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Molecular evaluation of victims of sudden death: a promising approach for excluding criminal responsibility

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Abstract. Sudden death constitutes one of the most frequent causes of death in adults. In order to look for genetic markers that could play a role in the sudden death in adults (SDA), the common deletions of 4977 bp in the mitochondrial DNA and variations in the apoB gene were studied. The correlation of mtDNA common deletion with SDA victims might offer a rapid and simple tool to provide additional information in complex SDA investigations

Keywords: Mitochondrial DNA; Sudden death; 4977-bp deletion; ApoB gene

1. Introduction

One of the most frequent causes of death is the so-called sudden death in adults (SDA). The victims die by no apparent causes and without clinical information that might establish its cause. Data for some countries show approximately 88% SDA are of cardiac origin. An epidemiological study on coronary heart disease (CHD) has indicated that mother's age at death was more significant predictor of age at sudden CHD death than father's age [1]. This suggests that the maternally transmitted mitochondrial genotype may influence the susceptibility to adult-onset disease. In contrast to the nuclear DNA, mitochondrial DNA (mtDNA) have rendered 10–20 times more vulnerable to mutation and oxidative damage. MtDNA mutations have been proposed to contribute to the aging process. The occurrence in human tissues, with age-associated manner of a particular large mtDNA 4977-bp deletion (mtDNA4977-deletion) was first reported in 1990 [2]. This deletion accumulates preferentially in post-mitotic tissue such as muscle and brain [3]. Previous studies showed high levels of mtDNA4977-deletion in cardiac muscle from patients suffering from CHD. Recently, it was hypothesized that mtDNA damage is a common mechanism by which risk factors such as diabetes mellitus, hypertension, hypercholesterolemia and age contribute to atherogenesis [4]. ApoB is the main lipid transport protein in the blood. Several studies have shown that

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genetic variation at the apoB gene locus is associated with altered circulating cholesterol, apoB levels and risk of CHD [5]. Until now, the association studies between apoB gene polymorphism and risk of CHD have reported contradictory results among populations.

In order to evaluate some mutations and polymorphisms potentially linked with SDA, the common mtDNA4977-deletion, the nuclear mutation Arg3500Gln/apoB and the polymorphism *Msp*I/apoB in codon 3611 were selected to analyze cardiac muscle samples from SDA and controls.

2. Materials and methods

2.1. Samples

Genomic DNA was isolated from a set of 13 cardiac muscle tissue from victims of SDA (18–70 years), and 9 muscle tissue sample from corpses not affected by SDA (22–71 years).

The extracted DNA was quantified by spectrophotometric analysis.

2.2. 4977-bp 'common' deletion

The dilution-PCR method was used to estimate the proportion of the total mtDNA harboring the mtDNA4977-deletion. DNA was prior digested with *Pst*I and *Eco*RI to avoid amplification of wild-type products. Serial dilutions of the digested were performed for the amplification of D-loop and mtDNA 4977-bp deletion. D-loop: primers 5′ caccattagcacccaaagct3′ (forward) and 5′ tgatttcacggaggatggtg3′ (reverse) amplify a 440-bp fragment and were used to quantify the total mtDNA. In order to detect mtDNA 4977-bp deletion, the 5′ ctcagagcccactgtaaagc3′ (forward) and 5′ cttgtcagggaggtagcgat3′ (reverse) primers amplify a 389-bp fragment. The PCR products were separated on 2% agarose gels and detected using a Foto/Analyst® and quantified using an Image Quant 5.1 software.

With all samples, the decline in optical density (OD) of the PCR products for each dilution series versus the amount of mtDNA fitted a semi-logarithmic plot according to a regression line, $r^2>0.9$. The percent of deleted mtDNA with respect to total mtDNA was determined by the ratio of the DNA dilutions that reduced the PCR product OD of the deleted mtDNA and total mtDNA curves to the same level. All PCR products were sequenced by the Big Dye terminator System.

2.3. Arg3500Gln/apoB and polymorphism MspI/apoB

This method is based on the selective creation of an artificial *Msp*I restriction site in the wild-type allele, but not in the mutant allele, to observe the Arg3500Gln. In the same fragment, there is a polymorphism *Msp*I/apoB (RFLP) in codon 3611showing the allele M− or M+. PCR was carried using the forward 5′ cttacttgaattccaagagcaccc3′ and reverse 5′ tggtaggatgatatttttgaggaa3′ primers.

The PCR product was digested with *Msp*I and were separated in a high resolution gel, GeneAmp™ Detection Gel and silver stain detection.

3. Results and discussion

MtDNA4977-deletion percent (%) (mean ± S.D.) was higher in victims of SDA (0.00488 ± 0.0011) than those not affected by SDA (0.00223 ± 0.0037) ($P<0.05$).

Moreover, when SDA victims were divided into two subgroups according to age distribution, no differences were found in mtDNA4977-deletion percent between victims < 39 and >51 years. By sequence analysis, we confirmed in all samples affected that a single breakage point was detected between positions 8482 and 13460, which defines the deletion of the 4977-bp fragment.

Regarding ApoB gene, none of the samples tested showed the Arg3500Gln/apoB mutation. Detection of *Msp*I/apoB polymorphisms shows that 3 out of 13 SDA and 2 out of 9 not SDA presented the rare allele M − .

This preliminary investigation was mainly focused to search an association between SDA and oxidative damage, evidenced by an increase of mtDNA4977-deletion.

Our results indicate that samples from SDA have a significant percent increase of mtDNA4977-deletion in comparison to samples from victims not affected by SDA. Mitochondrial disease can be due to either a homoplasmic or a heteroplasmic mutation. In the case of a heteroplasmic mutation, the disease state develops only after a threshold level of mutation has been achieved. When the percent-mutated DNA far exceeds the threshold level, then an acute (and sometimes lethal) condition will be observed [3]. These results are probably related to the high incidence of CHD among SDA; in turn, cardiac ischemia is associated with high oxidative stress.

In this study, no differences were found in the percent of mtDNA4977-deletion between samples from young and old victims of SDA. The fact that samples from young victims showed high level of such deletion would indicate that this deletion may be associated with SDA, irrespective of age. On the other hand, it was considered necessary to study other genetic variations related to CHD. Herein we proposed some apoB gene variation such as Arg3500Gln and *Msp*I polymorphism. As expected, applying both mutation and polymorphisms of ApoB gene to a small sample tested did not correlate neither with SDA victims tissues nor with control samples. In order to correlate genetic variations with sudden death as an association study, a bigger sample would be required. The correlation of mtDNA common deletion with SDA victims might offer a rapid and simple tool to provide additional information in complex SDA investigations. Although the cause of sudden death may be probably due to multiple genetic effect, energy reduction led by anomalous (faster) mutated mtDNA replication may play a remarkable role in the SDA.

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