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HDL-Associated Paraoxonase 1 Gene Polymorphisms as a Genetic Markers for Wide Spread Diseases

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1. Introduction

The story of paraoxonase 1 (PON1) begins in 1946, when Abraham Mazur reported the presence of an enzyme in human and rabbit tissues which was able to hydrolyse organophosphate compounds [1]. In 1950s, enzyme was named "paraoxonase" according to its ability to hydrolyse paraoxon, the toxic metabolite of the organophosphate insecticide parathion [2,3]. Later it was discovered that it exhibits a broad spectrum of activities and has diverse substrates. Mackness and colleagues linked PON1 to cardiovascular diseases in 1991 and demonstrated that PON1 could prevent the accumulation of oxidized lipids in low-density lipoprotein (LDL) [4]. However, despite intensive research over sixty years the exact physiological function of PON1 is still unclear.

2. Body

2.1. Paraoxonase 1

The paraoxonase (PON) family of the enzymes consists of three members, PON1, PON2 and PON3 that share approximately 65% similarity at the amino acid level. These were named in order of their discovery, but according to the structural homology and predicted evolutionary distance between them it seems that PON2 is the oldest and PON1 is the youngest family member [5].

PON1 and PON3 enzymes are secreted from liver cells and associate with HDL in the circulation [6]. Low levels of PON1 may be expressed in a number of tissues, primarily in epithelia. PON2 in humans is more widely expressed and is found in nearly every human tissue including heart, kidney, liver, lung, placenta, small intestine, spleen stomach, testis [6-

8]. Also, human PON2 mRNA is detected in the cells of the artery wall, including endothelial cells, smooth muscle cells and macrophages and is undetectable in HDL, LDL or the media of cultured cells [6, 7]. Of the three PON proteins, PON3 is the most recently identified and the least characterized.

PON1 is the most studied and best understood. This calcium-dependent esterase is consisting of 354 amino acids with a molecular mass of approximately 45 kDa [9, 10] and requires calcium ions for structural stability and enzymatic activities [11]. It is capable of hydrolyzing organophosphates such as oxon metabolites of insecticides parathion, diazinon and chlorpyrifos and nerve agents sarin and soman, aromatic esters such as phenyl acetate (arylesterase activity) and a variety of aromatic and aliphatic lactones (lactonase activity) [12-18]. Beside its protective role against dietary and environmental lactones, PON1 also catalyzes the reaction of lactonization of γ - and δ -hydroxycarboxylic acids [18]. It is capable of hydrolyse the oxidised lipid derivatives 5-hydroxy-eicosatetraenoic acid lactone acid (5-HETEL) and 4-hydroxy-docosahexaenoic acid (4-HDoHE) which are potent triggers of an inflammatory response and therefore determinants of atherosclerotic disease [16,19].

2.2. Paraoxonase 1 gene polymorphisms

Genes that code for three PON proteins (*pon1*, *pon2* and *pon3*) are located to each other on the long arm of chromosome 7 in humans (7q21.3-22.1) and share approximately 70% similarity at the nucleotide level [8]. Earlier studies on different human populations showed that the hydrolytic activity of serum PON1 was polymorphically distributed [20-22] and a number of research demonstrated that the molecular basis of these differences were Q192R, L55M and C(-107)T polymorphisms in *pon1* gene.

The *pon1* gene contains functional polymorphisms in both the coding and promoter regions. In the coding region, two common polymorphism are a glutamine (Q) to arginine (R) substitution at codon 192 (Q192R) and a leucine (L) to methionine (M) substitution at position 55 (L55M). In Q192R polymorphism the exchange of codon CAA to CGA in exon 6 of *pon1* gene determines isoforms of the enzyme which differ greatly in the rate of hydrolysis a number of substrates. Paraoxon is hydrolysed at a far greater rate by the R192 isoform compared to the Q192, but some organophosphates and lactones are hydrolyzed faster by Q192 [12,13]. Recent study showed that R isoform of the enzyme has higher lactonase activity and increased antiatherogenic potential [23].

L55M polymorphism (exchange of codon TTG to ATG in exon 3) is correlated with blood enzyme level with isoform L55 associated with higher serum enzymatic activity. Still, it is not clear whether this is because of a decreased stability of the M55 alloenzyme [24] and/or because of the linkage disequilibrium with -107/-108 T allele [25, 26]. Isoform M55 showed lower stability and loses activity more rapidly and to a greater extent than the L isoform [24]. This is due to the key role of L55 in packing in the propeller's central tunnel, and of its neighboring residues which ligate calcium ions [27].

At least five polymorphisms have been detected in the human *pon1* gene promoter region: C(-107/-108)T, G(-126)C, G(-162)A, G(-832)A and G(-909)C, but only C(-107T) appear to affects expression level of PON1 enzyme [25,26]. This single nucleotide polymorphism (SNP) is within stimulating protein-1 (Sp1) binding site with allele T that disrupts the recognition sequence for Sp1 and results in decreased affinity for it [28].

The frequencies of alleles of Q192R, L55M and C(-107)T polymorphisms are different among populations worldwide (Table 1). Data for European population showed predominance of Q192 and -107C alleles over R192 and -107T alleles. Spanish and Serbian populations showed higher frequency of the -107T allele. For codon 55 polymorphism, populations worldwide show predominance of L55 over M55 allele. In Asia allele Q192 is more frequent only in Indian Punjabis and Iranians and allele -107C is predominant among examined populations. Afro-Americans and Amerindian tribes showed higher frequency of allele R compared with allele Q and predominance of allele-107C. Only Mexicans showed higher frequency of -107T allele. There is a very little data from „black“ continent and it concerns only Q192R polymorphism frequency with higher frequency of allele R only in Beninese (Table 1).

More than 200 single nucleotide polymorphisms (SNPs) have been identified in the human *pon1* gene but only these three have been associated with a number of pathophysiological conditions.

2.3. Pon1 variants and oxidative stress-related disorders

The central role of HDL is in the process of reverse cholesterol transport (RHC). Also it has antioxidative, antiinflammatory and antifibrinolytic functions that contribute to its antiatherosclerotic effects. Mackness and coworkers were the first that showed that HDL acted at a specific point in the oxidation cascade: it metabolises oxidized phospholipids on LDL [29]. Although several other HDL-associated proteins such as apo AI, lecithin:cholesterol acyltransferase (LCAT) and platelet-activating factor acetyltransferase (PAFAH) also have antioxidant properties, PON1 seems to be the predominant antioxidant enzyme [4, 29-31]. HDL isolated from the blood of PON1 knock-out mice or from avian species which naturally lack PON1, has at best, no effect on LDL-oxidation and at worst promotes LDL-oxidation [32,33]. Conversely, HDL isolated from mice overexpressing human PON1 completely abolishes LDL-oxidation [34]. Several human studies have shown an inverse linear relationship between the concentration of oxidised-LDL in the circulation and PON1 activity, strongly implicating PON1 in the metabolism of oxidised-LDL *in vivo* [35,36].

Enzymatic and nonenzymatic systems of antioxidative protection are included in scavenging free radicals and their metabolic products and in maintaining normal cellular physiology. Increased level of free radicals and impairment of antioxidant status are processes underlying pathophysiologic mechanisms in a variety of diseases including

atherosclerosis, diabetes mellitus, cancer, chronic liver impairment, several neurological diseases, many infectious diseases and association studies have identified links between *pon1* gene polymorphisms and susceptibility and outcome of these diseases.

<i>pon1</i> polymorphisms	Q192R		L55M		C(-107)T		References
	Q	R	L	M	C	T	
<i>Populations of Europe</i>							
Finnish	0.69	0.31	0.67	0.33	-	-	76
Dutch	0.68	0.32	0.63	0.37	-	-	77
Spanish	0.7	0.3	0.63	0.37	0.46	0.54	78
Italians	0.65	0.35	0.66	0.34	0.57	0.43	79
English	0.78	0.22	0.7	0.3	0.52	0.48	80
Turkish	0.69	0.31	0.7	0.3	-	-	81
Croatian	0.77	0.23	0.66	0.34	0.54	0.46	82
Czechs	0.54	0.46	0.69	0.31	0.59	0.41	83
Serbian	0.77	0.23	0.68	0.32	-	-	84
<i>Populations of Asia</i>							
Asian Indians Punjabis	0.74	0.26	0.81	0.19	0.52	0.48	85
Japanese	0.4	0.6	0.94	0.06	0.48	0.52	86
Koreans	0.38	0.620	0.94	0.06	-	-	87
Chinese	0.42	0.58	0.95	0.05	0.57	0.43	88, 89
Iranian	0.69	0.31	0.59	0.41	-	-	90
<i>Populations of America</i>							
Caucasian- Americans	0.73	0.27	0.64	0.36	0.5	0.5	26
Canadians	0.73	0.27	0.64	0.36	0.48	0.52	91, 28
African- Americans	0.37	0.63	0.79	0.21	0.85	0.15	92
Amazonian Amerindian tribes	0.27	0.730	0.967	0.033	-	-	93
Caribbean- Hispanics	0.540	0.460	0.71	0.29	0.65	0.35	92
Mexicans	0.510	0.490	0.84	0.16	0.45	0.55	94
Peruvians	0.539	0.461	-	-	0.61	0.39	95
<i>Populations of Africa</i>							
Beninese	0.388	0.612	-	-	-	-	96
Ethiopians	0.592	0.408	-	-	-	-	96
Egyptians	0.67	0.33	-	-	-	-	97

Table 1. The allele frequencies of *pon1* gene polymorphisms Q192R, L55M and C(-107)T in populations worldwide

According to World Health Organization (WHO data for 2010), 95% of mortality in Serbia is caused by chronic noncontagious diseases, wherefrom 58% of it is caused by cardiovascular diseases (CVD) [37]. Although patients with CVD commonly have at least one identifiable risk factor, many ischemic events occur in the absence of any of it [38]. Atherogenesis, one of the main risk factors for CVD, is initiated by oxidation of the low-density lipoprotein (LDL) and by impairment in oxidative stress-antioxidant balance.

Enhanced oxidative stress such as in diabetes, leads to the development of accelerated atherosclerosis. Atherosclerosis in patients with diabetes tends to occur earlier and be more aggressive. People with type 2 diabetes have a 3–4 fold increased risk of developing atherosclerosis compared to people without type 2 diabetes. Serbia falls into the group of European countries with the highest diabetes mortality rates where diabetes is the fifth leading cause of death and the fifth cause of the burden of disease [39]. At least a half of the persons with non-insulin dependent diabetes mellitus (NIDDM) have not been diagnosed and are not aware of their disease [40,41].

Due to the abovementioned, there has been a marked interest in discovering additional markers of oxidative stress, including gene variants, which may have a role in predicting wide spread diseases risk. Because controversial results have been reported so far, the aim of studies performed in our laboratory was to evaluate possible interactions between *pon1* gene polymorphisms and clinical manifestations of atherosclerosis and diabetes mellitus type 2 in our population.

Allele and genotype frequencies for Q192R, L55M and C(-107)T did not show significant difference between cases with clinical manifestations of atherosclerosis (60 subjects) and controls (100 subjects) ($P > 0.05$). Although the M allele (L55M) has shown a somewhat higher risk (OR=1.23) and the T allele (-107C/T) has shown a 1.49 times lower risk of occurrence of the disease (OR=0.67) the difference did not reach statistical significance, most likely due to low number of subjects (Grubisa et al., unpublished data).

Also, we investigated the association between these polymorphisms and atherosclerosis in patients with type 2 diabetes mellitus (140 subjects). Our results have shown that R allele is a risk factor for atherosclerosis in these patients (OR=2.22, $P < 0.0001$). Although M allele has shown a little higher risk (OR=1.26) and allele T has shown a slightly lower risk (OR=0.85) the results obtained do not support an association between these *pon1* gene variants and atherosclerosis in NIDDM patients (Grubisa et al., unpublished data).

Lactones are hydrolyzed preferentially by either PON1 Q or R isoforms, depending of their structure. R192 is more efficient at hydrolyzing homocysteine thiolactone, while δ -valerolactone and 2-coumaranone are more rapidly hydrolyzed by PON1Q192 [12]. In 1990's the results obtained indicated that the Q192R polymorphism may play the role in coronary heart disease (CHD) etiology because this genotype is associated with LDL oxidation; the PON1-192 R isoform is less effective at hydrolysing lipid peroxides than the Q isoform [42,43]. It have been shown that position 192 is involved in HDL binding as a part of amphipathic helix H2 of active site [27]. Gaidukov and coworkers reported from *in*

vitro and sera tests that the PON1-192Q isoform binds HDL with a 3-fold lower affinity than the R isozyme and consequently exhibits significantly reduced stability, lipolactonase activity, and macrophage cholesterol efflux [27]. The higher lactonase activity is manifested by increased antiatherogenic potency: the observed rate of HDL-mediated cholesterol efflux from macrophages is 2.2-fold higher for the 192R [27]. Also it was shown that the affinity and stability of the PON1 on HDL was lower in sera of individuals with the Q192 variant than in individuals with the 192R variant [27]. Low levels of HDL particles is one of the strongest risk factors for coronary heart disease and one of the characteristic features of diabetic dyslipidemia and it seems that proteins on HDL play a major role in the protection against atherosclerosis-based cardiovascular diseases. HDL carrying apolipoprotein A-I binds PON1 with high affinity, stabilizes the enzyme and stimulates its lipolactonase activity [44].

PON1 is also an extracellular homocysteine-thiolactonase (Hcy-thiolactonase). Hcy-thiolactone is a toxic metabolite linked to immune activation and thrombogenesis in human cardiovascular diseases and is elevated under conditions predisposing atherosclerosis [45-47]. A small fraction of Hcy, a sulfur-containing amino acid, is metabolized to a Hcy-thiolactone in an error-editing reaction in protein biosynthesis when Hcy is mistakenly selected instead of dietary methionine (Met) [48]. Hcy-thiolactone is neutral at physiological pH and can diffuse out of the cell and accumulate in the extracellular fluids where is hydrolyzed to Hcy by extracellular Hcy-thiolactonase-paraoxonase 1 [49]

Hcy-thiolactonase activity is strongly associated with *pon1* genotype in diverse human populations [15]. High Hcy-thiolactonase activity is associated with L55 and R192 alleles, more frequent in blacks than in whites and low activity is associated with M55 and Q192 alleles, more frequent in whites than in blacks [15]. Despite the impact of *pon1* genotype on Hcy-thiolactonase activity, these genetic variations are not associated with atherosclerosis-based cardiovascular diseases. It seems that PON1 phenotype is better predictor [16,50].

Human clinical studies suggest that PON1 phenotype, i.e., paraoxonase activity is a much stronger predictor of cardiovascular disease status than PON1 genetic polymorphisms [51-55] a finding that has been confirmed in other studies [52,55]. Bhattacharyya and colleagues demonstrated that both the *pon1* Q192R polymorphism and serum PON1 activity are associated with prevalent coronary artery disease and incident adverse cardiovascular events [56]. This study complemented the study of Gaidukov, demonstrating that individuals with the arginine (R) at position 192 have higher serum levels of PON1 activity, lower systemic indices of systemic oxidative stress and corresponding reductions in both prevalent coronary artery disease and prospective cardiac events [56]. Plasma PON1 activity can vary up to 40 to 50-fold, and differences in PON1 protein levels up to 13–15-fold are also present within a single PON1 Q192R genotype in adults [57,58]. A number of studies indicated that measurement of an individual's PON1 function (serum activity) takes into account all polymorphism and other factors that might affect PON1 activity or expression. However, modulation of PON1 by alcohol, smoking, drugs, diet, certain physiological and pathological conditions should also be considered. These factors can increase or decrease PON1 activity [59] as well as HDL status.

However, PON1 activity is partially inactivated during the detoxification of lipid hydroperoxides [60]. This effect can be possibly related to displacement of calcium ions or inhibition through free radicals directly. It has been suggested that other antioxidant enzymes might prevent this inhibition of PON1 activity. Antioxidant enzymes, all show co-activity and might work in a collaboration against oxidative stress and elevation in oxidative stress might inhibit these enzymes [61].

Paraoxonases are important detoxifying and anti-oxidative enzymes, which establishes their role in organophosphate poisoning, diabetes, obesity, cardiovascular diseases, and innate immunity [62, 63]. Consequently, PON2 has been the focus of a great deal of research in recent years. Both PON1 and PON2 protect against atherosclerosis development and share ability to hydrolyze lactones with both overlapping and distinct substrate specificities [19]. Although PON1 is associated with circulating serum HDL and reduces oxidative stress in lipoproteins, macrophages in arterial walls and in atherosclerotic lesion by its ability to hydrolyze specific oxidized lipids, PON2 acts as an intracellular antioxidant [7,64-68] associated with plasma membrane [6-8]. The mechanism how PON2 modulates oxidative stress is still unknown, although Altenhöfer demonstrated that PON2 prevents superoxide generation, but was ineffective against existing radicals [69]. Oxidative stress affects PON2 expression too, but additional studies are needed to highlight the PON2 expression level under oxidative stress since controversial results both from *in vivo* and *in vitro* experiments have been reported [6,7,66,70-74].

3. Conclusion

Paraoxonase 1 is found to be associated with HDL particles within circulation and therefore promotes some of HDL's functions. There is no consistent evidence for involvement of *pon1* genotypes in atherosclerosis and diabetes mellitus type 2. Studies analyzed the role of *pon1* polymorphisms in oxidative stress-based diseases showed a great variation in ethnics, environmental background, age and gender of case and control groups. Allele frequencies appeared to be dependent on geographic locations, perhaps also due to genetic drift. Probably the effect of each polymorphism alone of the so called oxidative stress-associated genes is not strong enough to affect initiation and progression of atherosclerosis as well as PON1 enzyme status (activity levels and catalytic efficiency specified by the Q192R polymorphism) [75].

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