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RESEARCH ARTICLE

PARAOXONASE 3 GENE ALA99ALA POLYMORPHISM DISTRIBUTION IN BENINESE THREE ETHNIC POPULATIONS

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ABSTRACT

The paraoxonase has anti-atherogenic activity which may be altered by the coding gene polymorphisms. The purpose of this study was to assess the genotypes distribution and allelic frequencies in paraoxonase 3 gene Ala99Ala polymorphism in Beninese different ethnic groups. The paraoxonase 3 gene Ala99Ala polymorphism distribution of 228 subjects from three different populations, was studied respectively using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism technique and compared with those in other word populations. The alleles A and G frequencies were 78.0 percent and 22.0 percent respectively of our study population. The allelic frequencies were 98.0 percent and 2.0 percent respectively of Abomey Calavi population; 67.0 percent and 33.0 percent respectively of *Adja* ethnic group; 66.0 percent and 34.0 percent respectively of *Mahi* ethnic group. There were no significant ethnic differences for these allelic frequencies between *Adja* and *Mahi*ethnic groups ($p > 0.05$), but there were significant differences compared to Beninese Abomey-Calavi population and other word populations ($p < 0.05$).These ethnic variations in PON3 gene polymorphisms distribution can be used as an excellent genetic marker and as the basis for further investigation on the association of this polymorphism with the risk of inflammatory diseases.

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INTRODUCTION

The paraoxonases (PON) enzymes are associated with many inflammatory diseases, such as cardiovascular diseases, by protecting the cells again oxidative stress (Aviram and Rosenblat, 2004). They are associated with high-density lipoprotein (HDL), hydrolyzed lactones and inhibited the oxidation of low-density lipoprotein (LDL), a function that is believed to slow the initiation and progression of atherosclerosis (Seres *et al*, 2004 and Zhang *et al*, 2010). The paraoxonase gene has three isoforms, PON1, PON2 and PON3 (Draganov *et al*, 2005) located on chromosome 7q21.3–22.1 and covers approximately 136kb (Primo-Parmo *et al*, 1996).

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Various populations' studies have reported inter-ethnic differences in the allele frequencies for PON gene polymorphisms (Campo *et al*, 2004 and Wu *et al*, 2010). This variability suggests that ethnic differences, gene-gene interactions and susceptibility to environmental factors might modulate the relationship between PON polymorphisms and many inflammatory diseases (Wang *et al*, 2003). Many studies have been conducted on the relation between PON polymorphisms and genetic susceptibility to coronary heart disease (Erlich *et al*, 2006 and Zhang *et al*, 2013). The paraoxonase 3 (PON3) have a similar role to PON1 (Sanghera *et al*, 2008). The expression of PON3 gene occurs mainly in the liver and bind to HDL in blood stream (Reddy *et al*, 2001). The PON3 gene has five commons Single Nucleotide Polymorphisms (SNPs) Ala99Ala, Asp107Asn, Glu146Lys, Ala179Asp and Tyr233Cys (Robertson *et al*, 2003; Li *et al*, 2004; Pasdar *et al*, 2006 and Sanghera *et al*, 2008). Despite the

importance of the paraoxonase gene and its implications on the genetic susceptibility to coronary heart disease, no studies on African black populations have been reported. The purpose of this study was to assess the PON3 gene Ala99Ala polymorphism distribution in Beninese populations for further investigation of the association of this polymorphism with the risk of inflammatory diseases.

MATERIALS AND METHODS

Subjects

This study has been conducted on a total of 228 unrelated volunteers 70 men and 158 women, aged between 6 and 72 years old, were randomly selected from Beninese three different populations living in the south and central areas of the country. The characteristics of the study populations were shown on Table 1.

DNA extraction and genotyping

Venous blood samples were collected from each volunteer after written consent. Genomic DNA was extracted by phenol-chloroform method at Genetics and Biotechnologies Laboratory (GBL) at Abomey Calavi University. The polymerase chain reaction (PCR) primers sequences for the PON 3 Ala99Ala SNP (rs1053275) were designed as previously described by Wu *et al* (2010) and synthesized by SANGON Biotech (Shanghai, China). The sequences were: forward 5'-TCCAGGCATGCCAAACTTT-3' and reverse 5'-TTTCCCTCATTTCCCCCTT-3' were used to amplify 197 bp fragment containing the polymorphism site Ala99Ala on PON3 gene. PCR was performed using thermo cycler PTC 100™ (*Programmable Thermal Controller*; Perkin Elmer) in a final volume of 25 µL as follows: 3 µL (3 ng) DNA sample was added to a reaction mixture containing 2.5 µL of 10×PCR buffer, 1.5 µL of 25 mmol/L MgCl2, 1µL of 200 µmol/L dNTPs, 1U Taq DNA polymerase (1 µL) (Fermentas) and 2 µL of 0.2 µmol/L of each primer, dimethyl sulfoxide (DMSO) was added to a final concentration of 5% and ultra-pure water (Merck) to a final volume of 25 µL. The fragment amplification was performed under the following conditions: 10 min pre-denaturation at 94 °C followed by 30 cycles of 45 sec denaturation at 94 °C, 45 sec hybridization at 50 °C and 45 sec extension at 72 °C; and finished by 7 min extension at 72 °C. Then 10 µL of PCR products was digested with 2 µL of 10xNEB buffer, 2 µL of HhaI endonuclease (Promega) and ultra-pure water to a final volume of 20 µL. Tubes were incubated at 37°C for 4h before separation on a 2 percentaga rose gel and visualization by staining in ethidium bromide under UV trans-illumination.

Digestion recognition sequences were GCGC, thus the G allele version would be digested by the enzyme HhaI. The expected results for this polymorphism were the electrophoretic profile with 112 bp, 63 bp and 22 bp bands corresponded to the homozygote genotype G/G; 175 bp and 22 bp bands to the homozygote genotype A/A; and 175 bp, 112 bp, 63 bp and 22 bp bands to the heterozygote genotype G/A. The 63 bpand 22 bp bands were not visible on the agarose gel.

Statistical analysis

Alleles and genotypes frequencies were calculated by gene counting. The chi-square test on IBM SPSS 21 Statistics software was used both to estimate the Hardy-Weinberg equilibrium and to compare the allelic frequencies observed in Benin population with those reported in other word populations. Value of $p < 0.05$ was considered statistically significant.

RESULTSAND DISCUSSION

PON3 gene Ala99Ala polymorphism identification

The PON 3 gene Ala99Ala polymorphism (rs1053275) is a G to A substitution resulting a synonymous change, Ala (GCG) to Ala (GCA). Two-hundred-twenty-eight (228) samples from Beninese three different populations were used to amplify a 197 bp fragment, digested with HhaI. The figure 1 represents the electrophoretic profile of the different genotypes. The PCR products 197 bp fragment were represented on the gel by SG band. On the gel, the individuals A9, A12, M1 and M7 were genotyped G/A (with both fragment of 175 bp and 112 bp); the individuals A1, A3 and M2 were genotyped A/A (with only one fragment of 175 bp) and the individuals A19 and M22 were genotyped G/G (with only one fragment of 112 bp).

PON3 gene Ala99Ala polymorphism genotypes distribution and alleles frequencies

The genotypic and allelic frequencies of PON3 Ala99Ala polymorphism in Beninese ethnic populations were shown in Table 2.Two alleles and three genotypes were observed in Beninese different ethnic groups. But the homozygote individual G/G was not found in *Abomey-Calavi* population. The observed and expected frequencies for the polymorphisms have met Hardy-Weinberg equilibrium ($p > 0.05$). The comparison of PON3 Ala99Ala allelic frequencies within Beninese different ethnic groups was shown in Table 3. There was no significant ethnic difference between *Adja* and *Mah*i for PON3 gene Ala99Ala polymorphism allelic frequencies ($p > 0.05$). But significant differences were observed for PON3 Ala99Ala polymorphism allelic frequencies in these tow ethnic groups compared to Beninese *Abomey-Calavi* population respectively ($p < 0.05$). The comparison of PON3 gene Ala99Ala allelic frequencies in Beninese ethnic populations with those in other word populations was shown on Table 4.There were significant differences in PON3gene Ala99Ala polymorphism allelic frequencies in Beninese ethnic populations compared to Chinese *Li* minority and British *Caucasians*.

DISCUSSION

The present study has determined the genotypes distribution and the allelic frequencies of PON3 gene Ala99Ala polymorphism in Beninese ethnic populations. Two alleles and three genotypes were observed. These genotypic and allelic frequencies of PON3 gene Ala99Ala polymorphism in Beninese ethnic populations were compared with previously described frequencies in others word populations (Chinese and British). Inter-ethnic variations in PON3 gene Ala99Ala polymorphisms were observed in Beninese population.

Table 1. The characteristics of the study population

Ethnic groups	numbers	sex		Age	City/ area in Benin	Study periods
		M	F			
*AC population	84	24	60	22 -71	*AC /central south	Dec 2012
Adja	50	13	37	17-72	Lokossa/southern-west	June 2015
Mahi	94	33	61	6 - 70	Savalou/central	Nov. 2015
Total	228	70	158	6 - 72	South and central	

* Abbreviation of Abomey-Calavi

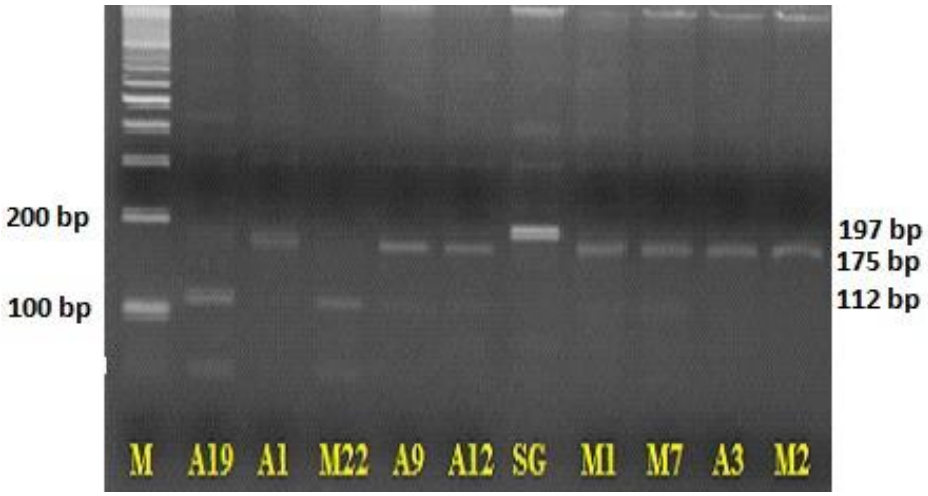

Figure 1. Electrophoretic profile of the different genotypes

Table 2. Genotypic and allelic frequencies

Ethnic groups	n	Genotypes			Alleles	
		A/A	A/G	G/G	A	G
ACpopulation	84	95.0	5.0	0.0*	98.0	2.0
Adja	50	44.0	46.0	10.0	67.0	33.0
Mahi	94	51.0	30.0	19.0	66.0	34.0
Total	228	66.0	24.0	10.0	78.0	22.0

*G/G genotype was not found in Abomey-Calavi population

Table 3. Comparison of PON3 Ala99Ala allelic frequencies within Beninese different ethnic populations

Frequencies (%)	*Adja*	*Mahi*	*AC* p*	*Adja* vs *Mahi*	*AC* p*vs *Mahi*	*AC* p* vs *Adja*
				pvalue	*pvalue*	*pvalue*
Allele	33.0	34.0	2.0	$p > 0.05$	$p < 0.05$	$p < 0.05$

* Abbreviation of *Abomey-Calavi*population

Table 4. Comparison of PON3 A99A allelic frequencies in Beninese ethnic populations with those in Chinese minority *Li* and British *Caucasians*

Ethnic groups	n	Allelic frequencies (%)		p *values*
		A	G	
ACpopulation	84	98.0	2.0	$p < 0.05$
Beninese *Adja*	50	67.0	33.0	$p > 0.05$*
Beninese *Mahi*	94	66.0	34.0	$p > 0.05$*
Beninese population	228	78.0	22.0	$p < 0.05$
Chinese *Li* [a]minority	150	75.0	25.0	$p < 0.05$**
British *Caucasians*[b]	450	52.0	48.0	$p < 0.05$**

*Significant ethnic differences were observed when compared to Beninese Abomey-Calavi population;
** Significant differences were observed when compared to Chinese and British;
[a] Wu *et al*. 2010 ; [b]Pasdar *et al*. 2006

There were significant differences in PON3 gene Ala99Ala polymorphism genotype distribution and allelic frequencies in Beninese *Adja* and *Mahi* ethnic groups compared to Beninese *Abomey Calavi* population respectively (Segbo *et al*, 2014). But no significant ethnic differences in PON3 gene Ala99Ala polymorphism genotypes distribution between *Adja* and *Mah*i ethnic groups were observed. Also the homozygote individual G/G was found in *Adja* and *Mahi* ethnic groups but not in *Abomey Calavi* population. These inter-ethnic variations in PON3 gene polymorphisms distribution may be explained by the fact that Beninese *Fon* is the major and the most heterogeneous ethnic group living in the south-central area (Abomey-Calavi city), the denser area in the country; while *Adja* and *Mahi* ethnic groups were considered as homogenous population within which the genetic traits transmission was much conservative. So the distribution of PON3 gene Ala99Ala polymorphism in the Beninese *Adja* and *Mahi*ethnic groups were much conservative and this polymorphism site may be used as an excellent genetic marker for DNA analysis in Benin. Our results were contrasted with those observed in British Caucasians (Robertson, 2003; Pasdar, 2006). But some similarities existed when compared to Chinese *Li* minority (Wu *et al*, 2010). No significant difference in PON3 gene Ala99Ala polymorphism allelic frequencies between Beninese population and Chinese *Li* minority was observed. And also the G/G genotype frequency in *Adja* ethnic group (10.0 %) was similar to that observed in Chinese *Li* minority (8.0 %). This similarity may be explained by the homogeneous *Adja* ethnic group. But additional studies in large cohorts from different ethnic groups in Benin may be needed to determine the real genotypes distribution of PON3 Ala99Ala polymorphism in Beninese population

Conclusion

In this study, the genotypes distribution and allelic frequencies of the PON3 gene Ala99Ala polymorphism in Beninese three ethnic populations were described and compared with those in other word populations. Inter-ethnic variations in PON3 gene polymorphisms distribution were observed in Beninese ethnic populations. The PON3 gene Ala99Ala polymorphism distribution in Beninese different ethnic populations were significantly different from that in other word populations. The ethnic variations of PON3 gene polymorphisms in Beninese different ethnic populations could be used as basis for further investigation on the association of this polymorphism with the risk of cardiovascular diseases and other inflammatory diseases.

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