Association of myeloperoxidase gene variation with carotid atherosclerosis in patients with essential hypertension

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Abstract. The aim of this study was to investigate the association between myeloperoxidase (MPO) gene polymorphisms and carotid atherosclerosis (AS) in patients with essential hypertension (EH). A total of 214 patients with EH were divided into an AS and a non-atherosclerosis (non-AS) group. MPO gene polymorphisms in EH were detected using PCR. The frequency of increased intima-media thickness and the occurrence of plaque in the carotid artery in the AS group was higher compared to the non-AS group. The frequency of the GC genotype in the AS group was significantly higher compared to the non-AS group. The frequency of carotid AS is higher in patients with hypertension, which is related to the MPO gene polymorphism.

Introduction

Atherosclerosis (AS) is an inflammatory disease. In the disease development process, macrophages, which are cells of the immune system, are regarded as one of the inflammatory components of AS (1). Once macrophages are activated, phagocytes generate reactive oxygen species, which induce destructive oxidative stress to the vascular wall (2). In AS, chronic inflammation of the arterial wall causes an exaggerated phagocyte response and generates oxidative low density lipoprotein (LDL). LDL granules cause AS. Due to the continuous inflammation, phagocyte activity is believed to cause inflammatory reactions in the vascular wall, including cellular activation and proliferation, leading to further damage (3-6).

Myeloperoxidase (MPO) is a heme protease containing a heme prosthetic group secreted by neutrophilic granulocytes, monocytes and macrophages of certain tissues, and it is one of the heme peroxidase superfamily members (7). Nicholls and Hazen (8) found that although MPO was capable of achieving a certain immune function, MPO also caused a harmful effect to the artery wall, and oxidation products generated by its catalysis and macrophages were aggregated at the AS site. Eiserich *et al* (9) found that MPO was rapidly absorbed by endothelial cells through the cell transformation process and aggregated in the endothelial gap, participating in the effect of nitric oxide on the vascular wall. MPO weakened the nitric oxide-dependent vasodilatation reaction, reduced bioavailability of nitric oxide in mature cells and regulated the vascular inflammatory reaction by adjusting the bioavailability of nitric oxide.

At present, it has been found that MPO is associated with the incidence of leukemia, nephritis, polyangitis, tumors and AS (10). New studies suggest that MPO is one type of inflammatory marker, and its level in plasma may predict the incidence risk of future coronary heart disease in a healthy population (11). It may also reflect the extent of heart failure severity (12) in patients with chronic heart failure and the risk of adverse cardiac events (13). With the increased amount of MPO research, it has been shown that MPO gene polymorphisms cause differences in the susceptibility of individuals to certain diseases, and that MPO is closely related to the occurrence and development process of a variety of human diseases (14). Our research found that MPO gene polymorphisms are associated with essential hypertension (EH), and this study further investigates the relationship of carotid atherosclerosis (AS) of EH patients with MPO gene polymorphisms.

Patients and methods

Patients. This study was a single center case-control study. The study participants were patients hospitalized and examined at the People's Hospital of Gansu Province (December 2007-September 2008). There were 214 cases of hypertension, including 128 male and 86 female cases. The average age of the patients was 58.96±12.92 years. According to ultrasound findings of the common carotid artery, the patients were divided into AS and non-atherosclerosis (non-AS) groups. Hypertension diagnosis complied with the 'Chinese Hypertension Prevention and Cure Guideline in 2005', and patients with secondary hypertension, cardiomyopathy, congenital heart disease, rheumatic valvular heart disease, coronary heart disease, lung cancer, Alzheimer's disease, chronic granulomatous disease, benzolism and leukemia were excluded. This study was conducted in accordance with the declaration of Helsinki, and

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was conducted with approval from the Ethics Committee of the People's Hospital of Gansu Province. Written informed consent was obtained from all participants.

Detection of clinical biochemical indicators. Each patient fasted for 12 h, and 3 ml anterior cubital vein blood was drawn in the morning and placed into a dry tube. The standard ELISA method was used to detect plasma total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and LDL.

Color doppler ultrasound measurement indicators and methods. All patients lay on their backs, and conventional ultrasonography of the carotid artery was conducted. A ProSound α10 (Hitachi Aloka, Tokyo, Japan) Color Doppler ultrasound imaging instrument was used. The probe frequency was 7.5 Hz, and axial resolution was 0.1 mm (15). The measured AS indicators included: intima-media thickness (IMT) detection of the common carotid artery. Longitudinal ultrasound images of the common carotid artery posterior wall showed two parallel light lines, and the distance between the two lines was the IMT of the carotid artery. Normal common carotid artery had an IMT of <1 mm. Determination of plaques: plaques were defined as restricted acoustic echo from the lumen, IMT ≥1.3 mm (16). Internal diameter (D) of carotid artery: the vertical distances between anterior and posterior intima surfaces at the bilateral thickest positions of IMT. The mean was obtained.

DNA extraction. A total amount of 3 ml anterior cubital vein blood was drawn in the morning and placed into a dry tube, and anticoagulation was conducted with 2% EDTA to separate leukocytes. In addition, the conventional phenol/chloroform method was used to extract DNA.

MPO genotyping. It was necessary for this research to obtain the upstream base sequence of the MPO gene using the National Center for Biotechnology Information of America (NCBI) GenBank. Primer 5.0 software was used to design the primers with restriction sites complying with amplification. Primer sequences: upstream primer, 5'-CGG TAT AGG CAC ACA ATG GTG AG-3'; downstream primer, 5'-GCA ATG GTT CAA GCG ATT CTT C-3'. The primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). A DNA fragment containing the *AciI* restriction site was amplified, and the length of the amplified DNA fragment was 350 bp. The total PCR system (50 μl) included 10X reaction buffer 5.0 μl, dNTP 200 μmol/l, $MgCI_2$ 2.5 mmol/l, primers 15 pmol, template 200-500 ng and Taq DNA polymerase 1.0 units (Promega, Madison, WI, USA), respectively. Amplification parameters: predenaturation for 4 min at 94˚C, denaturation for 1 min at 94˚C, annealing for 1 min at 56˚C, extension for 1 min at 72˚C for 35 cycles, and extension for 7 min at 72˚C. Subsequently, 10 μl PCR product, 0.5 μl restriction enzyme *AciI* (BioLab, Beijing, China), 2 μl NE buffer 10X and 7.5 μl sterile deionized double distilled water were digested by enzymes at 37˚C overnight. Restriction products were electrophoresed with 2.5% agarose gel (containing 0.5 μg/ml ethidium bromide), and a gel imaging system was used to capture images to determine the genotypes. In this study, a total of three genotypes were detected: GG, GA and AA. For the GG genotype, the sizes of three obtained fragments following restriction with the AciI incision enzyme were 169, 120 and 61 bp, respectively. For the GA genotype, the sizes of the four obtained fragments following restriction were 289, 61, 169 and 120 bp, respectively. For the AA genotype, the sizes of the two obtained fragments following restriction were 289 and 61 bp, respectively (Fig. 1).

Table I. Comparison of MPO gene in patients with hypertension between AS and non-AS groups (n, %).

Group	Case	GG	AA + GA	F-value	P-value
AS	172	120 (69.8)	52 (30.2)		
Non-AS	42	26 (61.9)	16 (38.1)	61.56	0.00

MPO, myeloperoxidase; AS, atherosclerosis; non-AS, non-atherosclerosis.

Table II. Comparison of MPO allele between AS and non-AS groups (n, %).

Group	G	A	P-value
AS	292 (0.846)	104 (0.154)	
Non-AS	68 (0.768)	32 (0.232)	0.046

MPO, myeloperoxidase; AS, atherosclerosis; non-AS, non-atherosclerosis.

Statistical analysis. The genotype distributions in all patients should comply with the Hardy-Weinberg balance. SPSS 11.0 statistical software was used, and all data were expressed as the means ± standard deviation (means ± SD. The homogeneity test for variance was used for data with normal distribution, t-test was used for comparison between the two groups, and one-way ANOVA was used for comparison among multiple groups. In addition, χ^2 test of four-grid table or of paired data was used for count data. P<0.05 was considered to indicate a statistically significant difference.

Results

Gene comparison of patients with hypertension between the AS and the non-AS groups. Carotid artery intima-media thickening frequency of hypertension patients of the AS group markedly increased, and the GG genotype distribution frequency of the AS group was significantly higher compared to that of the non-AS group. There was a significant difference between the two groups (F=61.56, P=0.00) (Table I).

Comparison of alleles between AS and non-AS groups. For comparison of the allele frequency distribution between the AS and non-AS groups, the A allele frequency of the AS group was lower compared to that of the non-AS group, and there was a significant difference between the two groups (0.154 vs. 0.232, P=0.046) (Table II).

Table III. Association of gene variation with clinical variables, such as carotid artery ultrasound in AS and non-AS groups mean ± SD).

	GG		AA + GA	
Variables	AS (n=120)	Control (n=26)	AS (n=52)	Control (n=16)
BMI (kg/m^2)	24.12±2.29	23.76±2.18	24.31±2.66	23.38±2.18
SBP (mmHg)	144.15±15.72	139.16±13.97	142.88±14.13	143.27±14.97
DBP (mmHg)	93.83±9.70	93.62±8.58	94.16±11.01	95.58±10.32
TC (mmol/l)	5.04±0.85	4.84±0.92	5.10±0.95[a]	4.98±0.93
TG (mmol/l)	2.08±1.26[a]	1.84±0.86	1.98±0.86	1.89±0.90
HDL-c (mmol/l)	1.39±0.30	1.45±0.32	1.36±0.25	1.41±0.29
LDL-c (mmol/l)	3.22±0.78	3.03±0.83	3.27±0.84	3.12±0.85
CCA (mm)	7.57±0.77[a]	6.79±0.87	7.09±1.30[a,b]	6.85±0.81
IMT (mm)	0.98±0.26[a]	0.79±0.23	0.97±0.17[a]	0.77±0.15

[a]P<0.05 vs. the normal control group; [b]P<0.05 vs. the GG genotype group. AS, atherosclerosis; non-AS, non-atherosclerosis; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; CCA, common carotid artery; IMT, intima-media thickness.

Table IV. Comparison of carotid artery plaque between AS and non-AS groups (n, %).

Group	Case	Control	AS	F-value	P-value
Plaque	162	45 (25.9)	120 (74.1)		
Without	112	69 (61.8)	43 (38.2)	31.804	0.00

AS, atherosclerosis; non-AS, non-atherosclerosis.

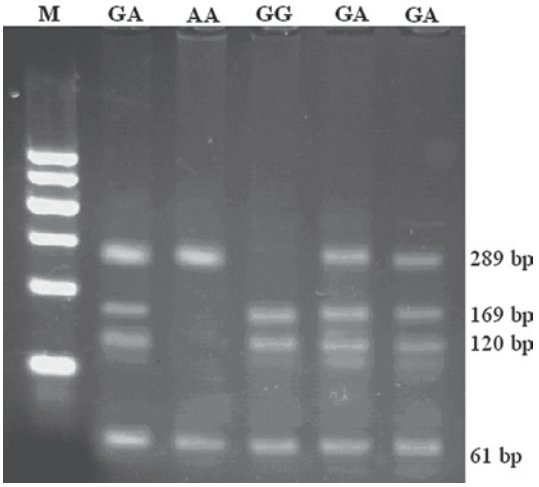

Figure 1. Myeloperoxidase (MPO)-463G/A polymorphism digested by enzymes. Genotype GG included 169, 120 and 61 bp DNA fragments; genotype GA included 289, 61, 169 and 120 bp DNA fragments; genotype AA included 289 and 61 bp DNA fragments. M, marker.

Relationship of MPO-463G/A gene variation with internal D of the carotid artery, IMT and other clinical variables. In patients with the GG genotype, TG level, internal D of the carotid artery and IMT of the AS group were higher than those of the control group, and there were significant differences (P<0.05). In the AA and GA genotypes, TC, internal D of the carotid artery and IMT were higher than those of the control group, and there were differences between the 2 groups (P<0.05). Among different genotypes, TG level, internal D of carotid artery and IMT of the AS group were higher compared to those of the non-AS group, and there were significant differences between the 2 groups (P<0.05) (Table III).

Comparison of carotid artery plaque of EH patients between AS and non-AS groups. The incidence rate of carotid artery plaque in the AS group significantly increased, while the incidence rate of carotid artery plaques in the AS group significantly reduced, and there was a difference between the two groups (P=0.00) (Table IV).

Discussion

The human MPO gene is located in q23 to q24 of chromosome 17, containing 12 exons and 11 introns, and its length is approximately 14,638 bp and its gene expression is regulated by growth factor III. It is known that the MPO gene firstly expresses a precursor protein with a relative molecular weight of 89x10^3. Following translation, it is cut into α and β subunits. Subsequently, the two subsets polymerize into the mature MPO molecule. After the sugar chain is bound, it finally forms functional MPO. The MPO gene DNA sequence changes due to defects of MPO during the expression process, and its activity is influenced. Polymorphisms of the MPO gene also affect the transcription and expression, and have an influence on the susceptibility of the body to a variety of diseases.

Since MPO gene polymorphisms were first reported in 1993, research on these gene polymorphisms has increased. MPO gene polymorphisms are related to the occurrence and development of a variety of diseases. Skuladottir *et al* (17) showed

that the MPO gene 463G/A polymorphism AA/GA genotype was associated with a decreased susceptibility to lung cancer. Hung *et al* (18) found that the GG genotype was involved in the incidence of bladder carcinoma. Certain studies indicated that MPO gene polymorphisms were associated with the incidence of gastric cancer and breast carcinoma (19,20). Asselbergs *et al* (21) reported that the patients with the G/G genotype and coronary heart disease suffered from more cardiovascular events than the patients with the G/A and A/A genotypes and suggested that the G/G genotype coronary heart disease and family history were the main risk factors predicting cardiovascular events. Sugiyama *et al* (22) indicated that the pathogenic mechanism of MPO gene polymorphisms possibly lies in the fact that MPO participated in LDL oxidation, and high-level MPO increased plaque vulnerability to cause stable plaques to become instable plaques and increase AS incidence. Mäkelä *et al* (23) found that the MPO gene 463G/A polymorphism had an age-dependent relationship with aorta fibrous degeneration and calcified atherosclerotic disease. It was found from single factor and linear regression analyses that for males aged less than 53 years old, the MPO gene (A/A and A/G) had low expression in aorta fibrous degeneration and calcified atherosclerotic disease, while it had high expression in male patients aged 53 years or greater suffering from thoracic aorta and abdominal aorta AS. Nikpoor *et al* (24) observed that French Canadians more highly expressed MPO alleles G and A. It was thought that the potential role of MPO in the development of coronary arteriosclerosis possibly promoted the effect of the MPO gene 463G/A polymorphism on the incidence of coronary artery diseases. Exner *et al* (25) found that when the HDL level was less than 49 mg/dl, MPO was associated with the occurrence of carotid AS. Hoy *et al* (26) reported that MPO content in plasma was not associated with the MPO gene 463G/A polymorphism, while the subjects carrying the A allele presented more high-level risk factors causing AS than the subjects carrying the G allele, such as serum TG, TC, LDL and apolipoprotein B. Mäkelä *et al* (27) reported that the MPO gene was not associated with type 2 diabetes mellitus. In the control group, the comparison result of carotid artery IMT showed that the MPO gene AG/AA genotype was approximately 7.3% higher than the GG genotype, indicating that MPO gene variation changed carotid artery IMT. Mateo *et al* (28) reported that the recent hypertension guidelines added carotid artery IMT as the marker of end organ damage. In particular, average IMT of the carotid artery could better evaluate AS. Hypertension is the main risk factor promoting AS occurrence and development, while stenosis caused by renal arteriosclerosis may cause secondary hypertension. Therefore, hypertension and AS have an interactional relationship. Hypertension and AS have similar arterial inflammatory pathological changes, and both cause inflammation and endothelial cell damage to the vascular wall (29). As the main risk factor of AS, hypertension is capable of quickening the occurrence and development of AS, while the relationship of MPO gene polymorphisms with carotid AS of EH patients is not still reported.

The morphology of common carotid artery is straighter, location is more superficial, and common carotid artery is more parallel to the skin surface. IMT is also easily measured, the measurement value is reliable and repeatability is good. Therefore, IMT may be widely used in the clinic. An epidemiological study (30) and an intervention test (31) have proven that the IMT of carotid artery is an important marker of AS by means of high-resolution ultrasound detection. Moreover, ultrasound detection of carotid IMT has been used to predict apoplexy and myocardial infarction (32). Many experiments, but not all, (33,34) have proven that blood pressure plays an important role in the occurrence and development of IMT.

This study carried out fractional analysis of hypertension patients and showed that the IMT of the carotid artery and plaque frequency of EH patients significantly increased, and the genotype GG distribution frequency of patients in the AS group was higher compared to that of the non-AS group. In this study, it was found that IMT of the common carotid artery of EH patients of the AS group was thicker compared to that of the non-AS group, and there was a significant difference for frequency distribution between the GG and AA+GA genotypes. The incidence rate of hypertension patients with the GG genotype was high. In addition, further comparison of carotid plaques of hypertension patients showed that the carotid plaque incidence rate of the MPO gene GG genotype patients in the AS group increased. Thus, it may be observed that carotid AS is related to MPO gene polymorphism.

In conclusion, MPO gene polymorphisms in AS are closely related to the occurrence and development of cardiovascular disease. Although our study suggests that hypertension genetic susceptibility of Chinese patients is related to MPO gene polymorphisms, research results in different ethnicities and populations may be inconsistent. This study analyzed the relationship of EH and MPO gene polymorphisms. As there are fewer genotypes, it is necessary to define the association of genotypes with EH by analyzing larger sized samples.

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