Human PON Promoters: From Similarity to Prediction of Polymorphic Positions within Transcription Factor Elements

M. Najafi* and Z. Jangravi

Biochemistry Department, Iran University of Medical Sciences, Tehran, Iran

Abstract: The human paraoxonases (PON) are a group of anti-oxidative enzymes that catalyze important reactions in body. Some polymorphic variations within the PON genes have been reported to relate with several diseases. In this article, the polymorphisms of the PON upstream regions were evaluated to predict their positions within elements of transcription factors by similarity tools. However, taxonomy studies suggested the PON2 duplication on the chromosome 7 but pairwise alignments did not show vast similarity among the fragments of PON promoters. Multiple sequence alignment (MSA) tool showed the PON1 T-107C and PON3 C-31T positions are conserved within several transcription elements. Based on these tools, the review assumes that the PON upstream regions have no similarity and only two polymorphisms are considered to interact with several transcription factors.

Keywords: PON, similarity, polymorphism, element, promoter, transcription factor.

INTRODUCTION

The paraoxonases are known as a group of anti-oxidative enzymes that hydrolyze lactones, esters, thiolactones, carbonates and phosphotriesters [1]. These enzymes also catalyze important reactions including detoxification of organophosphates in the nervous tissue [2] and protection of lipids from oxidation in body [3, 4].

Association between the paraoxonases and atherosclerosis, diabetes, sepsis, Alzheimer's dementia, Parkinson and cancer has been reported in several studies [5, 6]. Based on these studies, the PON tissue levels are dependent on various factors including the polymorphic variations within coding and non-coding regions [7, 8]. In this article, we focus on the short DNA stretches covering polymorphic sites within the 5' regulatory regions of PON genes and evaluate these fragments as the elements of transcription factors.

METHODS

The alignment tools were applied to identify the nucleotide similarity among the PON promoters. The following are the stages for prediction of polymorphic positions in the transcription elements: 1) The polymorphisms within 5' regulatory regions of the PON genes were searched and evaluated to be clinically associated with diseases. 2) The DNA fragments covering the polymorphic sites (wild type) were continually shortened and searched by MatInspector program to find the transcription elements despite decreased sensitivity for the shorter fragments. 3) The MSA tool was used to predict the polymorphic positions within the transcription elements and also the frequency of their interaction with transcription factors. The tissue specific patterns of the transcription factors were checked at Symatlas data set (http:// symatlas.gnf.org).

*Address correspondence to this author at the Biochemistry Department and Cellular & Molecular Research Center, Tehran, Iran;
Tel/Fax: + 982188058742; E-mail: nbsmmsbn@iums.ac.ir

PARAOXONASE GENE CLUSTER

The human PON gene family has three members, PON1 (HGNC: 9204), PON2 (HGNC: 9205) and PON3 (HGNC: 9206), clustered on the reverse strand of chromosome 7q21.3-22.1 (Fig. **1**).

Based on the taxonomy studies, the PON2 is the oldest member of this family [9] and its similarity to the other members of PON family is about 65% (Ensembl) (Fig. **2**).

SIMILARITY AMONG UPSTREAM REGIONS OF PON GENES

Upstream fragments (2.4 kb) of the PON genes (Ensembl) aligned by MSA tool (Fig. **3**). The results showed that the percent of nucleotide mismatches within consensus sequence is up to 23%. Based on the pairwise comparison, Dot plots also showed no similarity between 5'regulatory regions so that the longest similarities between PON2 vs. PON1 and PON2 vs. PON3 promoter sequences were 11nt and 16 nt, respectively (Fig. **4**).

POLYMORPHISMS WITHIN PON1 PROMOTER REGION

Human PON1 enzyme expresses widely in the liver and then transports to the cell membrane where it can be integrated with high-density lipoprotein (HDL) [10].

Based on population studies, the Q192R (rs662) and M55L (rs854560) polymorphisms are associated with several diseases [11-15]. Brophy reported that the M55L polymorphism is responsible for 15.3% of variations in the serum PON1 activity, but this dropped to 5% after adjusting the C-107T and the Q192R polymorphisms [16].

Primary reports suggested that -107, -126, -162 and -907 positions may affect PON1 mRNA levels [7, 16-18] (Fig. **5**). Further studies have shown that the distribution of polymorphisms within the regulatory region of PON1 gene is different among ethnic groups (MIM: 168820) so that the -107,

1389-5575/10 $55.00+.00

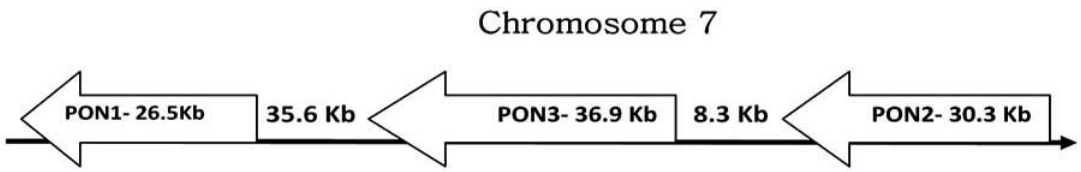

Fig. (1). PON gene cluster.

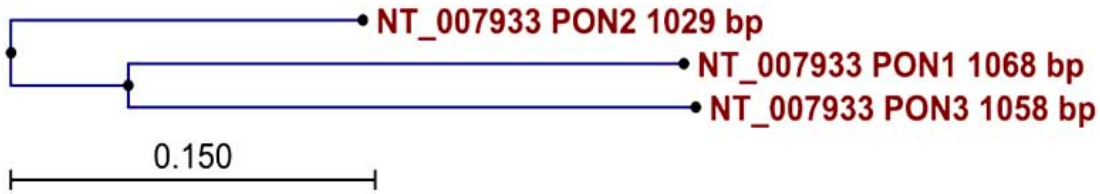

Fig. (2). Nucleotide similarity among PON coding regions. Result is obtained by CLC sequence viewer (ver. 6.3).

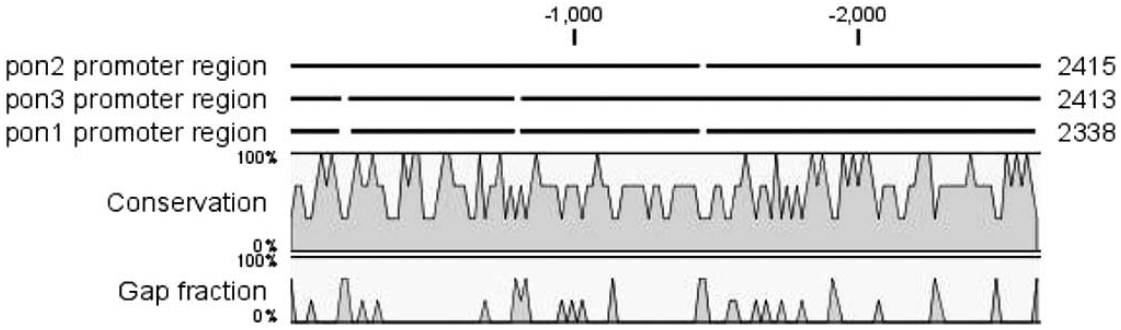

Fig. (3). Nucleotide similarity among PON upstream regions. Gap open cost =10; Gap extension cost = 1 and End gap cost was free. (CLC sequence viewer).

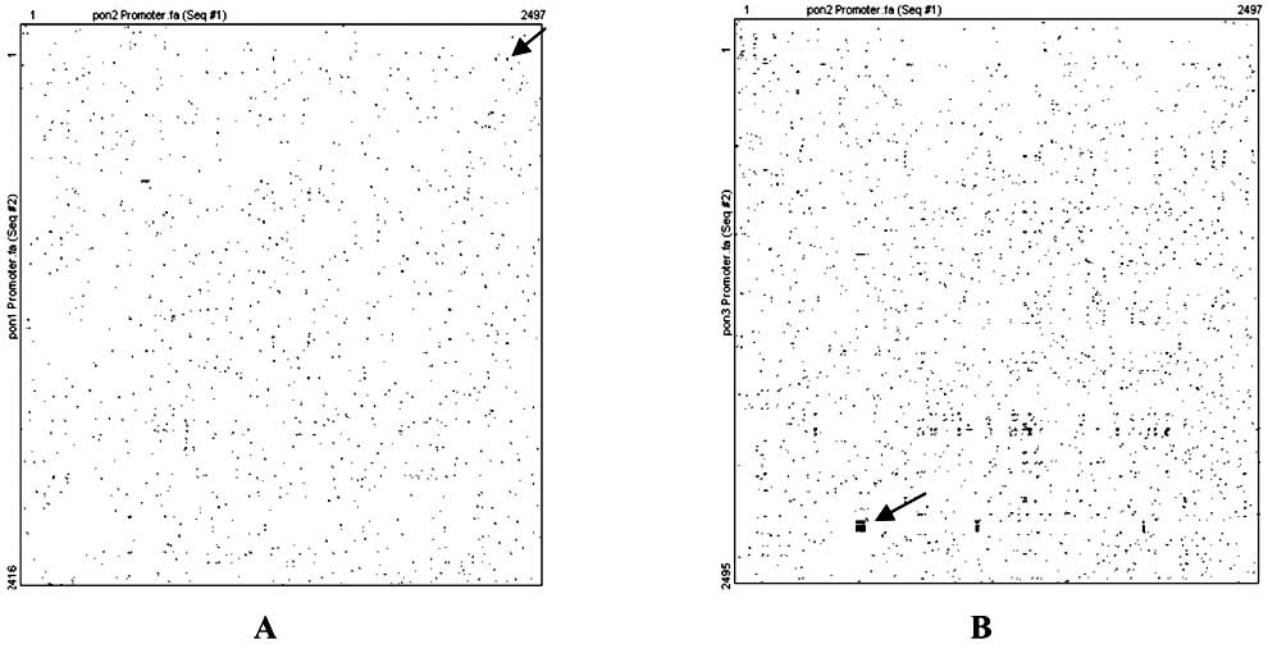

Fig. (4). Pairwise alignment of upstream regions of PON genes. Dot plot A, PON 2 vs. PON1 promoter regions. The longest similarity (11nt) is between 2390-2401(PON2 promoter seq.) and 96-107 (PON1 promoter seq.) fragments. Dot plot B, PON 2 vs PON3 promoter regions. The longest similarity (16nt) is between 606-922 (PON2 promoter seq.) and 2218-2234 (PON3 promoter seq.) fragments. Minimal length of alignment is 7 nt and arrows show the longest alignment regions.

-162 and -907 polymorphisms are more related with diseases. Tables **1-3** show the association of these polymorphisms with several diseases and the C-107T site is considered more important as compared to other sites [19-29].

C-107T POLYMORPHISM

The PON1 serum concentration and activity are associated with the −107C variant as suggested by Deakin. The

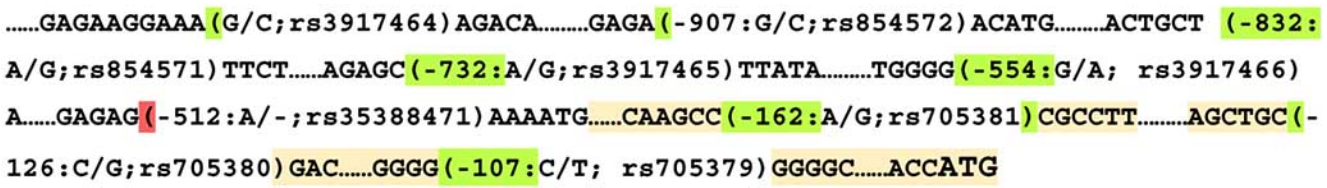

Fig. (5). Polymorphisms within upstream region of the PON1 gene.

Table 1. Allele Distribution of PON1 C-107T Polymorphism

P	T	C	Case/Control	Disease/Country
Sig.	0.61	0.39	Case	**Dementia** France [19]
	0.49	0.51	Control	
Sig.	0.52	0.48	Case	**SALS** Australia [22]
	0.57	0.43	Control	
NS	0.42	0.58	Control	**Diabetes** Czech [24]
	0.45	0.55	Type 1	
NS	0.46	0.64	Type 2	
Sig.	0.56	0.44	+Heart Disease	**Diabetes** Switzerland [26]
	0.49	0.51	-Heart Disease	
NS	0.57	0.43	Case	**CHD** China [28]
	0.56	0.44	Control	
Sig.	0.47	0.53	Case	**Stroke** USA [29]
	0.48	0.52	Control	

P; P value: Sig. <0.05 and NS > 0.05. SALS; Sporadic Amyotrophic Lateral Sclerosis.

variant has greater affinity for Sp1 transcription factor and is indicated as high-expresser variant [30]. The results have been confirmed by others (Table **1)**.

We aligned a short fragment (GGGCGGG) covering the -107 position with the transcription elements and found nine with high similarity. Multiple sequence alignment (MSA) showed that −107C variant is highly conserved within the elements. The results also showed that in this region, several elements are probably involved in gene expression and may act synergistically in elevation of the PON1activity in the serum (Fig. **6**).

A-162G POLYMORPHISM

The A-162G polymorphism has been studied in several diseases (Table **2**). Brophy reported the position has approximately a two-fold effect on the PON1 mRNA level as compared to the -126 and -907 polymorphisms [16]. We found three transcription elements similar to fragment (GGTGC) covering -162 site on reverse strand, but the position did not stand within the core (conserved region within the transcription element) of elements (Fig. **7**). Based on the similarity and epidemiological studies, the fragment is not effectively involved in the PON1 gene expression.

G-907C POLYMORPHISM

Some studies have reported that the −907G variant is related with the serum PON1 concentration and activity in several diseases [17, 20] (Table **3**). We observed five transcription elements similar to fragment (AGACA) covering -907 site. The effect of −907G variant on the PON1 gene expression was not clearly discernable since the polymorphic site was not located within the core of transcription elements (Fig. **8**).

HAPLOTYPES

The −107C, −824A and −907G haplotype is related with the serum PON1 concentration and activity as reported by Leviev. This study has also shown the -907G allele is associated with reduced risk of vascular disease in younger patients but not in older patients probably due to a reduction in the PON1 activity [27].

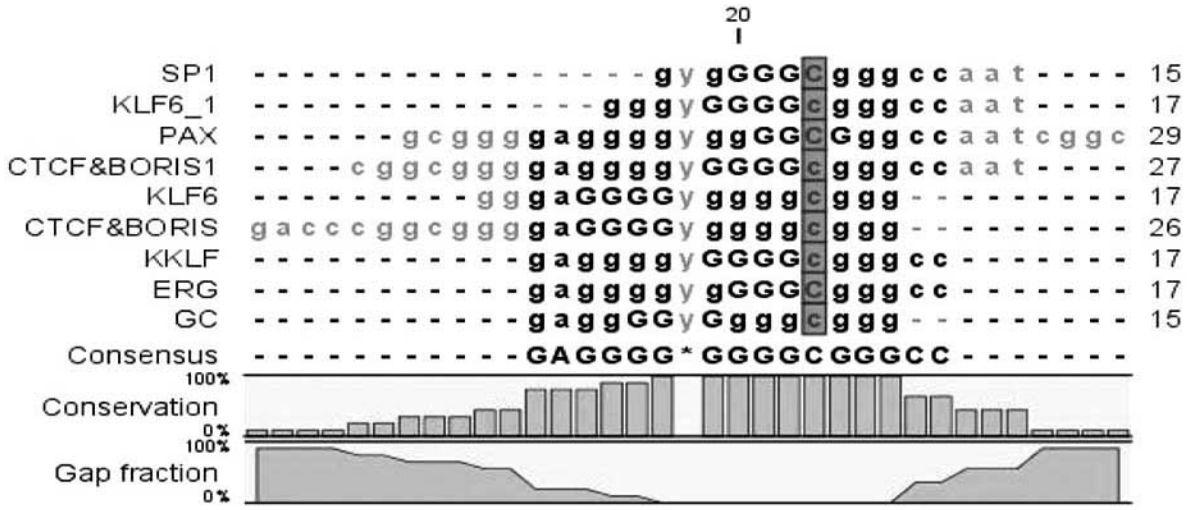

Fig. (6). Multiple sequence alignment of nine transcription elements similar to fragment (GGGCGGG) covering PON1 C-107T position. The C nucleotide (Wild type) in gray boxes shows the −107C position within the elements. Graphs show the nucleotide similarity among elements. Capitals (4 letters) show core of the transcription elements. The elements are found and aligned by MatInspector program and MSA tool. SP1; GC-Box factors SP1/GC, KLF6_1; Krueppel like transcription factors, PAX; PAX-5 B-cell-specific activator protein, CTCF & BORIS; CTCF & BORIS gene family, transcriptional regulators, KLF6; Krueppel like transcription factors, KKLF; Krueppel like transcription factors, EGR; EGR/nerve growth factor induced protein C & related factors, GC; GC-Box factors SP1/GC.

Table 2. Allele Distribution of PON1 A-162G Polymorphism

P	G	A	Case/Control	Disease/Country
NS	0.78	0.22	Case	**Dementia** France [19]
	0.81	0.19	Control	
NS	0.77	0.23	Case	**SALS** Irish [21]
	0.71	0.29	Control	
Sig.	0.60	0.39	+ Micro albuminuria	**Diabetes** Australia [25]
	0.75	0.25	- Micro albuminuria	
Sig.	0.84	0.16	Case	**CHD** China [28]
	0.90	0.10	Control	
NS	0.21	0.79	Case	**Stroke** USA [29]
	0.26	0.74	Control	

P; P value: Sig. <0.05 and NS > 0.05. SALS; Sporadic Amyotrophic Lateral Sclerosis.

The indirect haplotype analyses in Chinese Han women showed that the PON1 C-107T and PON2 S311C (rs7493) polymorphisms are significantly related to coronary heart disease (CHD) [31]. We reported the role of haplotypes (-107, -162 and -907 sites) in CHD patients. Our findings showed a significant association between the number of -107C alleles within two allelic haplotypes and stenotic arteries [32, 33]. In addition, there was a significant association between PON1 arylesterase activity and the -107C variant [34].

POLYMORPHISM WITHIN PON2 PROMOTER REGION

Human PON2 enzyme known as an intracellular antoxidative enzyme is expressed in a variety of tissues including the liver, lung, heart, kidney, placenta and testis. PON2

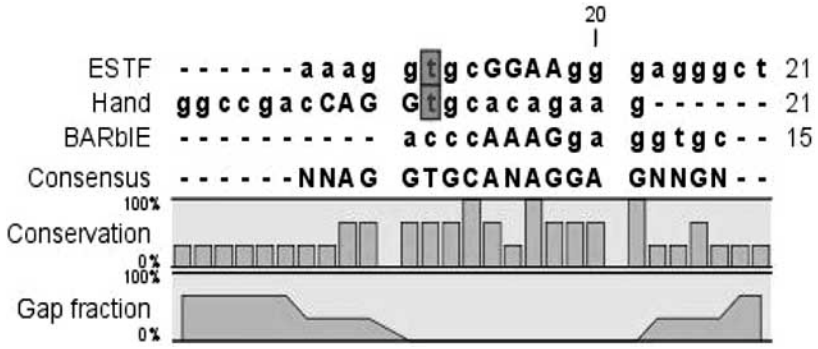

Fig. (7). Multiple sequence alignment of three transcription elements similar to fragment (GGTGC) covering PON1 A-162G site on reverse strand. T nucleotide (Wild type) in gray boxes shows the −162 position within the elements. Graphs show the nucleotide similarity among elements. Capitals (4 letters) show core of the transcription elements. The elements are found and aligned by MatInspector program and MSA tool. ETSF; Human and murine ETS1 factors, HAND; Twist subfamily of class B bHLH transcription factors, BARBIE; Barbiturate-inducible element box from pro+eukaryotic genes.

Table 3. Allele Distribution of PON1 C-907G Polymorphism

P	G	C	Case/Control	Disease/Country
NS	0.46	0.54	Case	**SALS** Australia [22]
	0.53	0.47	Control	
NS	0.29	0.71	Control	**Diabetes** Czech [24]
	0.70	0.30	Type 1	
NS	0.70	0.30	Type 2	
Sig.	0.41	0.59	+ Micro albuminuria	**Diabetes** Australia [25]
	0.51	0.49	- Micro albuminuria	
NS	0.53	0.47	Case	**Stroke** USA [29]
	0.54	0.46	Control	

P; P value: Sig. <0.05 and NS > 0.05. SALS; Sporadic Amyotrophic Lateral Sclerosis.

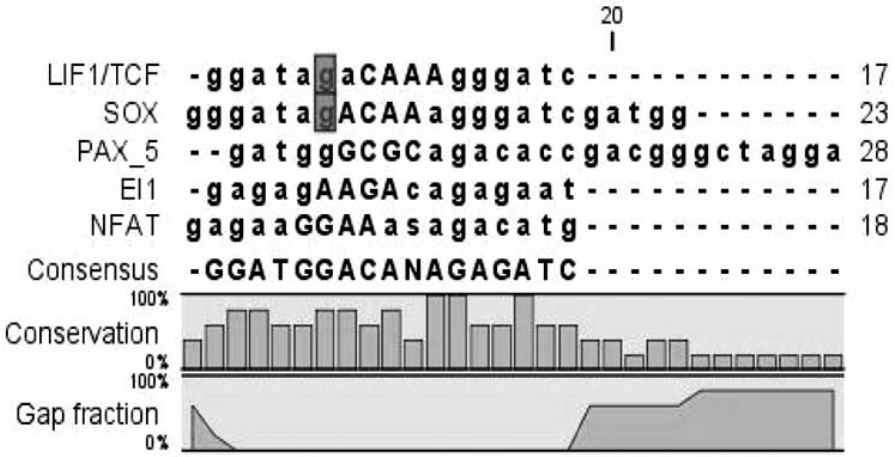

Fig. (8). Multiple sequence alignment of five nucleotide elements similar to fragment (AGACA) covering PON1 G-907C site. G nucleotide (Wild type) in gray boxes shows the −907 position within the elements. Graphs show the nucleotide similarity among elements. Capitals (4 letters) show core of the transcription elements. The elements are found, aligned by MatInspector program and MSA tool. LEF1/TCF. SOX; SOX/SRY-sex/testis determining and related HMG box factors. PAX-5; PAX-5 B-cell-specific activator protein. EI1; EVI1-myeloid transforming protein. NFAT; Nuclear factor of activated T-cells.

.....AGCTACTCA(-635G/A rs13243101)GAGGCTGAGGC.....GTACTCCA(-558G/C rs13242987)CCTA(-553G/Ars13242985)GCAACACAGC.....CCCCGACGC(-123A/G rs7805636)GGGACTCG.....GCTCCC(-5C/T rs17876183)CGCCATG

Fig. (9). Polymorphisms within upstream region of the PON2 gene.

enzyme prevents LDL cell mediated oxidation so that it reduces intracellular oxidative stress (MIM: 602447), indicating its association with several diseases [35, 36].

Two important polymorphisms have been reported in the coding region of PON2 gene, G148A (rs12026) and C311S (rs7493). However, some studies have indicated that C311S polymorphism is associated with pathophysiology of coronary artery disease, ischemic stroke [37-40], diabetes mellitus [41] and Alzheimer's disease [42, 43] but the association of promoter variations with above diseases is not widely studied. We evaluated the transcription elements similar to short sequences covering G-5A (rs17876183), A-123G (rs7805636), G-553A (rs13242985) and G-558C (rs13242987) positions (Fig **9**) and found only the A-123 variant within the core of CTCF element (CAGA).

POLYMORPHISMS WITHIN PON3 PROMOTER REGION

Human PON3 is expressed primarily in the liver and kidney and has an anti-oxidative effect similar to that described for the PON1 so that it can preserve HDL particles against oxidation (MIM: 602720) and may participate in etiology of atherosclerosis [44, 45].

Campo has suggested the S311T and G324D polymorphisms in the PON3 coding region having no effect in the enzyme activity [46]. In addition, Sanghera experienced six PON3 tagging single nucleotide polymorphisms (tagSNPs) and showed that A2115T and A10340C polymorphisms cause 1% and 2% variation in the serum PON1 activity, respectively [47]. We evaluated the nucleotide similarity of short DNA fragments (5-6 bases) covering C-31T (rs17886586), C-498A (rs2072200) and C-506G (rs624695565) sites (Fig. **10**) with the transcription elements, and observed that the C-31variant (rs17886586) is close to a conserved region within several elements (Fig. **11**).

CONCLUSION

Taxonomy studies, however, showed that PON2 gene is the oldest member of family, but pairwise comparisons did not show vast similarities among the promoter regions, suggesting the role of different regulatory factors in regulation of the PON genes expression.

.....GGTGTT(-749C/T rs17882539)ATCAGGAGAGGGGAA.....AAGGAAA(-665T/C rs11770903)GA....CGCCTTAACTATGGGGACAGAC(-567G/A rs11764079)CAGCTA.....AGGAGA(-506 C/A rs624695565)AGGTTCC(-498C/G rs2072200)GTGCCC.....CTCTCGGCG(-31C/T rs17886586)CGC....ACCCCGAGACCATG

Fig. (10). Polymorphisms within upstream region of the PON3 gene.

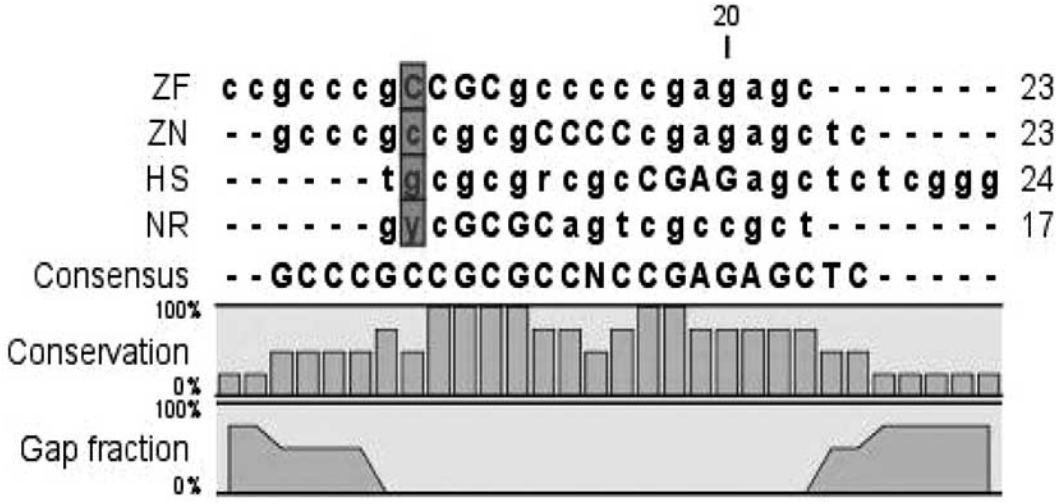

Fig. (11). Multiple sequence alignment of four transcription elements similar to fragment (CGCCGC) covering PON3 C-31T site. The C nucleotide in gray boxes shows the position of C−31 variant within the element. Graphs show the nucleotide similarity among elements. Capitals (4 letters) show core of the transcription elements. The elements are found, aligned by MatInspector program and MSA tool.ZF; Myeloid zinc finger 1 factors. ZN; Zinc binding protein factors. HS; Heat shock factors, NR; Nuclear respiratory factor 1. Graphs show conserved sites and gaps within elements.

In this study, the SNPs within the promoter regions of PON genes are reported to be associated with diseases, evaluated by the similarity tools to improve our understanding on the function of SNPs. Based on the similarity results, the PON1 T-107C and PON3 C-31T sites are supposed to conserve within the transcription elements and may be important in gene expression. However, the PON2 A-123G site is conserved within core of element CTCF but it is not probably a functional element in the promoter because its factor has 11 highly conserved zinc finger domains.

In conclusion, the similarity results can represent the suppositions for function and primary structure of macromolecules and may be applied as starting point for experimental studies. In addition, the alignment tools may support and improve our knowledge of experimental works. Based on the similarity results, the PON upstream regions had no similarity and only two polymorphisms in this region were considered to interact with several transcription factors.

REFERENCES

[1] Aviram, M.; Rosenblat, M. Paraoxonases (PON1, PON2, PON3) analyses *in vitro* and *in vivo* in relation to cardiovascular diseases. Methods. *Mol. Biol*., **2008**, *477*, 259-276.

[2] La, Du, B.N. In: *Human serum paraoxonase/arylesterase*. Kalow, W.; Ed. Pharmacogenetics of Drug Metabolism. New York, NY: Pergamon Press, **1992**, pp. 51-91.

[3] Mackness, M.I.; Arrol, S.; Abbot, C.; Durrington, P.N. Protection of low density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. *Atherosclerosis*, **1993**, *104*, 129-135.

[4] Watson, A.D.; Berliner, J.A.; Hama, S.Y.; La, Du, B.N.; Faull, K.F.; Fogelman, A.M.; Navab, M. Protective effect of high density lipoprotein associated paraoxonase: inhibition of the biological activity of minimally oxidized low density lipoprotein. *J. Clin. Invest*., **1995**, *96*, 2882-2891.

[5] Li, H.L.; Liu, D.P.; Liang, C.C. Paraoxonas gene polymorphisms,oxidative stress and diseases. *J. Mol. Med*, **2003**, *81*, 766-779.

[6] Ng, C.J.; Shih, D.M.; Hama, S.Y.; Villa, N.; Navab, M.; Reddy, S.T. The paraoxonase gene family and atherosclerosis. *Free Radic. Biol. Med*., **2005**, *15*,153-163.

[7] Leviev, I.; James, R.W. Promoter polymorphisms of human paraoxonase PON1 gene and serum paraoxonase activities and concentrations. *Arterioscler. Thromb. Vasc. Biol*., **2000**, *20*, 516- 521.

[8] Soran, H.; Younis, N.N.; Charlton-Menys, V.; Durrington, P. Variation in paraoxonase-1 activity and atherosclerosis. *Curr. Opin. Lipidol*., **2009**, *20*, 265-274.

[9] Draganov, D.I.; La, Du, B.N. Pharmacogenetics of paraoxonases: a brief review. Naunyn Schmiedeberg's *Arch. Pharmacol*., **2004**, *l369*, 8- 88.

[10] Durrington, P.N.; Mackness, B.; Mackness, M.I. Paraoxonase and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol*., **2001**, *21*, 473-480.

[11] Adkins, S.; Gan, K.N.; Mody, M.; La, Du, B.N. Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: glutamine or arginine at position 191, for the respective A or B allozymes. *Am. J. Hum. Genet*., **1993**, *52*, 598-608.

[12] Humbert, R.; Adler, D.A.; Disteche, C.M.; Hassett, C.; Omiecinski C.J.; Furlong, C.E. The molecular basis of the human serum paraoxonase activity polymorphism. *Nat. Genet*., **1993**, *3*, 73-76.

[13] Blatter, Garin, M.C.; James, R.W.; Dussoix, P.; Blanche, H.; Passa, P.; Froguel, P.; Ruiz, J. Paraoxonase polymorphism Met-Leu54 is associated with modified serum concentrations of the enzyme: a possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. *J. Clin. Invest*., **1997**, *99*, 62-66.

[14] Leviev, I.; Negro, F.; James, R.W. Two alleles of the human paraoxonase gene produce different amounts of mRNA: an explanation for differences in serum concentrations of paraoxonase associated with the (Leu-Met54) polymorphism. *Arterioscler. Thromb. Vasc. Biol*., **1997**, *17*, 2935-2939.

[15] Mackness, B.; Mackness, M.I.; Arrol, S.; Turkie, W.; Durrington. P.N. Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. *FEBS. Lett.*, **1998**, *423*, 57-60.

[16] Brophy, V.H.; Jampsa, R.L.; Clendenning, J.B.; McKinstry, L.A.; Jarvik, G.P.; Furlong, C.E. Effects of 5′ regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. *Am. J. Hum. Genet*., **2001**, *68*, 1428- 1436.

[17] Brophy, V.H.; Hastings, M.D.; Clendenning, J.B.; Richter, R.J.; Jarvik, G.P.; Furlong, C.E. Polymorphisms in the human paraoxonase (PON1) promoter. *Pharmacogenetics*, **2001**, *11*, 77- 84.

[18] Suehiro, T.; Nakamura, T.; Inoue, M.; Shiinoki, T.; Ikeda, Y.; Kumon, Y.; shindo M.; Tanaka, H.; Hashimoto, K. A polymorphism upstream from human paraoxonase (PON1) gene and association with PON1 experession. *Atherorosclerosis*, **2000**, *150*, 295-298.

[19] Helbecque, N.; Cottel, D.; Codron, V.; Berr, C.; Amouyel, P. Paraoxonase 1 gene polymorphisms and dementia in humans. *Neurosci. Lett*., **2004**, *18*, 41-44.

[20] Cellini, E.; Tedde, A.; Bagnoli, S.; Nacmias, B.; Piacentini, S.; Bessi, V.; Bracco, L.; Sorbi, S. Association analysis of the paraoxonase-1 gene with Alzheimer's disease. *Neurosci. Lett*., **2006**, *20*, 199-202.

[21] Cronin, S.; Greenway, M.; Prehn, J.H.; Hardiman, O. Paraoxonase promoter and intronic variants modify risk of sporadic amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry*, **2007**, *78*, 984-986.

[22] Morahan, J.M.; Yu, B.; Trent, R.J.; Pamphlett, R. A gene-environment study of the paraoxonase 1 gene and pesticides in amyotrophic lateral sclerosis. *Neurotoxicology*, **2007**, *28*, 532-540.

[23] Kovács, T.J.; Harris, S.; Vas, T.K.; Seres, I.; Short, C.D.; Wittmann, I.K.; Paragh, G.; Mackness, M.I.; Mackness, B.; Durrington, P.N.; Nagy, J.M.; Brenchley, P.E. Paraoxonase gene polymorphism and serum activity in progressive IgA nephropathy. *J. Nephrol*., **2006**, *19*, 732-738.

[24] Flekac, M.; Skrha, J.; Zídková, K.; Lacinová, Z.; Hilgertová, J. Paraoxonase 1 gene polymorphisms and enzyme activities in diabetes mellitus. *Physiol. Res*, **2008**, *57*, 717-726.

[25] Hofer, S.E.; Bennetts, B.; Chan, A.K.; Holloway, B.; Karschimkus, C.; Jenkins, A.J.; Silink, M.; Donaghue, K.C. Association between PON1 polymorphisms, PON activity and diabetes complications. *J. Diabetes. Complications*, **2006**, *20*, 322-328.

[26] James, R.W.; Leviev, I.; Ruiz, J.; Passa, P.; Froguel, P.; Garin, M.C. Promoter polymorphism T(-107)C of the paraoxonase PON1 gene is a risk factor for coronary heart disease in type 2 diabetic patients. *Diabetes*, **2000**, *49*, 1390-1393.

[27] Leviev, I.; Poirier, O.; Nicaud, V.; Evans, A.; Kee, F.; Arveiler, D.; Morrisson, C.; Cambien, F.; James, R.W. High expressor paraoxonase PON1 gene promoter polymorphisms are associated with reduced risk of vascular disease in younger coronary patients. *Atherosclerosis*, **2002**, *161*, 463-467.

[28] Wang, X.; Fan, Z.; Huang, J.; Su, S,; Yu, Q.; Zhao, J.; Hui, R.; Yao, Z.; Shen, Y.; Qiang, B.; Gu, D. Extensive association analysis between polymorphisms of PON gene cluster with coronary heart disease in Chinese Han population. *Arterioscler. Thromb. Vasc Biol*., **2003**, *1*, 328-334.

[29] Ranade, K.; Kirchgessner, T.G.; Iakoubova, O.A.; Devlin, J.J.; DelMonte, T.; Vishnupad, P.; Hui, L.; Tsuchihashi, Z.; Sacks, F.M.; Sabatine, M.; Braunwald, E.; White, T.J.; Shaw, P.M.; Dracopoli, N.C. Evaluation of the paraoxonases as candidate genes for stroke: Gln192Arg polymorphism in the paraoxonase 1 gene is associated with increased risk of stroke. *Stroke*, **2005**, *36*, 2346-2350.

[30] Deakin, S.; Leviev, I.; Brulhart-Meyne,t M.C.; James R.W. Paraoxonase-1 promoter haplotypes and serum paraoxonase: a predominant role for polymorphic position −107, implicating the Sp1 transcription factor. *Biochem. J*., **2003**, *372*, 643-649.

[31] Su, S.Y.; Chen, J.H.; Huang, J.F.; Wang, X.L.; Zhao, J.G.; Shen, Y.; Qiang, B.Q.; Gu, D.F. Paraoxonase gene cluster variations associated with coronary heart diseases in Chinese Han women. *Chin. Med. J.*, **2005**, *118*, 1167-1174.

[32] Najafi, M.; Firoozrai, M.; Gohari, H.L.; Zavarehie, A.; Basiri, G. Direct haplotyping of bi-allelic SNPs using Arms and RFLP analysis techniques. *Biomol. Eng*., **2007**, *24*, 609-612.

[33] Najafi, M.; Gohari, H.L.; Firoozrai, M. Paraoxonase 1 gene promoter polymorphisms are associated with the extent of stenosis in coronary arteries. *Thromb*. *Res*., **2009**, *123*, 503-510.

[34] Najafi, M.; Gohari, H.L.; Firoozrai, M.; Zavarehee, A.; Basiri H.A. Association between Paraoxonase -1 Gene Pro-moter T (-107) C Polymorphism and Coronary Artery Disease. Iranian. *J. Public. Health*., **2008**, *37*, 108-113.

[35] Primo-Parmo, S.L.; Sorenson, R.; Teiber, J.; La, Du, B.N. The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. *Genomics*., **1996**, *33*, 498-507.

[36] Ng, C.J.; Wadleigh, D.J.; Gangopadhyay, A.; Hama, S.; Grijalva, V.R.; Navab, M.; Fogelman, A.M.; Reddy, S.T. Paraoxonase-2 is a ubiquitously expressed protein with antioxidant properties and is capable of preventing cell-mediated oxidative modification of low density lipoprotein. *J. Biol. Chem*., **2001**, *276*, 44444-44449.

[37] Chen, Q.; Reis, S.E.; Kammerer, C.M.; McNamara, D.M.; Holubkov, R.; Sharaf, B.L.; Sopko, G.; Pauly, D.F.; Merz, C.N.; Kamboh, M.I. Association between the severity of angiographic coronary artery disease and paraoxonase gene polymorphisms in the National Heart, Lung, and Blood Institute-sponsored Women's Ischemia Syndrome Evaluation (WISE) study. *Am. J. Hum. Genet*., **2003**, *72*, 13-22.

[38] Pan, J.P.; Lai, S.T.; Chiang, S.C.; Chou, S.C.; Chiang, A.N. The risk of coronary artery disease in population of Taiwan is associated with Cys-Ser 311 polymorphism of human paraoxonase (PON)-2 gene. *Zhonghua Yi Xue Za Zhi*. (Taipei), **2002**, *65*,415-421.

[37] Martinelli, N.; Girelli, D.; Olivieri, O.; Stranieri, C.; Trabetti, E.; Pizzolo, F.; Friso, S.; Tenuti, I.; Cheng, S.; Grow, M.A.; Pignatti, P.F.; Corrocher, R. Interaction between smoking and PON2 Ser311Cys polymorphism as a determinant of the risk of myocardial infarction. *Eur. J. Clin. Invest*., **2004**, *34*, 14-20.

[40] Sanghera, D.K.; Aston, C.E.; Saha, N.; Kamboh, M.I. DNA polymorphisms in two paraoxonase genes (PON1 and PON2) are associated with the risk of coronary heart disease. *Am J. Hum. Genet*., **1998**, *62*, 36-44.

[41] Wang, X.Y.; Xue, Y.M.; Wen, S.J.; Zhang, N.L.; Ji, Z.; Pan, S.Y. The association of paraoxonase 2 gene C311S variant with ischemic stroke in Chinese type 2 diabetes mellitus patients. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*., **2003**, *20*, 215- 219.

[42] Janka, Z.; Juhasz, A.; Rimanoczy, A.A.; Boda, K.; Marki-Zay, J.; Kalman, J. Codon 311 (CysYSer) polymorphism of paraoxonase-2 gene is associated with apolipoprotein E4 allele in both Alzheimer's and vascular dementias. *Mol. Psychiatry*., **2002**, *7*, 110-112.

[43] Erlich, P.M.; Lunetta, K.L.; Cupples, L.A.; Huyck, M.; Green, R.C.; Baldwin, C.T.; Farrer, L.A. Polymorphisms in the PON gene cluster are associated with Alzheimer disease. *Hum. Mol. Genet*., **2006**, *15*, 77-85.

[44] Draganov, D.I.; Teiber, J.F.; Speelman, A.; Osawa, Y.; Sunahara, R.; La, Du, B.N. Human paraoxonases (PON1, PON2, and PON3) are lactonases with overlapping and distinct substrate specificities. *J. Lipid Res*., **2005**, *46*, 1239-1247.

[45] Teiber, J.F.; Draganov, D.I.; La, Du, B.N. Lactonase and lactonizing activities of human serum paraoxonase (PON1) and rabbit serum PON3. *Biochem. Pharmacol*., **2003**, *66*, 887-896.

[46] Campo, S.; Sardo, A.M.; Campo, G.M.; Avenoso, A.; Castaldo, M.; D'Ascola, A.; Giunta, E.; Calatroni, A.; Saitta, A. Identification of paraoxonase 3 gene (PON3) missense mutations in a population of southern Italy. *Mutat. Res*., **2004**, *546*, 75-80.

[47] Sanghera, D.K.; Manzi, S.; Minster, R.L.; Shaw, P.; Kao, A. Bontempo F and Kamboh MI. Genetic variation in the paraoxonase-3 (PON3) gene is associated with serum PON1 activity. *Ann. Hum. Genet*., **2007**, *72*, 72-81.

Received: April 10, 2010 Revised: June 12, 2010 Accepted: June 17, 2010