The Egyptian Journal of Medical Human Genetics 18 (2017) 359–363

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The Egyptian Journal of Medical Human Genetics

journal homepage: www.sciencedirect.com

Original article

Association assessment of *platelet derived growth factor B* gene polymorphism and its expression status with susceptibility to coronary artery disease

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ARTICLE INFO

Article history:
Received 28 February 2017
Accepted 14 March 2017
Available online 21 April 2017

Keywords:
Coronary artery disease
PDGF-B
Polymorphism
Genotyping
mRNA expression

ABSTRACT

Background: Coronary artery disease (CAD) is the most frequent cause of morbidity and mortality in the world and it is known as a multifactorial disorder which is influenced by both genetic and environmental factors. Based on different assays, the *platelet derived growth factor B* (PDGF-B) gene is shown to be amongst the inflammation inducers involved in different pathological conditions such as atherosclerosis.
Aim: In this case-control study we have examined the association of the functional *PDGF-B* +286 and +1135 polymorphisms and its expression status with susceptibility to CAD.
Subjects and methods: Study groups included 369 patients with CAD and 413 healthy individuals. Genotypic and allelic frequencies of *PDGF-B* +286 and +1135 polymorphisms were determined by the SSP-PCR method. *PDGF-B* expression status was identified by quantitative real-time PCR.
Results: The analysis of genotyping results revealed CAD patients had an increased frequency of *PDGF-B* +286A>G A/A genotype in comparison with healthy individuals. Furthermore, we found that the *PDGF-B* expression level in CAD patients group is nearly 2-folds greater than its level in control group.
Conclusion: There is probably a relationship between variations in *PDGF-B* gene and CAD influence. The increase in *PDGF-B* gene expression may has a role in susceptibility to CAD.
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1. Introduction

Coronary artery disease (CAD) is the most frequent cause of disability and the world's major cause of death. The CAD is a complex chronic multifactorial disorder that results from obvious genetic susceptibility and environmental risk factors and their interactions [1] and starts during adolescence and slowly progressing during life. Family history is regarded as one of the important autonomous CAD risk factors, and identifying the susceptibility genes for this frequent and complex disease, is a crucial goal [2]. CAD is defined as narrowing of epicardial coronary arteries induced by a build-up of atherosclerotic plaques. Inflammation is considered as one of the main processes contributing to atherogenesis [3]. According to National Heart, Lung, and Blood Institute in 2010 the prevalence of CAD was lower in women with age <60 years than men with same age. The CAD rate increases in both genders after age 60 years, especially in men who are living in the '80 s [4]. Among the different risk factors associated with CAD, age, gender, cigarette smoking, hypertension and hyperlipidemia are more effective. The manifestation of fibrous atherosclerotic plaque results from modification or acceleration of multiple inflammatory-fibro proliferative responses. The risk factors and the vascular smooth muscle cells are the central cell component involved in these responses. CAD is approximately 40–60% heritable, but its genetics mechanism is not completely clear. Genetics-epidemiological and twin studies have revealed the role of genetics factors in the atherosclerosis development. Moreover, case-control studies on polymorphisms have identified genes associated with increased risks to CAD [5,6].

The *Platelet derived growth factor B* (PDGF-B) gene locus is located on 22q13.1 chromosome, producing a cationic hydrophilic 30 KD protein which binds to specific receptors, and now is considered as one of the best characterized growth factor-receptor systems. PDGF-B was described approximately 40 years ago as a potent mitogen and chemo-attractant serum factor, and was elicited from the alpha-granules of the platelets. This protein stimulates the proliferation of mesenchymal original cells such as fibroblasts and vascular smooth muscle cells. Different assays have

Peer review under responsibility of Ain Shams University.
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http://dx.doi.org/10.1016/j.ejmhg.2017.03.004
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shown that PDGF contributes to different pathological conditions such as atherosclerosis, fibrosis, restenosis, glomerulonephritis, liver cirrhosis, pulmonary fibrosis and cancer. The conventional PDGF-A and PDGF-B, and more recently discovered PDGF-C and PDGF-D polypeptides, constitute four members of PDGF family [7,8]. Four disulfide-bonded homodimers of PDGF protein including PDGF-AA, PDGF-BB, PDGF-CC and PDGF-DD, and one heterodimer, PDGF-AB are biologically active proteins. These proteins act through structural activation of two tyrosine kinase receptors, PDGFRα and PDGFRβ [9,10]. PDGF is expressed by cells which related to mesenchymal cells expressed PDGF receptors, including megakaryocytes that are the precursor of platelets cells. These expressional patterns suggest that PDGF mediates paracrine signaling. Although some recent case-control studies have been performed on gene polymorphisms in CAD at populations with different geographic and genetic diversity [5,6]. No studies have been examined the role of these *PDGF-B* functional polymorphisms in susceptibility to CAD in Iranian population yet. Therefore, we conducted a case-control study to analyze the association of the *PDGF-B* functional polymorphisms (positions of +286 and +1135 from transcription start site) and *PDGF-B* expression level with susceptibility to CAD in Iranian population.

2. Subjects and methods

2.1. Study subjects

In this prospective study, we randomly screened 369 CAD patients and 413 control individuals (mean age 30–60) from the northeast of Iran to analyze the genetic variations in the PDGF-B gene +286 and +1135 loci. For selecting the CAD patients, two expert cardiologists confirmed the occurrence of CAD, according the clinical and paraclinical findings, invasive angiography, as ≥70% stenosis in at least 1 major epicardial coronary artery. Control subjects were selected randomly among healthy individuals with a normal angiography (≤15% stenosis) and with no visible autoimmune or inflammatory disorders. This work has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans, and was approved by the local Ethical Committee. The informed written consent was obtained from all recruited individuals. None of the approached subjects refused to participate. Expression assay also was randomly performed on 80 CAD patients and 80 control individuals among the same individuals with the same age condition.

2.2. DNA extraction and genotyping

Genomic DNA was extracted from 2 ml peripheral whole blood by a standard protocol [11]. Briefly, red blood cells were lysed three times with a buffer containing ammonium chloride, potassium dihydrogen phosphate, and disodium hydrogen phosphates (Sigma, Munich, Germany). Then, SDS (10%), EDTA, and 10 μl proteinase K (Sigma, Munich, Germany) were added to the pellet that was incubated for 1 h at 65 °C. After incubation, a phenol/chloroform/isoamyl alcohol mix (Sigma, Munich, Germany) was added to samples. The mix was then centrifuged. Isopropanol and sodium acetate (Merck, Kenilworth, US) were added to the supernatant to visualize and precipitate the DNA, and DNA was extracted after centrifugation. Using a spectrophotometer (Techne, Staffordshire, UK), the amount of DNA for each sample was determined by measuring the optical density at 260 nm wavelength. DNA samples were aliquoted in graded distilled water and stored at −80 °C until used. The sequence-specific primer-polymerase chain reaction (SSP-PCR) method was used for genotyping. To detect the PDGF-B polymorphism at +286 and +1135 position, 100 ng of genomic DNA was amplified in a 15 ml reaction mix with 13 μl master mix containing 20 μm dNTP, 1X ready-load PCR buffer, DMSO, one unit Taq polymerase (GENETBIO, Daejeon, South Korea), 6 μm human growth hormone primer (Eurofins, Ebersberg, Germany) as internal control, and 30 μM of each specific primer (Eurofins, Ebersberg, Germany). The PCR reaction for SNPs of +286 and +1135 were performed in a Thermal Cycler (Techne, Staffordshire, UK), with the one program that described previously [12]. The primer sequences used for both polymorphisms are shown in Table 1. The PCR products were electrophoresed on a 1.5% agarose gel (Merck, Germany), and bands were visualized with a gel documentation system (Uvitec, Cambridge, UK).

2.3. Quantitative real-time PCR

Total RNA was extracted from cultured leukocytes of 6 ml of peripheral whole blood. The transcriptor First Strand cDNA Synthesis Kit (Roche, Grenzach-Wyhlen, Germany) was used to converting 1 μg of total RNA into cDNA. Quantitative real-time polymerase chain reaction of the PDGF-B gene was performed in duplicates in ABI Prism 7300 Sequence Detection System using SYBR Green (Applied Biosystems, Foster City, US) according to the manufacturer's instructions with the following optimized PCR conditions (94 °C 10 min, and 94 °C 30 s, 58 °C 1 min, 72 °C 45 s for 35 cycles, then 10 min extension at 72 °C). All thresholds cycle (Ct) of PDGF-B gene normalized with PGK1 cDNA. The used primers are shown in Table 1. Primers were designed based on sequences from the GenBank and to span exon-intron junctions to prevent amplification of genomic DNA. The mRNA level was calculated using the $2^{-\Delta Ct}$ method [13].

2.4. Statistical analysis

After entering data in a SPSS v-16 program, the means of parametric variables were calculated. Data are presented as Mean ± SD for parametric variables and as percentages for non-parametric values. Allele and genotype frequencies were calculated and compared between groups by non-parametric tests followed by Fisher's exact analysis using STATA v-8 (CA, US). P-values were determined, and those less than 0.05 were considered to be significant.

3. Results

A total of 369 CAD patients and 413 healthy individuals were successfully genotyped for SNPs, PDGF-B +286A>G and PDGF-B +1135A>C. In both SNPs, deviation from the Hardy-Weinberg equation was not observed in either patient group (PDGF-B +286A>G: $\chi2 = 0.263$, df = 1, p = 0. 607 and PDGF-B +1135A>C: $\chi2 = 0.119$, df = 1, p = 0. 730), or the control group (PDGF-B +286A>G: $\chi2 = 0.096$, df = 1, p = 0.765 and PDGF-B +1135A>C: $\chi2 = 0.864$, df = 1, p = 0.352). Complementary data including the allele and genotype frequencies of PDGF-B +286 are shown in Table 2. According to the data analysis, there is a significant association between A/A genotype and CAD disease [OR (95%CI): 1.58 (1.05–2.36), p = 0.032]. The results also indicated that allele A is significantly associated with the CAD disease [OR (95%CI): 1.26 (1.03–1.54), p = 0.021]. As the Table 3 showed, allelic and genotypic frequencies of PDGF-B +1135 polymorphism did not show any significant statistical association with CAD. To investigate the inheritance model of SNPs at *PDGF-B* gene, three models of recessive, dominant and co-dominant were considered and no significant association was seen in inheritance models, in both SNPs (Tables 2 and 3). Combined genotypes distribution of PDGF-B

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