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PERSPECTIVES

MCP-1 –2518 A/G gene polymorphism is associated with blood pressure in ischemic heart disease asymptomatic subjects

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Abstract: Monocyte chemoattractant protein-1 (MCP-1), one of the key inflammatory chemokines, plays an important role in the initiation of atherosclerosis, and represents a risk for coronary artery disease and myocardial infarction. A recent animal study showed that MCP-1 gene might be a candidate gene for salt-sensitive hypertension in Dahl salt sensitive rats. This effect has not been yet studied in asymptomatic humans.
We tested the MCP-1 –2518 A/G single nucleotide polymorphism (SNP) in 66 hypertensive ischemic heart disease asymptomatic subjects. Inflammatory markers, classic risk factors and absolute cardiovascular risk (SCORE system) were also investigated in these subjects.
Our results showed that both, systolic and diastolic values of blood pressure were associated with MCP-1 –2518 A/G SNP at the level of both, genotype and allele frequencies. Subjects with mutant G allele had higher levels of both values of blood pressure, systolic (p = 0.035) and diastolic (p = 0.040) than subjects with allele A. Statistically significantly higher levels of both values of blood pressure, systolic (p = 0.037) and diastolic (p = 0.021) were found also in IHD asymptomatic subjects with AG and GG genotypes. Subjects with AG and GG genotypes had also an increased absolute cardiovascular risk (1.62% vs 3.17%; p = 0.004) and an increasing trend for elevated plasma level of high-sensitive CRP (2.858 vs 2.062 mg/l; p = 0.076). We did not find any significant correlation between the serum level of MCP-1 and blood pressure.
To our best knowledge, this is the first study concerning the association between MCP-1 polymorphism and arterial blood pressure in IHD asymptomatic subjects. These results indicate that the expression of MCP-1 may be increased before the onset of hypertension but further observations from larger cohorts are needed to confirm this finding *(Tab. 6, Ref. 41)*. Full Text in free PDF *www.bmj.sk.*
Key words: cardiovascular risk, CRP, hypertension, inflammation, ischemic heart disease, MCP-1, cytokine polymorphism.

Monocyte chemoattractant protein-1 (MCP-1/CCL2; gene name CCL2) plays an important role in the initiation of atherosclerosis. It promotes the recruitment of monocytes to the endothelium, its differentiation to foam cells and proliferation of smooth muscle cells (1, 2). Murine models of atherosclerosis showed that the deletion of CCL2 resulted in the reduction of size of atherosclerotic plaque (3) or the overexpression of MCP-1 resulted in its increase (4). In humans, the plasma levels of MCP-1 have been associated with an increase in the risk of myocardial infarction (MI), sudden death, coronary angioplasty and stent restenosis (5–7). Framingham Heart Study reported that CCL2 polymorphisms were associated with serum levels of MCP-1 and myocardial infarction (MI) (8). GG genotype of MCP-1 – 2518 single nucleotide polymorphism (SNP) in the MCP-1 regulatory region was associated with susceptibility to ischemic heart disease (IHD), angina pectoris (AP) and myocardial infarction (MI) (9–12).

Recent animal study showed that the gene encoding MCP-1 might be a candidate gene for salt-sensitive hypertension in Dahl salt sensitive rats (13). The association between MCP-1 genotype and hypertensive subjects without IHD has not been studied in humans.

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Subjects and methods

The original cohort of subjects was enrolled in 1999–2000 from cardiologic and general registers. Registers contain 6228 subjects of whom 1,281 had proven ischemic heart disease (IHD). Seven hundred subjects were randomized and 350 were invited to participate. Subjects in whom ischemic heart disease (according to below mentioned criteria) was diagnosed were considered

Tab. 1. Demographic characteristic of investigated subjects without ischemic heart disease.

Variables	Hypertensive	Normotensive	Significance
Number	42	64	
Sex (male)	32.8 %	47.6 %	0.126
Age	51.8±9.74	44.2±9.75	<0.005
BMI	30.50±4.97	26.3±5.22	<0.005
Systolic BP (mmHg)	162.0±24.1	124.7±13.2	<0.005
Diastolic BP (mmHg)	101.9±11.3	81.4±8.6	<0.005
Pulse BP (mmHg)	60.2±18.7;	43.3±10.4	<0.005
Cholesterol (mmol/l)	6.32±1.14	5.94±1.04	0.075
Triglycerides (mmol/l)	1.54±0.82	1.35±0.61	0.161
Smoking	28.6 %	48.4 %	0.122
Obesity	53.7 %	22.2 %	<0.005

BMI – body mass index, BP – blood pressure

patients whereas the apparently healthy subjects were considered controls. Ischemic heart disease (IHD) was defined as documented myocardial infarction or presence of documented myocardial ischemia (ECG, Holter ECG or exercise test) or pathological finding on coronary arteries during selective coronarography or intervention (PTCA or CABG). Arterial hypertension was defined as increased blood pressure ≥140/90 mmHg measured according to standard protocol or as normotension on antihypertensive therapy. Dyslipoproteinemia was defined as cholesterol =5 mmol/l or triglycerides ≥1.7 mmol/l or the use of hypolipidemic medication. Cardiovascular symptoms in control subjects were evaluated according to the standard of Rose´s questionnaire ([14) and additionally, in a substantial part of control subjects (55 %) by exercise electrocardiography.

Our case control study with determined MCP-1 polymorphism comprised 263 patients with IHD (118 males/145 females). In further analysis, we investigated the control group that comprised 106 healthy subjects from Velky Lom (Tab. 1). Sixty-six of them (36 males/30 females, mean age 50.33±10.690 years/ 49.71±9.353 years, p=0.802) were investigated for both MCP-1 –2518 SNP and blood pressure**.** All tested subjects were of Caucasian origin. The study was approved by the local Ethics Committee of the Medical School of Comenius University in Bratislava and all subjects signed an informed consent.

Complete personal and medical history was taken by qualified physicians who underwent standardization training. Blood pressure was measured in a separate quiet room after 5 minutes in a sitting position three times on the right arm by standard sphygnomanometer method using the method of auscultation. Only the last two measurements were analyzed within the study. Uric acid was measured by spectrophotometry (b 456 nm; Hitachi 911, Roche, Switzerland). Total antioxidant status in plasma (TAS) was quantified with colorimetry using original TAS kit (Randox, Cat. No, NX 2332, Randox Laboratories, Gromlech, UK). Blood sample for total glutathione (tGSH) and oxidized glutathione (GSSG) was deproteinated by 10 % sulfosalicylic acid (SSA) (400 µl of 10 % SSA in 750 µl of blood) and centrifugated in chilled centrifuge. The analysis was performed by spectrophotometry (450 nm; microassay reader TECAN (Spectra Fluor, Austria). Schiff base substance was measured by fluorometry (335 nm). Serum concentration of MCP-1 was tested by MCP-1 ELISA kit (RND, USA; Microtitrate plate reader MRX II – Dynex technologies, USA).

Genotyping

Genomic DNA was extracted using a standard salting-out procedure (15). MCP-1 wild type (A) and mutant (G) alleles were typed by polymerase chain reaction using sequence-specific primers (PCR-SSP). The two-reaction format with specific reactions either to A or G allele of MCP-1 –2518 SNP was used and an internal control was adopted from phototyping [16]. The sequences of specific primers included allele A, forward: 5´GTG GGA GGC AGA CAG CTA; allele G, forward: 5´ GTG GGA GGC AGA CAG ATG; constant reverse: 5´TGA GTG TTC ACA TAG GCT TC. The PCR mixture was according to phototyping (16). The cycling protocol was described earlier (17). MCP-1, namely 2518 genotypes were assessed from the presence/absence of PCR amplicons specific to the particular alleles in a standard 2 % agarose gel stained with ethidium-bromide.

Statistical analysis

Populations were tested for conformity to the Hardy-Weinberg equilibrium using a 2 x 2 χ^2 test between observed and expected data. Data with normal distribution were analyzed by Student t-test. The data, which did not satisfy the Gauss distribution, were transformed and analyzed, and the results are displayed in an untransformed way. As no transformation was suitable, we used nonparametric test (Mann-Whitney test). In analysis of variance, we used post hoc tests. Because of the rarity of homozygosity for the G allele and small numbers of available subjects, the data from subjects with G allele in homozygous or heterozygous state (AG + GG) were analyzed together and were compared with subjects homozygous for the common A allele. For categorical variables, χ^2 was used to test the statistical significance. We calculated odds ratio (OR) and its 95 % approximate confidence intervals (95 % CI) as estimator of the relative influence on blood pressure for –2518 A/G SNP. A binary logistic analysis was performed for the determination of independent predictors for MI. A two-tailed p-value <0.05 was considered statistically significant.

Results

The demographic and clinic characterization of studied groups are demonstrated in Table 1. We found significant differences in age ($p<0.005$), body mass index ($p<0.005$), systolic values ($p<0.005$), and diastolic values ($p<0.005$) as well as pulse values of blood pressure ($p<0.005$). Levels of cholesterol and triglyceride did not differ significantly between normotensive and hypertensive groups. There was also no variance in prevalence of smoking but obesity was more prevalent in the hypertensive group ($p<0.005$).

Tab. 2. Systolic, diastolic and pulse blood pressures in healthy subjects in association with MCP-1 -2518 A/G SNP.

	No of subjects	Systolic BP (mmHg)	Diastolic BP (mmHg)	Pulse BP (mmHg)
Genotypes				
MCP-1 AA	37 (57.06 %)	133.24±23.084	85.11±13.38	48.13±14.35
MCP-1 AG + GG	29 (42.94 %)	147.48±31.205	93.83±16.579	53.65±20.82
Sign.		p=0.037	p=0.021	p=0.208
Allele frequency (%)				
Allele A	99 (75 %)	137.71±26.540	87.53±14.91	50.18±16.56
Allele G	33 (25 %)	150.92±32.160	94.68±17.32	56.24±21.22
Sign.		p=0.035	p=0.040	p=0.125

BP – blood pressure, SNP – single nucleotide polymorphism

Tab. 3. Percents of subjects without IHD with hypertension categorized according to genotype.

MCP-1 genotype	Normotensive	Hypertensive
AA	24 (64.9 %)	13 (35.1 %)
AG	13 (52.0 %)	12 (48.0 %)
GG	2 (50.0 %)	2 (50. 0 %)

Chi square = 0.937; p=0.626

Tab. 4. Association between MCP genotype and risk for cardiovascular event (ESC) in subjects without IHD.

Variables	AA	AG + GG	Significance
Number	37	29	
Sex (male)	32.4 %	60.0 %	0.024
Age	44.97±8.56	51.07±2.208	0.041
Systolic BP (mmHg)	133.24±23.084	147.48±31.205	0.037
Cholesterol (mmol/l)	6.02±1.08	5.91±1.14	0.705
Smoking	45.9 %	40.0 %	0.755
ACR, score (%)	1.62±4.329	3.17±3.413	0.004
Thrombocytes (x10^9/liter)	256.1±58.4	237.4±75.5	0.030
Uric acid (mmol/l)	286.02±84.01	306.18±97.94	0.368
Glutathione oxidized	31.323±20.372	33.928±31.919	0.784
Total antioxidant status	1.296±0.147	1.316±0.170	0.661
hsCRP (mg/l)	2.062±2.344	2.858±2.513	0.076
MCP -1 (ng/ml)	215.24±58.209	242.69±75.06	0.310

ACR – absolute cardiovascular risk, BMI – body mass index, BP – blood pressure, MCP-1 – monocyte chemoattractant protein 1

The distributions of genotypes in both, normotensive and hypertensive groups meet the conditions of Hardy-Weinberg equilibrium. The MCP-1 –2518 A/G gene polymorphism was significantly associated with blood pressure. Both, systolic value (147.5±31.21 vs 133.2±23.084 mmHg; p=0.037) and diastolic value of blood pressure (93.8±16.58 vs 85.1±13.38 mmHg; p=0.021) in the group of subjects without IHD with AG or GG genotypes were significantly elevated. For pulse blood pressure, there were no significant differences. In allele frequency analysis, a significant association between the presence of G allele and both, systolic and diastolic values of blood pressure was confirmed (Tab. 2). Comparing groups of subjects with AA, AG and GG genotypes we found an increased number of hypertensive subjects with G allele, however the difference did not reach the statistical significance (Tab. 3).

Subjects without IHD with AG and GG genotypes have also an increased absolute cardiovascular risk (1.62 vs 3.17 %; p=0.004) and increasing trend of serum level of hsCRP elevation (2.858 vs 2.062 mg/l; p=0.076) (Tab. 4). In order to disclose the potential confounder on the relationship between MCP-1 polymorphism and hypertension we have built binary logistic regression models with systolic and diastolic values as a dependent variable while MCP-1 polymorphism, BMI, age, smoking, diabetes mellitus, cholesterol were considered independent variables. MCP genotype (AA vs AG+GG) remained as a significant predictor of hypertension only when controlling for BMI (systolic BP p=0.037; diastolic BP p=0.022). After adding the age data and other variables to the model, MCP genotype became non-significant (Tab. 5).

There was no significant correlation between the levels of MCP-1 protein and blood pressure (systolic, r=0.071; p=0.690; diastolic, r=0.091; p=0.607; pulse, r= -0.068; p=0.701) (Tab. 6). MCP-1 genotype was neither associated with levels of uric acid, oxidized glutathione nor with total antioxidant status as markers of oxidation (Tab. 4).

Subjects with AG and GG genotype had also a decreased number of thrombocytes (256.1±58.4 vs 237.4±75,5; p=0.030) (Tab. 4).

Discussion

Various studies showed that the increased inflammatory activity predisposes to the development of hypertension (18, 19). Hypertensive, salt-sensitive patients have higher levels of P--selectin, E-selectin and MCP-1 even after the control for arterial blood pressure and age. There is proof that renin-angiotensin system (RAS) increases the level of MCP-1 and prevalence of systemic arterial hypertension (20). Indeed, a recent study indicates that the expression of CCL2 may be increased before the onset of hypertension (13). Other works too gave evidence that renal infiltration of immune cells leads to elevation of arterial pressure and renal damage as well as possibly decreases the glomerular filtration rate in rats (21). Tubulointerstitial inflammation in conjunction with microvascular disease may be crucial in the

Tab. 5. Binary regression modeling using systolic and diastolic blood pressure as dependent variables (analysis preformed in subjects without IHD).

Variables	Systolic blood pressure				Diastolic blood pressure			
	B-coeff.	SE	Sign.	95% CI.	B-coeff.	SE	Sign.	95% CI.
(Constant)	5.405	23.737	0.821	-42.093 - 52.902	24.733	14.466	0.093	-4.214 - 53.680
MCP Genotype	6.197	5.670	0.279	-5.149 - 17.544	5.266	3.456	0.133	-1.649 - 2.181
Age	1.193	0.283	<0.0005	0.628 - 10.759	0.429	0.172	0.016	0.084 - 0.774
BMI	2.130	0.531	<0.0005	1.068 - 3.192	1.081	0.323	0.001	0.434 - 10.728
Diabetes mellitus	4.072	6.152	0.511	-8.237 - 16.382	3.853	3.749	0.308	-3.649 - 11.355
Smoking	2.037	6.041	0.737	-10.052 - 14.125	2.312	3.682	0.532	-5.055 - 9.679
Cholesterol	1.469	2.585	0.572	-3.703 - 6.641	0.837	1.575	0.597	-2.316- 3.989

B-coeff – beta coefficient, SE – standard error, CI – confidence interval

Tab. 6. Correlation between blood pressure and MCP - 1 in subjects without IHD.

	Systolic BP		Diastolic BP		Pulse BP	
	Correlation	Significance	Correlation	Significance	Correlation	Significance
MCP - 1	0.071	0.690	0.091	0.607	-0.068	0.701

MCP-1 – Monocyte chemoattractant protein-1

genesis of hypertension (22). MCP-1 promotes atherosclerosis mainly by recruiting monocytes into vascular endothelium (1). Its role was supported in animal models since MCP-1 knockout mice decreased the formation and development of atherosclerosis (3). Clinical usefulness of evaluation of MCP-1 gene polymorphism was demonstrated also in asymptomatic patients in association with occult ischemia and CAD risk factors (23). Inconsistent results came from studies of subclinical atherosclerosis in peripheral arteries and MCP-1 polymorphism (24, 25). The role of monocytes in the development of hypertension supports the finding that silica, a selective toxin to monocytes has an antihypertensive effect and inhibits the left ventricular hypertrophy in rats (26). In addition, peripheral blood monocytes in patients with essential hypertension were found to be preactivated (27).

The role of inflammation in arterial hypertension is broadly discussed. Despite the findings that higher hsCRP levels represent an elevated risk for the development of hypertension (19), this fact is not widely accepted because not all observations are so conclusive (28). The analysis of the British Women's Heart and Health Study discusses the view that inflammation and CRP may be independent risk factors for hypertension (29). In this study "Mendelian randomization" was applied, which is an approach avoiding the residual confounding and reverse causation. The study revealed a strong cross-sectional correlation between CRP and blood pressure, however the genetic polymorphism associated with an increase in CRP by 30 % was not connected to blood pressure. These results indicate that the reverse causation even confounding rather than causative effect stand beside the relationship between inflammation and blood pressure.

Our results stand in contradiction to these findings. Because even on our small asymptomatic group without signs of atherosclerotic complications (coronary artery disease, stroke, and myocardial infarction) we found a distinct difference in blood pressures in patients that carried G allele of MCP-1 –2518 polymorphism. The adjustment for age and other possible confounding factors caused that the association between MCP-1 polymorphism and blood pressure became unsignificant. Nevertheless, the small size of the study group has not allowed us to make clear conclusions about the possible confounding factors.

The precise ways by which G allele might raise the risk of CAD and MI remains unclear. There is much evidence that circulating MCP-1 has been associated with an increased risk of MI (5), stent restenosis (30) coronary angioplasty (6) and inflammation in cardiovascular diseases (31)]. These papers also reported a strong association with risk factors for CAD as obesity, hypertension, diabetes mellitus, hypercholesterolemia as well as with age, smoking and family history of CAD. But not all authors reported such significant associations with ischemia and MI (23, 32). There still remains the question as to how much the MCP-1 genotype affects the level of circulating MCP-1 protein and inflammation (31).

Inflammation decreases the elasticity and increases the stiffness of arterial walls even in subjects without overt cardiovascular disease. Despite these findings and reported effect especially on pulse blood pressure, we have not observed any connection between MCP-1 polymorphism and pulse blood pressure. Thus, the reported connection between CCL2 polymorphism and renal damage may be responsible for blood pressure elevation in a greater part than the elevated stiffness of arterial wall (22).

The polymorphism of CCL2-2518 A/G is associated with a raised level of lipoprotein A and GG genotype predisposing to ischemic heart disease (12). These findings were confirmed recently in patients undergoing coronary artery bypass graft surgery (33)] and by Framingham Heart study, where G allele was independently associated with prevalent myocardial infarction even after controlling for risk factors for CAD (8).

The blockage of renin-angiotensin system is a potent way to prevent diabetes and damage of renal function. According to recent study, candesartan is an effective and well-tolerated treatment of prehypertensive patients in order to reduce the risk of further onset of hypertension (34). Patients with metabolic syndrome, visceral obesity and insulin resistance may profit greatly from the treatment. Similarly, insulin at physiologic concentrations shows an inhibitory effect on MCP-1, indicating an anti-inflammatory and possibly antiatherogenic effect of insulin (35).

In experimental animals as well as in humans, the salt loading is responsible for gradual elevation of blood pressure, renal damage and endothelial dysfunction. Apart from renin-angiotensin system, the oxidative stress is widely accepted as an underlying mechanism of these changes and organ damage. In hypertensive patients, an increased production of elevated reactive oxygen species and decreased antioxidant capacity were found (36). The antioxidant treatment decreases the renal superoxide production, urinary protein loss and glomerulosclerosis (37). In one study, there was an association between levels of uric acid, which reflect the antioxidant status in organism, and MCP-1 polymorphism (38). Despite this suggestion about the association between MCP-1 polymorphism and oxidative stress, we did not find any association between MCP-1 –2518 A/G SNP and the levels of uric acid, total plasmatic antioxidant status and oxidized form of glutathione, possibly because of the small number of investigated subjects.

There are distinct differences in frequencies of MCP-1 polymorphisms among different races. The distribution of gene variation may differ between various populations. Hence, ethnicity and patient's selection criteria might significantly influence the predictive power of genetic condition to chronic disease. The prevalence of G allele differs variously from 21.1–29 % in Caucasian population (33.39) to 65 % in Korean population (40). The power of G allele to predict atherosclerosis is related also to the character and extent of disease, race, age, environmental risk factors and their mutual interactions (41).

This was to our knowledge the first report dealing with the association between arterial hypertension and MCP-1 polymorphism in IHD asymptomatic adults. Considering the size of study group, we have been not able to rule out the possible confounding factors. Thus, further studies will be needed to confirm our findings. In recent years, much effort was made to classify the patients with pre-hypertension in order to select the group with higher risk for the development of hypertension. A precise qualification of these subjects is important because emerging evidence-based medication in this group could delay the onset of hypertension and thus reduce the damage of target organs. If our results are confirmed, MCP genotyping can be an important method in stratification of risk patients.

Conclusion

Our results indicate that MCP-1 –2518 A/G gene polymorphism is significantly associated with blood pressure. Group of subjects without IHD with AG or GG genotypes had significantly elevated systolic and diastolic values of blood pressure. We also found an increased association of AG and GG genotypes with absolute cardiovascular risk (1.62 vs 3.17 %; p=0.004) and increasing trend of hsCRP elevation (2.858 vs 2.062 mg/l; p=0.076). MCP genotype (AA vs AG +GG) remained as a significant predictor of hypertension only when controlling for BMI. After adding age and other variables to the model, the tested MCP -1 genotype became non-significant. However, we did not find a significant correlation between the level of MCP-1 protein and blood pressure. A very interesting finding is also the significant association of MCP-1 –2518 AG and GG genotypes with a decreased number of thrombocytes.

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