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System study of MPO promoter high-frequency polymorphic variants on transcription factor network

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ABSTRACT

The neutrophil myeloperoxidase (MPO) promotes the oxidative stress by the production of active chlorinated molecules. The aim of this study was to investigate the association between MPO promoter polymorphic variants (rs2243827 and rs2333227) and, its serum level in patients with the stenosis of coronary arteries. Furthermore, a system approach was applied to create the MPO transcription factor network. A total of one hundred fifty six subjects (controls, stenosis < 5%, n = 71 and patients, stenosis > 70%, n = 85) undergoing coronary angiography were recruited. The polymorphic haplotypes and serum MPO level were identified using ARMS-PCR and ELISA techniques, respectively. The MPO transcription factor network was primarily created with PSICQUIC and ChIP data and, was improved with the predicted transcription factors. The regression analyses did not show an association between the serum MPO level and the extent of stenosis in coronary arteries. The network showed that the predicted transcription factors at the flanking regions of polymorphic variants are not directly interacted to MPO. In conclusion, the population and prediction studies showed no association between the serum MPO level, the promoter high-frequency polymorphic frequencies and the extent of stenosis in coronary arteries. A gene sub-cluster with MYB as central node was suggested to be involved with MPO on the transcription factor network.

1. Introduction

Heart failure due to stenosis of coronary arteries is one of the most important clinical manifestations in the world. The numerous hypotheses have been presented to explain the molecular pathogenesis of stenosed coronary arteries (Libby et al., 2011). In this endeavor, the oxidative modifications of macromolecules are rather emphasized due to oxidant/antioxidant imbalance (Maiolino et al., 2013). According to oxidative modification hypothesis, the oxidized LDL particles activate cytokine-stimulating pathways within endothelial cells and, improve atherogenic plaques (Najafi et al., 2011). NAD(P)H oxidase (NOX), xanthine oxidase (XO), nitric oxide synthase (NOS), lipoxygenase (LOX) and myeloperoxidase (MPO) are the most important enzymes related to ROS (Reactive Oxygen System) within the sub-endothelial space. MPO (Entrez, Gene ID: 4353) is a vesicular heme-containing enzyme that catalyzes CL^- into 2e-oxidant HOCL within phagocytes. The MPO activity is coupled to NAD(P)H oxidase so that H_2O_2 generated from $O_2^{-\circ}$ can be catalyzed by MPO enzyme. The MPO/H_2O_2/CL^- system generates other oxidants and chlorinated molecules that are able to oxidize the lipid particles and, to convert macrophages into foam cells (Stocker and Keaney, 2004). Moreover, the MPO/H_2O_2/CL^- system may be involved in nitric oxide (NO) bioavailability (Szalai et al., 2014), APOA1 oxidation and metalloproteinase activation (Nizam et al., 2014).

The serum MPO level may be high due to chronic inflammatory events and, genetic failure so that some polymorphic sites (dbSNP, www.ncbi.nlm.nih.gov/SNP) within the MPO gene are reported to involve with coronary artery disease (CAD) (do Carmo et al., 2012; Wainstein et al., 2010).

Based on the high-throughput techniques, the MPO–protein interactions are reported in Proteomics Standard Initiative Common Query InterfaCe (PSICQUIC, www.ebi.ac.uk/Tools/webservices/psicquic). IntAct (www.ebi.ac.uk/intact) search showed the interactions between human MPO, Dynll1 and A2M proteins. ChIP Enrichment Analysis (ChEA, http://amp.pharm.mssm.edu/lib/chea.jsp) on binding proteins with DNA showed that several transcription factors significantly relate to the MPO 5′UTR region. The molecular interactions can also be predicted from other sources such as microarray and text-mining data. Furthermore, the transcription factors can be found by scanning the gene promoter with transcription factor element profiles (TRANSFAC and JASPAR).

Abbreviations: MPO, myeloperoxidase; MYB, v-myb avian myeloblastosis viral oncogene homolog; FOS, FBJ murine osteosarcoma viral oncogene homolog; NOX, NAD(P)H oxidase; XO, xanthine oxidase; NOS, nitric oxide synthase; LOX, lipoxygenase; CAD, coronary artery disease; PPI, protein–protein interaction; BMI, Body Mass Index; NO, nitric oxide

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In this study, the association between the MPO promoter high-frequency polymorphic variants and its serum level was investigated in patients with the stenosis of coronary arteries. Moreover, transcription factors were predicted at the flanking regions of polymorphic variants and, were evaluated on the transcription factor network.

2. Methods and materials

2.1. Subjects

A total of one hundred fifty six subjects undergoing coronary angiography were recruited and, excluded from those with kidney, liver and systemic diseases and also myocardial infarction in the last three months. The subjects were divided into control (stenosis < 5%, n = 71) and patient (stenosis >70%, n = 85) groups. The patient group was subdivided into single-vessel disease (SVD), two-vessel disease (2VD) and three vessel disease (3VD) based on the stenosis of the right coronary, left coronary and left anterior descending arteries. The study was approved by the ethics committee and informed consent was obtained from all subjects.

2.2. MPO, anti-MPO (pANCA) and DNA

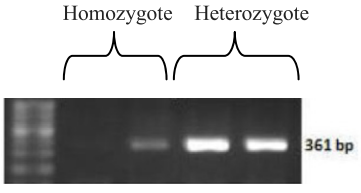
The serum MPO (Abcam, ab119605), anti-MPO (ORGENTEC Diagnostika, ORG 519) and lipid profile were measured in according to the manufacturer's instructions and the routine techniques. The genomic DNA was extracted from the whole blood samples collected in EDTA containing tubes using salting out method (Miller et al., 1988). All samples were rapidly stored at −80 °C.

2.3. Direct haplotyping technique

The polymorphic haplotypes were directly identified using ARMS-PCR (amplification refractory mutation system-PCR) and RF (restriction fragment) techniques as reported in other studies (Najafi et al., 2014). The direct haplotyping technique was performed by the following steps.

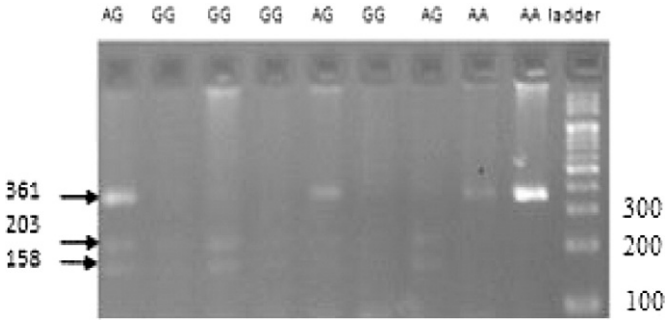
2.3.1. The chromosome separation and, rs2243827 identification

The ARMS-PCR reaction was performed in two microtubes (25 μl) containing the genomic DNA sample (0.2 μg), allele specific primer (1 μM, CCTTTCACAGGAACCCTGGATCAA and CCTTTCACAGGAACCCTG GATCAC), common primer (1 μM, CCCTAGCCTCTAGCCACATCATCAA), $MgCl_2$ (1.5 mM), and Taq polymerase (1 U). The temperature cycles (n = 35; 95 °C for 30 s, 56 °C for 50 s and, 72 °C for 60 s) were followed after incubation at 95 °C for 5 min and, a final extension for 7 min at 72 °C. The ARMS-PCR product (361 bp) was determined on the agarose gel (2%) so that in the rs2243827 heterozygote subjects (AC), it was detected in both microtubes while in the homozygotes (AA and CC) it was only amplified in a microtube.



2.3.2. The rs2333227 identification and haplotype detection

The ARMS-PCR product (361 bp) was digested by SsiI enzyme (Fermentas) so that in the AA homozygotes a fragment (361 bp), in the GG homozygotes two fragments (203 and 158 bp) and, in the AG heterozygotes three fragments (361, 203 and 158 bp) were detected for the rs2243827 AA and CC homozygotes on the agarose gel (2.5%).



2.3.3. Quality control

Two mismatches (bases 8 and 23) were substituted at the specific primer sequences to minimize non-specific reactions. The cutter efficiency (SsiI) was improved with the enzyme concentration gradients. Furthermore, one homozygote sample (GG) as control was always repeated in each run.

2.4. MPO transcription factor network

The transcription factor elements (similarity > 70%) were predicted at the flanking regions of polymorphic sites, based on the TRANSFAC and JASPAR profiles. The binary data on the MPO interaction was extracted from protein–protein interaction (PPI) databases. In addition, the transcription factors related to the MPO 5′UTR region were searched from ChEA database (containing ChIP-X experiments), and were used to seed the primary network. In order to create the curated network, the predicted transcription factors and the MPO interactions were limited for human data and, were merged with the primary network. The obtained scores from STRING database (string-db.org) were used for the network edges. Finally, the tab-limited data was imported and, was visualized with Cytoscape (3.1.1) plugins.

2.5. Statistics

Data analysis was performed using SPSS software (ver. 18 Inc., Chicago, IL, USA). The parametric distribution was evaluated with the Kolmogorov–Smirnov test. The group differences were evaluated with Student-t and chi-square tests. The linear and binary logistic regression analyses were performed on the study parameters. Odds ratio (OR) with 95% confidence interval (CI) adjusted for age, sex and BMI was calculated between the control and patient groups. P value less than 0.05 was considered to be significant.

Table 1
Characteristics of study population.

Parameter	Control (n = 71)	Patient (n = 85)	P-value
Sex (male/female)	23/48	63/22	0.0001
Age (year)	56.63 ± 12.52	62.00 ± 10.77	0.005
Body Mass Index (kg/m^2)	27.17 ± 6.77	25.30 ± 4.62	0.07
Systolic blood pressure (mm Hg)	126.46 ± 26.35	130.27 ± 20.65	0.31
Diastolic blood pressure (mm Hg)	77.56 ± 19.29	80.09 ± 13.44	0.33
Smoking (yes/no)	8/63	29/56	0.001
LDL (mg/dl)	99.39 ± 27.05	115.51 ± 31.09	0.001
HDL (mg/dl)	41.21 ± 7.44	40.61 ± 12.89	0.71
Triglyceride (TG) (mg/dl)	153.11 ± 75.47	171.10 ± 90.51	0.34
Total cholesterol (mg/dl)	174.85 ± 64.89	186.20 ± 85.44	0.05
MPO (ng/ml)	4.81 ± 1.18	4.58 ± 1.64	0.3
Anti-MPO[a] (ng/ml)	1.039–3.98	1.18–5.33	0.217[b]

[a] K–S test (P = 0.005).
[b] Mann–Whitney U test.