IVYSPRING INTERNATIONAL PUBLISHER

International Journal of Medical Sciences

2013; 10(7):840-847. doi: 10.7150/ijms.5728

Research Paper

Associations of MMP1, 3, 9 and TIMP3 Genes Polymorphism with Isolated Systolic Hypertension in Chinese Han Population

Rong Huang[1,3,5*], Liehua Deng[2*], AnNa Shen[1,3], Jian Liu[1], Hao Ren[1,3,4✉], Ding-Li Xu[1,3 ✉]

1. Department of Cardiology, Nanfang Hospital, Southern Medical University, Guangzhou, China;
2. Department of Critical Care Medicine, Affiliated Hospital, Guangdong Medical College, Zhanjiang, China;
3. Key Laboratory of Organ Failure, Ministry of Education, China;
4. Department of Nephrology, Nanfang Hospital, Southern Medical University, Guangzhou, China;
5. Gerontal Cardiology Department of People's Hospital of Xinjiang Uygur Autonomous Region, Urumqi, China.

* Rong Huang and Liehua Deng contributed equally to this work.

✉ Corresponding author: Professor Dingli Xu. Department of Cardiology, Nanfang Hospital, Southern Medical University, 1838 Northern Guangzhou Ave, Guangzhou, Guangdong 510515, China. E-mail address: dinglixu@fimmu.com. Telephone: +86-020-61641493. Fax: +86-020-61360416; or Associate Professor Hao Ren. Department of Nephrology, Nanfang Hospital, Southern Medical University, 1838 Northern Guangzhou Ave, Guangzhou, Guangdong 510515, China. E-mail address: renhao67@yahoo.com.cn. Telephone: +86-020-61641515. Fax: +86-020-61360416.

Received: 2012.12.18; Accepted: 2013.04.23; Published: 2013.04.30

Abstract

Background and aims: Large artery stiffness and endothelial dysfunction are the predominant characteristic of isolated systolic hypertension. Recently studies have revealed MMP1, 3, 9 and TIMP3 Genes polymorphism were associated with arterial stiffness, but the relationship with isolated systolic hypertension were not further studied. This study was to investigate the associations of MMP1,3,9 and TIMP3 Genes polymorphism with isolated systolic hypertension. **Methods:** We identified the genotype of the genes in 503 patients with isolated systolic hypertension, 481 essential hypertension patients with elevated diastolic blood pressure and 244 age-matched normotensive controls for 5 SNPs and detected the brachial–ankle pulse wave velocity, flow-mediated dilatation, endothelin-1 and nitric oxide among the participants. **Results:** Multinomial logistic analyses showed that the 5A allele of rs3025058(5A/6A) in MMP3 and the T allele of rs3918242(C-1562T) in MMP9 were significantly associated with isolated systolic hypertension after adjusted by age, triglyceride, low-density lipoprotein (P<0.001, $Pcorr$<0.003; P=0.009, $Pcorr$=0.027). The 5A/G/C and 6A/A/T haplotypes were significantly associated with isolated systolic hypertension (Permutation p=0.0258; Permutation p=0.000002). In addition, the brachial–ankle pulse wave velocity of different genotypes for the 5A/6A and C-1562T polymorphisms was significantly highest in 5A or T homozygotes (P<0.01), however, the flow-mediated dilatation and nitric oxide were markedly lowest in 5A or T homozygotes (P<0.01). **Conclusion:** MMP3 and MMP9 genes variant seem to contribute to the development of isolated systolic hypertension by affecting arterial stiffness and endothelial function.

Key words: artery stiffness, endothelial function, gene, isolated systolic hypertension, polymorphism.

Introduction

Isolated systolic hypertension is characterized by increased large artery stiffness and endothelial dysfunction. The factors that cause arterial stiffening and endothelial dysfunction are likely to be involved in

the development of ISH[1]. Large artery stiffness is influenced by the relative amounts of structural proteins (particularly elastin and collagens) and by smooth muscle tone[2]. The matrix metalloproteinases (MMP), a family of zinc-dependent enzymes with proteolytic activity against connective tissue proteins such as collagens, proteoglycans and elastin[3], Increased expression and activity of MMP have been identified in the change of relative amounts of structural proteins and vascular remodeling [4,5], whilst the activity of MMP is also influenced by TIMP (tissue inhibitors of MMP) which inhibit active MMP[6]. And also, there is a "functional" regulation of conduit artery stiffness by smooth muscle tone, which is influenced by circulating and endothelium-derived vasoactive mediators, including NO and endothelin-1[7].

Because of their major significance in vascular remodeling, some studies have demonstrated that MMP gene polymorphism are associated with artery stiffness and high blood pressure[8,9]. MMP-3 plays a pivotal role in matrix homeostasis in the large arteries. studies of the levels of MMPs-3 mRNA and protein in ex-vivo tissues including vascular tissues from individuals of different genotypes for the 5A/6A polymorphism(rs3025058) showed that the levels were highest in 5A homozygotes, intermediate in heterozygotes and lowest in 6A homozygotes[10], furthermore, the individuals with 5A allele homozygotes have greater large artery stiffness and higher blood pressure compared with 5A/6A heterozygotes and 6A homozygotes[9]. In agreement, plasma concentrations and different genotypes for –1562 C>T polymorphism(rs3918242) of MMP9 were not only associated with pathogenesis of coronary disease and prognosis of patients with cardiovascular disease[11,12], but also, T-1562 allele carriers had significantly greater aortic stiffness and higher brachial systolic and pulse pressure as well as carotid systolic and pulse pressure[13]. Yasmin et al. had demonstrated that MMP9 levels was related to aortic stiffness, not only in ISH patients[14], but also in younger and healthy individuals[8]. Furtherly, studies have revealed that MMP9 polymorphism(–1562 C>T) seemed to play a key role in the early stages of hypertensive vascular remodeling and the process of large artery stiffening[15, 16]. Recently, Armstrong C et al. had revealed that MMP9 R279Q(rs17576) was associated with internal carotid artery bulb IMT, and MMP1 A-519G(rs494379), TIMP3 T-1296C(rs5749511) were significantly associated with hypertension and artery stiffness[17]. As a result, MMP-1, MMP-3, MMP-9 and TIMP-3 Genes may be considered as functional candidate genes for ISH.

Pulse wave velocity (PWV) is a known marker of arterial stiffness and defined as the speed in which the pulse wave travels along the length of an artery[18]. Endothelial function, defined as flow mediated dilatation (FMD), is estimated as the percentage increase in vessel diameter from baseline conditions to maximum vessel diameter during hyperemia and used as a measure of endothelium-dependent vasodilatation[19]. Clinical investigation have demonstrated the presence of impaired endothelium-dependent vasodilation in patients with hypertension and the flow-mediated dilatation correlated inversely with PWV[1]. Nitric oxide(NO) and endothelin-1(ET-1) were the major endothelium-derived relaxing and contracting factors to regulate endothelium-dependent vasodilation, and involved in the pathogenesis of artery stiffness and hypertension[20,21].

We hypothesized that MMP-1, MMP-3, MMP-9 and TIMP-3 Genes polymorphism would be involved in the development of ISH by affecting baPWV, FMD, ET-1 or NO. The aim of this study was to test these hypotheses in a group of subjects with ISH, EH subjects (no ISH) and age-matched normotensive control subjects.

Materials and Methods

Overall, 1228 unrelated subjects were recruited from three cities (Guangzhou, Zhanjiang of the Guangdong province, Urumqi of Xinjiang Uygur Autonomous Region) from January 2008 to December 2011. They were divided into 3 distinct groups: 503 patients with ISH (mean age: 65.7±15.3 years; range:58 to 77 years), 481EH(no ISH) subjects (mean age: 64.1±14.2 years; range:55 to 79 years), and 244 aged-matched NT(normotension control) subjects (mean age: 64.1±14.3 years; range: 57 to 75 years). ISH was defined as SBP≥140 mm Hg and DBP <90 mm Hg, EH as SBP≥140 mmHg and DBP ≥90 mmHg while controls as SBP <140 mmHg and DBP<90 mmHg as stated by the 2007 ESH/ESC Guidelines for the management of arterial hypertension[22]. All subjects with a clinical history of secondary hypertension, coronary heart disease and diabetes were excluded from this study. Ethical approval for conducting the present study was granted by the local bioethical committee and informed consent was obtained from each participant beforehand. All of them were detected for baPWV, FMD, ET-1 and NO.

Blood Pressure Measurement

Blood pressure was measured after 10 minutes of rest in a sitting position. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) values were calculated as the means of three consecutive physician-obtained measurements.

Biochemical analysis

Serum concentrations of glucose, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol and creatinine were measured using the methods from the Department of Clinical Laboratory in Nanfang Hospital, in affiliation with Southern Medical University. Plasma concentrations of MMP1, MMP3, MMP9, TIMP and ET-1 were measured by ELISA in cardiology lab of Nanfang Hospital. The plasma NO concentration was measured with spectrophotometry using R&D Systems' Total Nitric Oxide Assay kit, catalogue number DE 1600. The measurements of plasma NO concentration were made by using transformation of NO into nitrate (III) and nitrate (V)catalyzed by nitrate reductase. The total transformed nitrate (III) was then detected spectrophotometrically using the Griess reaction.

Measurement of Arterial Stiffness

Arterial stiffness were determined by brachial-ankle pulse wave velocity (baPWV). The baPWV was measured with individuals in a supine position, after at least 5 minutes of rest using an automatic device (form PWV/ABI; Colin, Komaki Japan). Briefly, pressure waveforms of the brachial and tibial arteries were simultaneously recorded by placing occlusion cuffs connected to a plethysmographic sensor around the brachia and ankles. The time delay (T) of the two waveforms between both feet was measured. The lengths of the paths from the suprasternal notch to the brachium (Lb) and from the suprasternal notch to the ankle (La) were automatically calculated according to the height of each individual. The values of baPWV (right brachial to right ankle) were calculated for measurements made for an average time lag of 10s.

Measurement of Conduit Vessel Endothelial Function

Endothelial function was determined by recording the diameter changes in the brachial artery in response to increased blood flow generated during reactive hyperemia (FMD), which were measured by high-resolution ultrasound. After the patient rested 30 minutes in a supine position in a quiet, air-conditioned room, ultrasound evaluation was done with a 7.0 MHz linear-array ultrasound probe (Accuson 128XP/10, Accuson) by a single dedicated physician. Scans of the brachial artery were obtained ≈5cm proximal from the elbow in the longitudinal section in the right arm, and the probe was maintained in a fixed position at a fixed angle during the evaluation. Arterial flow was determined with a pulsed-Doppler signal at the beginning, 10 to 15 seconds after cuff release, and at the end of the study. Increased blood flow was induced by a blood pressure cuff placed around the forearm, with a 5-minute inflation at 50mm Hg above the subject's systolic blood pressure, followed by rapid deflation. Baseline images before cuff inflation and then for 2 minutes after cuff deflation were recorded. Reactive hyperemia was calculated as the maximal percentage change in flow from baseline.

Genotyping

In this study, we chose 5 SNPs (MMP1 rs494379 (A-519G), MMP3 rs3025058 (5A/6A), MMP9 rs17576 (R279Q), MMP9 rs3918242 (C–1562T), TIMP3 rs5749511 (T-1296C)) according to the literature, may be functional in arterial stiffness

Genomic DNA was extracted from peripheral blood leukocytes using a commercial blood DNA Extract kit (Genomic DNA purification kit; TaKaRa Biotechnology (Japan), Ltd.) and was stored at -20°C until use for genotype testing. All 5 SNPs were genotyped using the polymerase chain reaction (PCR)/ligase detection reaction (LDR) assay[23]. Primers were synthesized from Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. Each set of LDR probes was comprised of one common probe and two discriminating probes for the two types (Supplementary Material: Table S1)

The target DNA sequences were amplified using a multiplex PCR method. PCR reactions for each subject were carried out in a final volume of 10μl containing 1×PCR reaction buffer, 3.0 mMol/L MgCl2, 2.0 mMol/L of dNTPs, 0.4 μl of primers, 0.2 μl of Qiagen hotstar Taq polymerase (QIANGEN China Co. Ltd.), 4μl of 1×Q-solution and 10-20ng of genomic DNA. Thermal cycling was performed for rs494379, rs5749511and rs3025058 in Gene Amp PCR system 9600 (Perkin Elmer, USA) with an initial denaturation of 15 min at 95°C, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 56°C for 1 min, and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min. Next, the protocol for rs17576 and rs3918242 amplification consisted of an initial denaturation of 15 min at 95°C, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 59°C for 1 min, and extension at 72°C for 1 min, followed by a final extension at 72 °C for 7 min.

The ligation reaction for each subject was carried out in a final volume of 10μl containing 1×NEB Taq DNA ligase buffer, 12.5 pmol of each probe mix, 0.05 μl of Taq DNA ligase (NEB Biotechnology (USA), Ltd.) and 1μl of Multi–PCR product. A total of 35 cycles for LDR was performed using 35 cycles at 95°C for 2 min, 94°C for 30s and 60°C for 2 min. The fluorescent products of LDR were differentiated by ABI

sequencer 377 (ABI, USA).

Statistical analysis

Values were expressed as the mean±SD. All statistical analyses were conducted using SPSS software version 13.0 (SPSS, Chicago, IL). Tests for Hardy–Weinberg equilibrium were conducted using chi-square test. Differences in baseline characteristics among the three groups and the differences of baPWV, FMD, ET-1 and NO in different genotypes for gene polymorphisms (which were significantly associated with ISH) were assessed by one-way ANOVA. Associations between genotypes and hypertensive category were calculated by multinomial logistic regression analyses that included genotypes (major/heterozygous/minor), age, TG and LDL as covariates. Since we assessed the associations of 5 polymorphisms with hypertension in three groups, we adopted the Bonferroni correction for multiple testing. Statistical power calculations were performed using SamplePower (SPSS Inc). Haplotype analysis was performed with SNPAlyze7.0 (Dynacom)

Results

The baseline characteristics of the three groups are shown in Table1. The age, SBP, DBP, TG, LDL in ISH and EH groups are significantly higher than those in NT group. The baPWV, and ET-1 in ISH patients were significantly higher compared with EH patients and NT controls (P<0.01, P_{corr}<0.05), however, the FMD and NO in ISH patients and EH patients were markedly lower than NT controls (P<0.01, P_{corr}<0.05), although FMD and NO in ISH patients were not significantly different with EH patients. There were no other significant differences in the baseline demographics.

The associations between ISH and MMP-1, MMP-3, MMP-9 and TIMP-3 Genes Polymorphism are shown in Table 2. We compared allele frequencies of the 5 SNPs among ISH patients, EH patients and NT controls, the 5A allele of SNP rs3025058(5A/6A) in MMP3 gene was significantly associated with ISH because 26.6% of ISH patients carried 5A allele compared with 17.9% of the EH patients (OR, 1.67; 95%CI, 1.34-2.07; P<0.001, Pcorr<0.003) and 10.5% of NT controls (OR, 3.11; 95%CI, 2.28-4.25; P=0.013, Pcorr=0.039). The results of logistic analyses also showed that the 5A allele of SNP rs3025058 was significantly associated with ISH after adjusted by age, TG and LDL(P<0.001, Pcorr<0.003). And also, the T allele of SNP rs3918242(C-1562T) in MMP9 gene was markedly associated with ISH with 25.1% of T allele carriers in ISH patients versus 18.1% of carriers in EH patients(OR, 1.67; 95%CI, 1.34-2.07; P=0.002, Pcorr=0.006) and 15.0% of carriers in NT controls(OR, 1.90; 95%CI, 1.43-2.52; P=0.001, Pcorr=0.003), the logistic analyses revealed that T allele of SNP rs3918242 was significantly associated with ISH even after adjusted by age, TG and LDL(P=0.009, Pcorr=0.027). The other three SNPs in MMP1, MMP9 and TIMP3 were not significantly associated with ISH (P>0.05).

Table 1. Characteristics of Study Participants.

	NT	EH	ISH	F value	*P* value
Subjects	244	481	503		
Age(years)	64.11±15.59	64.08±14.16	65.77±15.32*	4.77	0.009
SBP(mmHg)	121.11±7.17	167.84±13.58*	165.87±13.47*	1303.87	0.000
DBP(mmHg)	71.71±6.73#	99.63±8.31#	80.12±6.12#	1521.77	0.000
Pulse(beats/min)	74.51±8.22	74.25±7.93	74.18±8.12	0.14	0.869
SG(umol/L)	5.76±1.67	5.94±2.96	5.96±1.79	0.64	0.528
TG(mg/dL)	1.28±0.76	1.37±0.89	1.45±1.23*	3.44	0.032
Scr(mg/dl)	0.67±0.06	0.67±0.06	0.67±0.06	0.99	0.372
TC(mg/dl)	5.37±1.37	5.18±1.38	5.22±1.26	1.73	0.178
LDL (mg/dl)	3.08±0.97	2.95±0.98	3.11±0.95*	3.57	0.028
HDL (mg/dl)	1.78±1.16	1.71±0.76	1.72±1.02	0.63	0.53
baPWV(cm/s)	980.94±109.75#	1523.78±103.75#	1791.97±56.01#	6854.66	0.000
FMD(%)	14.02±12.17#	10.78±10.7#	7.86±7.24#	367.87	0.000
ET-1(pg/ml)	4.99±0.97#	13.01±4.17#	23.13±2.42#	3150.12	0.000
NO(umol/L)	43.75±21.16#	14.56±5.21#	6.22±1.08#	810.59	0.000

Values are expressed as mean values ± s.d.NT, normotension; EH, essential hypertension excluding isolated systolic hypertension ; ISH, isolated systolic hypertension; baPWV, brachial–ankle pulse wave velocity; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein; HDL, high-density; FMD, flow mediated dilatation; ET-1, endothelin-1; NO, nitric oxide. # P<0.001 between each other, *P<0.001 vs NT

Table 2. SNP Genotype and Allele Frequencies in Patients With EH, ISH and NT Controls.

		NT	EH	ISH	NT vs EH			NT vs ISH			EH vs ISH		
Subjects		244	481	503	χ2 *P*, OR, 95% CI	*P*	P_{corr}	χ2 *P*, OR, 95% CI	*P*	P_{corr}	χ2 *P*, OR, 95% CI	*P*	P_{corr}
refSNP ID	Genotype												
MMP1	AA	63(25.8%)	123(25.6%)	110(21.8%)									
rs494379	AG	87(35.7%)	171(35.6%)	202(40.1%)									
A-519G	GG	94(38.5%)	187(38.8%)	192(38.1%)	0.995	0.922	>0.05	0.372	0.562	>0.05	0.243	0.416	>0.05
	Allele												
	A	213(43.6%)	417(43.3%)	422(41.9%)									
	G	275(56.4%)	545(56.7%)	584(58.1%)	0.913, 1.01, 0.81-1.26			0.533, 1.07, 0.86-1.33			0.531, 1.06, 0.88-1.26		
MMP3	5A5A	15(6.1%)	39(8.1%)	81(16.1%)									
rs3025058	5A/6A	21(8.6%)	94(19.5%)	106(21.1%)									
5A/6A	6A6A	208(85.3%)	348(72.4%)	316(62.8%)	0.000	0.000	<0.003	0.013	0.000	<0.003	0.000	0.000	<0.003
	Allele												
	5A	51(10.5%)	172(17.9%)	268(26.6%)									
	6A	437(89.5%)	790(82.1%)	738(73.4%)	0.000,1.86, 1.34-2.59			0.000, 3.11, 2.28-4.25			0.000, 1.67, 1.34-2.07		
TIMP3	CC	10(4.1%)	28(5.8%)	20(4.0%)									
rs5749511	CT	11(4.5%)	22(4.6%)	21(4.2%)									
T-1296C	TT	223(91.4%)	431(89.6%)	462(91.8%)	0.614	0.507	>0.05	0.974	0.836	>0.05	0.378	0.119	0.357
	Allele												
	C	31(6.4%)	78(8.1%)	61(6.1%)									
	T	457(93.6%)	884(91.9%)	945(93.9%)	0.231, 1.30, 0.84-2.00			0.827,0.95, 0.60-1.48			0.077, 0.73, 0.51-1.03		
MMP9	AA	22(9.0%)	40(8.3%)	71(14.1%)									
rs17576	AG	47(19.3%)	88(18.3%)	100(19.9%)									
R279Q	GG	175(71.7%)	353(73.4%)	332(66.0%)	0.889	0.653	>0.05	0.119	0.040	0.12	0.008	0.002	0.006
	Allele												
	A	91(18.6%)	216(22.5%)	242(24.1%)									
	G	397(81.4%)	746(77.5%)	764(75.9%)	0.094, 1.26, 0.96-1.66			0.02, 1.38, 1.05-1.81			0.400, 1.09, 0.88-1.34		
MMP9	TT	14(5.8%)	54(11.2%)	75(14.9%)									
rs3918242	CT	45(18.4%)	66(13.7%)	102(20.3%)									
C–1562 T	CC	185(75.8%)	361(75.1%)	326(64.8%)	0.022	0.031	0.093	0.001	0.009	0.027	0.002	0.000	<0.001
	Allele												
	T	73(15.0%)	174(18.1%)	252(25.1%)									
	C	415(85.0%)	788(81.9%)	754(74.9%)	0.134, 1.25, 0.93-1.69			0.000, 1.90, 1.43-2.52			0.000, 1.51, 1.21-1.87		

NT, normotension; EH, essential hypertension; ISH, isolated systolic hypertension; χ^2 *P*-value, chi-square. *P*-value; OR, Odds Ratio. *P* values were calculated by logistic analyses that included genotype (major/heterozygous/minor), and age as covariates. P_{corr} indicates *P*-value after Bonferroni correction.The statistical power was calculated by the latter model: ※ statistical power>0.60; *statistical power >0.70; †statistical power>0.80; #statistical power>0.90.

With the SNP rs3025058 in MMP3 and the SNP rs3918242 in MMP9 significantly associated with ISH, and the SNP rs17576 in MMP9 high Odds Ratios to ISH(OR=1.38), the three SNPs were combined to construct haplotypes, The haplotype-based associations were tested with a 1000000 iterated permutation test. Five major haplotype (each frequency >5%) were observed in ISH patients (Table 3). A global haplotype comparison showed significant differences among NT, EH and ISH groups (NT vs ISH, Permutation p=0.00484; EH vs ISH, Permutation p<0.001). The 5A/G/C and 6A/A/T haplotypes were more prevalent in ISH patients compared with EH patients and NT controls (NT vs ISH, Permutation *P*=0.0258; EH vs ISH, Permutation *P*=0.4821; NT vs ISH, Permutation *P*=0.000002; EH vs ISH, Permutation *P*<0.001)

The baPWV of different genotypes for the 5A/6A and C-1562T polymorphisms were significantly highest in 5A or T homozygotes, intermediate in heterozygotes and lowest in 6A or C homozygotes (*P*<0.01, P_{corr}<0.05).By contrast, the FMD and NO were markedly lowest in 5A or T homozygotes, intermediate in heterozygotes and highest in 6A or C homozygotes (*P*<0.01, *Pcorr*<0.05), however, there were no difference for ET-1 in different genotype of 5A/6A and C-1562T polymorphisms (Table 4).

Table 3. Associations Study with Haplotypes Consisting of Pairwise Combination of 3SNPs among NT, EH and ISH Patients.

Haplotype	MMP3 5A/6A	MMP9 R279Q	MMP9 C-1562T	Frequency			NT vs ISH			NT vs EH			EH vs ISH		
				NT (n=243)	ISH (n=503)	EH (n=479)	χ2	*p*-value	Permutation *p*-value	χ2	*p*-value	Permutation *p*-value	χ2	*p*-value	Permutation *p*-value
1	6A	G	C	0.7652	0.6271	0.6908	0.64	0.482	0.496	0.99	0.3181	0.3963	0.39	0.6658	0.6937
2	6A	A	C	0.1472	0.1036	0.1214	6.02	0.0142	0.0527	1.90	0.1677	0.2423	1.51	0.2193	0.3248
3	6A	G	T	0.1106	0.0977	0.0895	0.59	0.4392	0.5365	1.65	0.1989	0.2708	0.41	0.5229	0.6064
4	5A	G	C	0.0689	0.1122	0.1046	6.97	8.2762E-3	0.0258	10.61	1.126E-3	5.592E-3	0.68	0.4063	0.4821
5	6A	A	T	0.0197	0.1091	0.0191	35.73	2.257E-9	2.0E-6	0.06	0.9374	0.9504	65.54	5.684E-16	0.000
Global							55.39 (df=7)	5.416E-4	4.842E-3	15.76 (df=7)	0.0274	0.15	75.31 (df=7)	1.236E-13	0.000

Permutation *p*-value generated 1000000 iterations.

Table 4. Demographic Characteristics of Patients According to rs3025058 (5A/6A) and rs3918242(-1562C/T) Genotypes.

refSNP ID	Genotype	Subjects	baPWV(cm/s)	FMD(%)	ET-1(pg/ml)	NO(ng/ml)
rs3025058	5A/5A	135	1929.81±259.04*	5.84±2.94*	15.64±6.93	4.96±1.41*
	5A/6A	231	1551.68±218.37*	9.65±2.69*	16.63±6.37	7.97±2.29*
	6A/6A	862	1038.10±167.04*	13.18±7.07*	15.35±7.92	9.65±4.92*
	F-value		15.26	15.19	0.658	15.19
	P-value		0.000	0.000	0.518	0.000
rs3918242	T/T	143	2137.11±277.41*	5.76±3.85*	17.56±4.65	5.04±1.34*
	C/T	213	1540.44±168.49*	9.48±2.89*	16.42±6.09	7.81±2.24*
	C/C	872	927.64±159.62*	13.30±3.05*	15.18±8.13	9.73±4.95*
	F-value		32.05	16.23	1.67	16.68
	P-value		0.000	0.000	0.190	0.000

Values are expressed as mean values ± s.d. baPWV, brachial–ankle pulse wave velocity; FMD, flow mediated dilatation; ET-1, endothelin-1; NO, nitric oxide. * $P<0.05$ between each other.

Discussion

The principle findings of this study were that rs3025058(5A/6A) in MMP3 and rs3918242(C-1562T) in MMP9 were significantly associated with Isolated Systolic Hypertension, In addition, rs3025058 and rs3918242 were markedly associated with artery stiffness and endothelial function, furthermore, the baPWV in 5A or T homozygotes carriers were significantly higher compared with heterozygotes and 6A or C homozygotes carriers, adversely, the FMD and NO in 5A or T homozygotes carriers were markedly lowest, however, the ET-1 were not associated with the SNPs.

The matrix metalloproteinase (MMP) family consists of over 20 secreted or cell surface enzymes that are capable of degrading extracellular matrix proteins. High expression of MMP and destruction of matrix homeostasis in vascular and cardiac tissues have been implicated in the pathogenesis of several cardiovascular conditions including atherosclerosis, aneurysms, artery stiffness and elevated blood pressure[24].MMP-3 plays a pivotal role in matrix homeostasis in the large arteries, In vitro and in vivo experiments have revealed that 5A/6A polymorphism of the MMP3 promoter appears to play an important role in regulating MMP3 gene and protein expression, and the 6A allele has a lower promoter activity than the 5A allele[5,25] Furtherly, Medley et al. reported that in elderly people (age≥61 years), aortic stiffness was greater in 5A allele homozygotes compared with heterozygotes and 6A allele homozygotes[10], Subjects of the 5A/5A genotype were found to have higher elevated blood pressure than subjects of the 5A/6A or 6A/6A genotype, and this association remained significant after adjusting for classic cardiovascular risk factors[9]. It is speculated that increased blood pressure in individuals of the 5A/5A genotype may be related to increased blood vessel stiffening. The present study showed that 5A allele of SNP rs3025058 (5A/6A) was significantly associated with ISH even adjusted by age, TG and LDL, moreover, the baPWV was significantly higher in 5A homozygotes and heterozygotes compared with 6A homozygotes. The FMD was identified as the endothelium-dependent vasodilation function[24], NO, as an endothelial vasodilator, its deficiency has been implicated in the pathogenesis of endothelial dysfunction and hypertension[21,26,27]. ET-1

was a powerful endothelial vasoconstrictor, some findings suggest that endothelin-1 could also potentially be involved in the development of endothelial dysfunction and essential hypertension[26,27]. In this study, it demonstrated that the FMD and NO in 5A homozygotes and heterozygotes were markedly lower than in 6A homozygotes. Therefore, it can be speculated that the association of 5A allele with ISH might be related to increased artery stiffness and endothelial dysfunction.

In agreement, MMP9 were significantly associated with coronary artery disease and artery stiffness [24]. Medley TL,et al. showed that coronary artery disease patients who carried the T-1562 allele had significantly greater aortic stiffness, In addition, T-1562 allele carriers had higher brachial systolic and pulse pressure as well as carotid systolic and pulse pressure[13]. Some studies demonstrated that MMP9 mRNA levels, MMP9 protein levels, and MMP9 activity were higher in T-1562 allele carriers than in non-carriers in aortic tissues[28,29].The study of the association of MM9 gene with arterial stiffness in healthy individuals has demonstrated that aortic PWV, serum MMP9, and elastases were higher in carriers of the rare alleles for the –1562C>T (rs3918242)and R279Q(rs17576) polymorphisms, and suggests that the genetic variation in this protein may be involved in the process of large artery stiffening[14], in particular, Yasmin et al[8]. reported that aortic stiffness is related to MMP9, not only in ISH, but also in healthy individuals and hypothesized that MMP9 might be involved in the process of arterial stiffening and development of ISH. Although it has been reported that MMP9 polymorphisms were significantly associated with artery stiffness and the serum MMP9 were higher in ISH patients and healthy individuals with higher aortic PWV, but whether the –1562C>T (rs3918242) and R279Q(rs17576) polymorphisms are associated with ISH and artery stiffness in ISH patients are unclear. In the current datas, we demonstrated that T-1562 allele was not only significantly associated with ISH, but also the baPWV was significantly higher in T homozygotes and heterozygotes compared with that in C homozygotes, additionally, the FMD and NO were remarkably lower in T homozygotes and heterozygotes than in C homozygotes. So, it is indicated that T-1562 allele might play a part role in the development of ISH not only by increasing artery stiffness but also by weakening endothelial-dependent function. Unfortunately, even though R279Q(rs17576) polymorphisms showed high Odds Ratios to ISH, we didn't find the association of R279Q(rs17576) polymorphisms with ISH, and artery stiffness or endothelial dysfunction in this study. Haplotype-based analysis demonstrated that the 5A/C/C and 6A/T/T haplotypes which contained the 5A allele in rs3025058 and T allele in rs3918242 were more prevalent in ISH patients compared with EH patients and NT controls, which might be enhanced the power of association of 5A allele and T allele with ISH.

Although recently studies revealed that MMP1A-519G(rs494379), TIMP3 T-1296C(rs5749511) were significantly associated with hypertension and artery stiffness[24,30,31]. Unfortunately, the current study did not showed rs494379 and rs5749511 were markedly associated with ISH, artery stiffness or endothelial dysfunction.

Artery stiffness and endothelial dysfunction are the main cause of ISH, all the factors which promoted artery stiffness and endothelial dysfunction might be involved in the development of ISH. In this study, We have demonstrated that the baPWV, ET-1 in ISH patients were significantly higher compared with EH patients and NT controls, as described in our last study previously[32], studies have reported that artery stiffness was reversely correlated with endothelial function in ISH patients, in the present study, we also found that FMD and NO in ISH patients and EH patients were markedly lower than NT controls, although those in ISH patients were not significantly different with EH patients, and suggested that artery stiffness and endothelial function might be affected each other.

In conclusion, the present results indicated that rs3025058(5A/6A) and rs3918242(C-1562T) were significantly associated with ISH. Also, rs3025058 and rs3918242 were independently associated with baPWV, FMD and NO, implying that rs3025058 and rs3918242 were markedly associated with artery stiffness as well as endothelial dysfunction. It is suggested that they might be play a part role in the development of ISH by affecting large artery stiffness and endothelial function.

Perspectives

Although we have shown for the first time that rs3025058 and rs3918242 were significantly associated with ISH, it is necessary to confirm these associations in gene knockout animals model, on the other hand, due to relatively low number of SNPs in MMP were studied in this contribution, future studies may require more MMP SNPs to confirm the association of MMP polymorphism with ISH.

Supplementary Material

Table S1. http://www.medsci.org/v10p0840s1.pdf

Acknowledgments

We thank the DNA donors and the supporting medical staffs for making this study possible and thank Y. Eugene Chen (MD, Ph.D, Associate Professor of Internal Medicine, Cardiovascular Center University of Michigan Medical Center) for revising this paper.

Competing Interests

The authors have declared that no competing interest exists.

References

1. Wallace SM, et al. Isolated systolic hypertension is characterized by increased aortic stiffness and endothelial dysfunction. *Hypertension* 2007;50:228-233
2. Safar ME., Levy BI., Struijker-Boudier H. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. *Circulation* 2003; 107:2864-2869.
3. Opdenakker G, Van den Steen PE, Dubois B, et al. Gelatinase B functions as regulator and effector in leukocyte biology. *J Leukoc Biol* 2001;69:851-859.
4. Godin D, Ivan E, Johnson C, et al. Remodeling of carotid artery is associated with increased expression of matrix metalloproteinases in mouse blood flow cessation model. *Circulation* 2000;102:2861-2866.
5. Ye S, Eriksson P, Hamsten A, et al. Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. *J Biol Chem* 1996; 271:13055-13060.
6. Tan J, Hua Q, Xing X, et al. Impact of the metalloproteinase-9/tissue inhibitor of metalloproteinase-1 system on large arterial stiffness in patients with essential hypertension. *Hypertens Res* 2007 ;30:959-963.
7. Beevers G, Lip GY, O'Brien E. ABC of hypertension:The pathophysiology of hypertension. *BMJ* 2001;322:912-916.
8. Yasmin, McEniery CM, O'Shaughnessy KM, et al. Variation in the human matrix metalloproteinase-9 gene is associated with arterial stiffness in healthy individuals. *Arterioscler Thromb Vasc Biol* 2006;26:1799-1805.
9. Beilby JP, Chapman CM, Palmer LJ, et al. Stromelysin-1 (MMP-3) gene 5A/6A promoter polymorphism is associated with blood pressure in a community population. *J Hypertens* 2005 ;23:537-542.
10. Medley TL, Kingwell BA, Gatzka CD, et al. Matrix metalloproteinase-3 genotype contributes to age-related aortic stiffening through modulation of gene and protein expression. *Circ Res* 2003; 92:1254-1261.
11. Higo S, Uematsu M, Yamagishi M, et al. Elevation of plasma matrix metalloproteinase-9 in the culprit coronary artery in patients with acute myocardial infarction: clinical evidence from distal protection. *Circ J* 2005;69:1180-1185.
12. Morgan AR, Zhang B, Tapper W, et al. Haplotypic analysis of the MMP-9 gene in relation to coronary artery disease. *J Mol Med* 2003;81:321-326.
13. Medley TL, Cole TJ, Dart AM, et al. Matrix metalloproteinase-9 genotype luences large artery stiffness through effects on aortic gene and protein expression. *Arterioscler Thromb Vasc Biol* 2004; 24:1479-1484.
14. Yasmin, McEniery CM, Wallace S, et al. Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. *Arterioscler Thromb Vasc Biol* 2005; 25:372.
15. Zhou S, Feely J, Spiers JP, et al. Matrix metalloproteinase-9 polymorphism contributes to blood pressure and arterial stiffness in essential hypertension. *J Hum Hypertens* 2007;21:861-867.
16. Flamant M, Placier S, Dubroca C, et al. Role of matrix metalloproteinases in early hypertensive vascular remodeling. *Hypertension* 2007;50:212-218.
17. Armstrong C, Abilleira S, Sitzer M, et al. Polymorphisms in MMP family and TIMP genes and carotid artery intima-media thickness. *Stroke* 2007;38:2895-2899.
18. Oliver JJ, Webb DJ. Noninvasive assessment of arterial stiffness and risk of atherosclerotic events. *Arterioscler Thromb Vasc Biol* 2003;23:554-566.
19. Bots ML, Westerink J, Rabelink TJ, et al. Assessment of flow-mediated vasodilatation (FMD) of the brachial artery: effects of technical aspects of the FMD measurement on the FMD response. *Eur Heart J* 2005;26:363-368.
20. Martin C, Cameron J, McGrath B. Mechanical and circulating biomarkers in isolated clinic hypertension. *Clin Exp Pharmacol Physiol* 2008;35:402-408.
21. Cockcroft JR. Exploring vascular benefits of endothelium-derived nitric oxide. *Am J Hypertens*. 2005, 18:177S-183S.
22. Mancia G, De Backer G, Dominiczak A, et al. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2007;25:1105-1187.
23. Xiao Z, Xiao J, Jiang Y, et al. A novel method based on ligase detection reaction for low abundant YIDD mutants detection in hepatitis B virus. *Hepatol Res* 2006;34:150-155.
24. Ye S. Influence of matrix metalloproteinase genotype on cardiovascular disease susceptibility and outcome. *Cardiovasc Res* 2006;69:636-645.
25. Tsukahara S, Shinozaki M, Ikari K, et al. Effect of matrix metalloproteinase-3 functional SNP on serum matrix metalloproteinase-3 level and outcome measures in Japanese RA patients. *Rheumatology (Oxford)* 2008;7:41-44.
26. Puddu P, Puddu GM, Zaca F, et al. Endothelial dysfunction in hypertension. *Acta Cardio* 2000;55:221-232.
27. Nichols WW, Epstein BJ. Actions of selected cardiovascular hormones on arterial stiffness and wave reflections. *Curr Pharm Des* 2009;15:304-320.
28. Medley TL, Cole TJ, Dart AM, et al. Matrix metalloproteinase-9 genotype luences large artery stiffness through effects on aortic gene and protein expression. *Arterioscler Thromb Vasc Biol* 2004; 24:1479-1484.
29. Jones GT, Phillips VL, Harris EL, et al. Functional matrix metalloproteinase-9 polymorphism (C-1562T) associated with abdominal aortic aneurysm. *J Vasc Surg* 2003; 38:1363-1367.
30. Rutter JL, Mitchell TI, Buttice G, et al. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. *Cancer Res* 1998;58:5321-5325.
31. Zhang B, Dhillon S, Geary I, et al. Polymorphisms in matrix metalloproteinase-1,-3,-9, and-12 genes in relation to subarachnoid hemorrhage. *Stroke* 2001;32:2198-2202.
32. Deng L, Huang R, Chen Z, et al. A Study on Polymorphisms of Elastin Gene in Chinese Han Patients With Isolated Systolic Hypertension. *Am J Hypertens* 2009; 22:656-662.