5178C. However, the triglyceride concentration in females carrying mtDNA 5178A was lower than that in females carrying mtDNA 5178C. Though many genetic polymorphisms are known to correlate with cardiovascular diseases (Fortunato and Di Taranto, 2007), no results have been reported on the possible association of Caucasian mtDNA variability and plasma lipid levels. This work was undertaken to approach the study of the correlation of lipidemia with Caucasian mitochondrial DNA variants. We found no differences in the sedentary population but interestingly, in the case of a population disciplined in nutritional habits and life style as sportsmen, LDL cholesterol (LDL-c) was significantly higher and lower in subject harbouring HV and JT mtDNA variant, respectively.

2. Materials and methods

2.1. Subjects

A total of 83 sportsmen and 61 sedentary men from Zaragoza participated in this study. The mean of age was 27.2 ± 0.5 years. The subjects were fully informed of the aims of the experiments before giving their informed written consent to participate. The study conforms to the code of Ethics of the World Medical Association (declaration of Helsinki) and was approved by the Ethics Committee of University of Zaragoza (Approval No. PI 04/13).

Fresh blood and serum samples were obtained at the same hour of the day (09:00–12:00 h) under similar environmental condition. Sedentary subjects were voluntary healthy persons that they do not practice any physical activity for longer than 30 min per day. Sportsmen group included long distance runners ($n = 8$), cyclists ($n = 16$), soccer referees ($n = 44$), volleyball, basketball, handball, water polo and soccer players ($n = 15$). All of them have national or international sport level.

2.2. Lipoproteins determination of subjects

Total cholesterol, HDL cholesterol (HDL-c) and triglycerides were determined from serum samples by enzymatic methods with the use of Sigma kit Infinity cholesterol reagent (n. 401), Sigma kit Infinity triglycerides reagent (n. 343), and Boehringer Mannheim kit HDL-c precipitant reagent (n. 543 004). LDL-c was calculated using the equation of Friedewald et al. (1972).

2.3. DNA extraction and molecular analysis

Total DNA was extracted from blood samples EDTA-tubes, by conventional methods using phenol/chloroform. Mitochondrial DNA (mtDNA) was haplogrouped by PCR amplification of short mtDNA fragments, followed by RFLPs analysis. We used the haplogrouping strategy as previously described (Torroni et al., 1996) with some modifications (Ruiz-Pesini et al., 2000). When RFLPs analysis failed to show clear results, haplogroups were confirmed determining polymorphisms of HVS-I sequence of mtDNA (Richards et al., 2000). Samples with incomplete or uninterpretable results, or without DNA available, were excluded. Full description of the oligodeoxynucleotides utilized and the PCR amplification conditions are available upon request.

2.4. Genetic analysis

mtDNA clades were determined as already described for European mtDNA branch of haplogroups which present four main Caucasian clusters (HV, JT, U and IWX), according to Macaulay and Richards (Macaulay et al., 1999; Richards et al., 2000, 2002) (see Supplementary information). Individuals harbouring haplogroups L (African) and M (Asian) and those that we could not ascribe to any of the known Caucasian haplogroups, were grouped in haplogroup O as it has been previously reported (De Benedictis et al., 1999; Ruiz-Pesini et al., 2000).

2.5. Statistical analyses

The distribution of the diverse mitochondrial variants among populations was assessed by the chi-square χ^2 inde-