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Inflammatory markers in men with metabolic syndrome genotyped to -455G/A polymorphism in beta-fibrinogen gene

Markery stanu zapalnego a polimorfizm -455G/A genu dla fibrynogenu u mężczyzn z zespołem metabolicznym

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Summary

Low-grade inflammation is a characteristic feature of metabolic syndrome and significantly contributes to cardiovascular risk in affected subjects. The level of inflammatory process development is influenced by different metabolic and genetic factors. In the present study the association between -455G/A genetic variants in fibrinogen gene and plasma levels of CRP and adhesion molecules was assessed in 61 men with newly diagnosed metabolic syndrome. DNA was amplified by polymerase chain reaction and incubated with the restriction enzyme. Circulating levels of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) were measured by enzyme-linked immunosorbent assay (ELISA). Serum high-sensitivity CRP was determined by immunoturbidimetric method. The -455G/A genotype frequencies were 60%, 22%, and 7% for GG, GA, and AA, respectively. It was found that fibrinogen, CRP and adhesion molecules concentrations were enhanced in A-allele carriers as compared to GG homozygotes, and these differences were not related to BMI, and serum lipids and glucose levels.

Key words: genetic variants, fibrinogen, CRP, adhesion molecules, metabolic syndrome

Streszczenie

Rozwój procesu zapalnego niskiego rzędu jest cechą charakterystyczną pacjentów z zespołem metabolicznym i w istotny sposób determinuje ryzyko wystąpienia incydentów klinicznych w obrębie układu sercowo-naczyniowego. Poziom rozwoju procesu zapalnego zależy od wielu czynników metabolicznych i genetycznych. W prezentowanej pracy oceniano związek między polimorfizmem -455G/A genu dla fibrynogenu a poziomem CRP i molekuł adhezyjnych. Badaniem objęto 61 mężczyzn z rozpoznaniem zespołem metabolicznym. Poziom cząsteczek adhezyjnych (VCAM-1 i ICAM-1) określano metodą immunoenzymatyczną (ELISA). Stężenie CRP oznaczono metodą immunoturbidymetryczną. Polimorfizm -455G/A genu dla fibrynogenu wykrywano metodą PCR z wykorzystaniem enzymu restrykcyjnego. W badanej grupie mężczyzn z zespołem metabolicznym częstość genotypu GG wynosiła 60%, genotypu GA – 33%, zaś genotypu AA – 7%. Zaobserwowano wyższe stężenia fibrynogenu, CRP i molekuł adhezyjnych u nosicieli allele A w porównaniu z homozygotami GG. Różnice te były niezależne od wartości BMI oraz stężenia lipidów i glukozy.

Słowa kluczowe: warianty genetyczne, fibrynogen, CRP, molekuły adhezyjne, zespół metaboliczny

INTRODUCTION

Metabolic syndrome (MS) as a constellation of risk factors of cardiovascular disease, has received increased attention in the last years, because growing prevalence of MS has been noticed in epidemiological and clinical observations (1, 2). MS development is strongly related to improper diet and low physical activity, therefore obesity is one of the important features of MS. However, there is no doubt that different metabolic and genetic factors may influence both metabolic syndrome development and the level of associated

cardiovascular risk (3, 4). A role for inflammation in cardiovascular diseases development has become well established over the past decade (5). It has been also accepted that low grade inflammatory process is a key feature of metabolic syndrome strongly influencing associated cardiovascular risk (6, 7). There is no doubt that the intensity of inflammation depends on many factors (7, 8). Plasma concentrations of fibrinogen have been found to be associated with cardiovascular risk (9). However, it is uncertain whether this association is causal or reflects residual confounding of other risk

factors. Genetic variants that influence lifelong differences in plasma fibrinogen levels have not been found to be strongly associated with CVD risk, but there is a lack of data describing potential effect of such variants on inflammatory process development. Therefore, the aim of the presented study was to assess the relationship between occurrence of -455G/A genetic variants in beta-fibrinogen gene and serum levels of C-reactive protein and adhesion molecules in patients with metabolic syndrome.

EXPERIMENTAL PROCEDURES

Patients with metabolic syndrome were recruited for the study among these referred to the Outpatient Clinic of Metabolic Diseases of National Food and Nutrition Institute, who underwent a complete physical examination. Metabolic syndrome was defined according to the Third Report of the National Cholesterol Education Program's Adult Treatment Panel. MS was recognized when any three or more of following criteria were found: waist circumference > 102 cm in men and > 88 cm in women, serum triglycerides \geq 150 mg/dl, blood pressure \geq 130/85 mmHg, HDL-cholesterol < 40 mg/dl in men and < 50 mg/dl in women, serum glucose \geq 110 mg/dl

Venous blood was collected from each individual after fasting. Plasma total and high-density lipoprotein, triglycerides and glucose were measured by routine methods. CRP concentrations were determined by ultra sensitive assay from Randox. Enzyme immunoassays were used to determine serum levels of ICAM-1 and VCAM-1 (Endogen). Fibrinogen levels were assayed by immunoturbidimetric method based on reagents from DAKO. The plasma levels of adiponectin and leptin were measured by ELISA using reagent kits from Linco Research and Bio Vendor, respectively. DNA was extracted from whole blood with a DNA Blood Mini Kit (A&A biotechnology). The -455G/A polymorphism in the promoter region of the beta-fibrinogen gene was genotyped by PCR amplification of genomic DNA, followed by restriction enzyme digestion (HaeIII) and agarose gel electrophoresis according to procedure described by Thomas et al (10). Data were analyzed using InStat computer package (GraphPad Software, USA).

RESULTS

The study group consisted of 61 men with newly diagnosed metabolic syndrome. Recruited subjects were referred to the Outpatient Clinic for proper diagnosis and treatment, and they did not use lipid lowering and antihypertensive drugs. Patients with diabetes or under any treatment were not recruited for the study. Among participant 21% of smokers, 35% of ex-smokers (stopped to smoke at least 5 years before the study) and 44% of non-smokers were recognized according to self-reporting. The basic characteristic of study participants is presented in table 1.

Most of the study subjects were homozygotes for the G variant in the -455G/A polymorphism in the pro-

Table 1. Basic characteristics of study participants.

n	61
Age (years)	52 \pm 12
BMI (kg/m ²)	34.4 \pm 3.9
SBP(mmHg)	140 \pm 10
DBP (mmHg)	87 \pm 9
Total cholesterol (mg/dl)	232 \pm 27
HDL-cholesterol (mg/dl)	37 \pm 9
Triglycerides (mg/dl)	229 \pm 21
Glucose (mg/dl)	105 \pm 23
Insulin (μ U/ml)	12.9 \pm 5.1
Adiponectin (ng/ml)	14.2 \pm 3.1
Leptin (ng/ml)	25.8 \pm 4.9

moter region of the beta-fibrinogen gene (fig. 1). Only 7% of AA homozygotes were found. Therefore, for further analyses the group was divided in two subgroups: one consisted of GG homozygotes and second consisted of carriers of A allele (AA + GA). No differences in basic characteristic between GG homozygotes and A-allele carriers were found. In both subgroups were notice similar levels of BMI (34.6 \pm 3.7 vs 34.2 \pm .9, respectively), cholesterol (236 \pm 29 vs 228 \pm 25 mg/dl), HDL-cholesterol (36 \pm 10 vs 37 \pm 8 mg/dl), triglycerides (226 \pm 18 vs 231 \pm 27 mg/dl), glucose (105 \pm 18 vs 104 \pm 22 mg/dl). insulin (13.2 \pm 4.6 vs 12.7 \pm 5.3 μ U/ml), adiponectin (14.4 \pm 3.5 vs 14.0 \pm 2.8 ng/ml) and leptin (26.8 \pm 4.7 vs 24.9 \pm 5.1 ng/ml).

In A-allele carriers (AA + GA genotype) significantly higher levels of plasma fibrinogen than in GG-homozygotes were found (3.58 \pm 1.4g/l vs 3.21 \pm 1.9 g/l, p < 0.02). The percentage of smokers and non-smokers was similar in both groups, therefore smoking habits did not influence the observed differences. In addition in patients with A-allele the mean serum levels of C-reactive protein (fig. 2) and adhesion molecules VCAM-1 and ICAM-1 (fig. 3) were found to be enhanced as compared to GG-homozygotes (5.4 \pm 2.5 g/l vs 4.8 \pm 2.3 g/l, 919 \pm 84 ng/ml vs 860 \pm 72 ng/ml, 517 \pm 47 ng/ml vs 411 \pm 34 ng/ml, respectively). However, only differences in ICAM-1 levels have reached statistical significance (p < 0.01).

DISCUSSION

The prevalence of obesity not only in adults but also in adolescents and children is dramatically increasing (11). Obesity is the key cause of insulin resistance and significantly increased the risk of development of metabolic disturbances defined as metabolic syndrome (1,3,8). MS as a cluster of major cardiovascular risk factors significantly increases myocardial infarction risk (12-14). In Poland the prevalence of MS among adults is approximately 20% according to NATPOL PLUS survey (15). MS development and its relation to cardiovascular risk results from interactions between genes and life style factors (16). In CVD risk assessment different

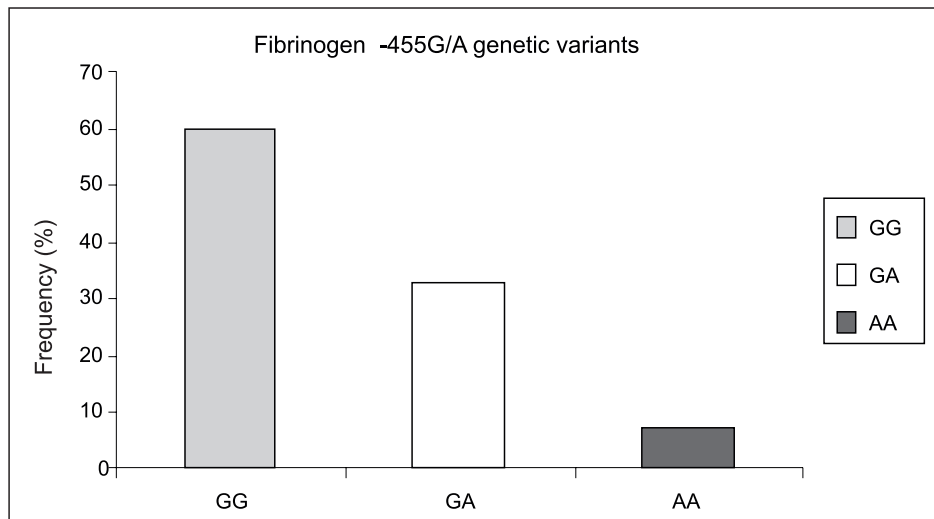


Fig. 1. Frequency of -455G/A variants in the promoter region of the beta-fibrinogen gene in men with metabolic syndrome (n = 61).

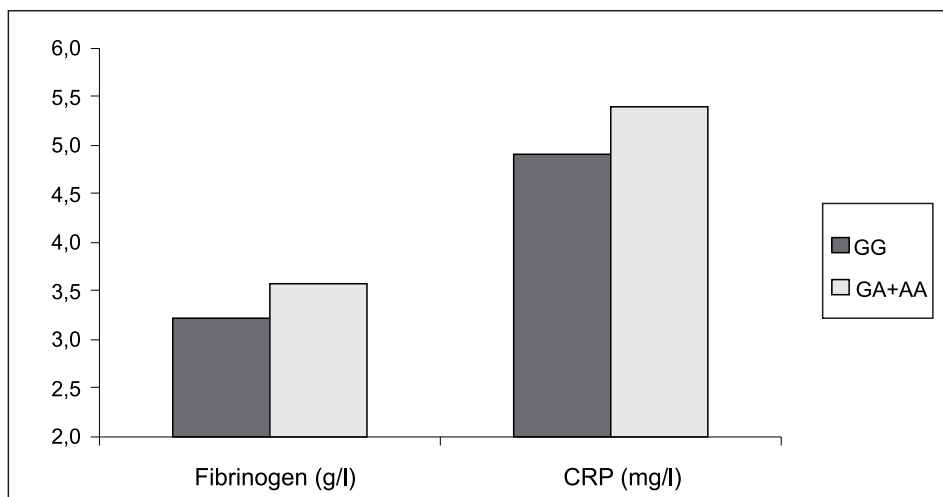


Fig. 2. Mean plasma fibrinogen and C-reactive protein levels in men with metabolic syndrome as a function of beta-fibrinogen -455G/A genotype.

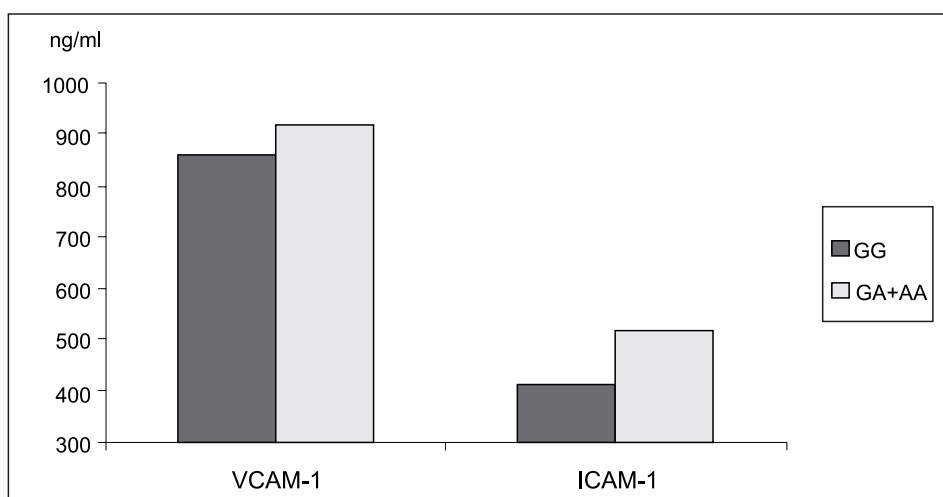


Fig. 3. Mean plasma adhesion molecules VCAM-1 and ICAM-1 levels in men with metabolic syndrome as a function of beta-fibrinogen -455G/A genotype.

markers are used and among them inflammatory markers, especially CRP has been recognized to be useful. Patients with metabolic syndrome are characterized by enhanced CRP levels (12-16). The observed CRP levels described intensity of inflammatory process, which results from the action of many factors. Among them polymorphisms of genes related directly and/or indirectly to inflammation may play an important role. In the presented study the potential influence of genetic variants of -455G/A polymorphism in beta-fibrinogen gene on the fibrinogen, CRP and adhesion molecules VCAM-1 and ICAM-1 concentrations in patients with metabolic syndrome was assessed by comparing the levels of these parameters in carriers of different genotypes, who did not differ in basic clinical characteristics. In carriers of A-allele as compared

to GG-homozygotes enhanced levels of fibrinogen, CRP, VCAM-1 and ICAM were found. The observed association between studied genetic variants and inflammatory markers was independent on BMI, blood pressure, serum lipids as well as leptin and adiponectin concentrations, because the levels of these factors were very similar in carriers of different genotypes. It may be, therefore, suggested that -455G/A polymorphism by influencing fibrinogen concentration modulate inflammatory process development. However, further studies are needed to confirm this association. Data of the role of polymorphisms in other genes and interactions between different genetic and environmental factors are also awaited to explain the role of genetic factors in cardiovascular disease risk in patients with metabolic syndrome.

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