

## CLINICAL STUDY

# The MCP-1 -2518 (A/G) single nucleotide polymorphism is associated with ischemic heart disease and myocardial infarction in men in the Slovak population

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**Abstract:** We investigated the MCP-1 -2518 (A/G) single nucleotide polymorphism (SNP) in Slovak cohort of patients with ischemic heart disease (IHD). Our study comprised 270 patients with IHD, out of them 92 with myocardial infarction (MI). We found that the frequencies of the mutant GG genotype in Slovak patients with IHD (10.7 %;  $p=0.019$ ) and MI (12.0 %;  $p=0.046$ ) were significantly higher than those in the control subjects (5.8 %). After subdividing the groups according to the sex, statistically significant difference was found only in men (IHD:  $p=0.013$ , MI:  $p=0.009$ ). We also found a higher rate of GG homozygous genotype in patients with early ( $\leq 50$  years of age) MI (18.4 %;  $p=0.004$ ) – statistically significant again only in men (23.1 %;  $p=0.002$ ). The frequencies of G alleles in IHD male patients (30.3 %,  $p=0.046$ ) and in early MI male patients (38.5 %,  $p=0.019$ ) were also statistically significantly higher than in control group. Our results confirm that IHD and MI are linked to MCP-1 -2518 (A/G) single nucleotide polymorphism (Tab. 4, Ref. 34). Full Text (Free, PDF) [www.bmj.sk](http://www.bmj.sk).  
Key words: chemokines, inflammation, ischemic heart disease, myocardial infarction, MCP-1 polymorphism, SNP.

Inflammation plays a key role in the process of atherosclerosis characterised by the migration of monocytes and lymphocytes into the vessel wall in response to chemokines (1–3). One of the most important chemokines involved in the early events of atherosclerosis and in myocardial infarct healing is monocyte chemoattractant protein (MCP)-1 (4–6).

Coronary artery disease (CAD) is a complex multifactorial and polygenic disorder that results from an interaction between the individual's genetic background, vascular inflammation and various environmental factors. Inflammation and genetics are both prominent mechanisms in the pathogenesis of atherosclerosis and atherothrombosis – one of the leading causes of CAD. Under-

standing the genetic basis of CAD may improve our management and prevention. A number of population studies have explored the association of CAD with gene polymorphisms of different inflammatory molecules (7–10). MCP-1 has already been proposed as a candidate gene for studies of genetic markers in CAD (11). The data on contribution of the MCP-1-2518 (A/G) single nucleotide polymorphism (SNP) to the pathogenesis of coronary atherosclerosis are not uniform in spite of the fact, that polymorphism of the receptor for MCP-1 – CCR2 has been implicated as susceptibility factor for myocardial infarction by several independent investigators (12–14). We therefore investigated the MCP-1 -2518 SNP in a cohort of Slovak patients with ischemic heart disease (IHD).

## Subjects and methods

The original cohort of patients was enrolled in 1999–2000. Patients with ischemic heart disease were recruited from cardiological register of Cardiological laboratory of 2nd Department of Internal Medicine, Comenius University in Bratislava, cardiological registers of two specialists in Nove Zamky and one register in Velky Lom (middle Slovakia). Randomization was made according to random tables by independent researchers. Basic characteristics (structure of sex, age and diseases) were analysed in both groups (patients recruited into project and patients excluded).

Ischemic heart diseases (IHD) was defined as documented myocardial infarction (hospitalization or coronarography), or

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**Tab. 1. Distribution of MCP-1 -2518 (A/G) genotype frequencies in Slovak patients with IHD, MI and in Slovak healthy control population.**

Study group	Genotypes			Statistics (significant values in bold)			
	AA	AG	GG	*P =	95% C.I.	OR/Etiologic fraction	$\chi^2$
Patients with:							
IHD (n = 270)	54.4% (n = 147)	34.8% (n = 94)	10.7% (n = 29)	<b>0.019</b>	1.112 - 3.423	1.951/8.1%	5.428
MI (n = 92)	56.5% (n = 52)	31.5% (n = 29)	12.0% (n = 11)	<b>0.046</b>	1.006 - 4.537	2.137/9.2%	3.906
Controls (n = 449)	57.5% (n = 258)	36.7% (n = 165)	5.8% (n = 26)				
Early MI (n = 38)	47.4% (n = 18)	34.2% (n = 13)	18.4% (n = 7)	<b>0.004</b>	1.548 - 10.103	3.955/20.8%	8.255
Late MI (n = 54)	63.0% (n = 34)	29.6% (n = 16)	7.4% (n = 4)	0.661	0.441 - 3.675	1.272/1.5%	0.195

Legend: IHD – ischemic heart disease, MI – myocardial infarction, 95% C.I. – 95% confidence interval, OR – odds ratio. \* P values, OR and etiologic fraction for comparisons of MCP-1-2518 GG genotype frequency between particular (sub)groups of IHD patients and healthy control group.

**Tab. 2. Distribution of MCP-1 -2518 (A/G) genotype frequencies in Slovak patients with IHD, MI and in Slovak healthy control population according to sex.**

Study group	Genotypes			Statistics (significant results in bold)			
	AA	AG	GG	P =	95% C.I.	OR / Etiologic fraction	$\chi^2$
Patients with:							
IHD in men (n = 122)	51.6% (n = 63)	36.1% (n = 44)	12.3% (n = 15)	<b>0.013</b>	1.201 - 4.720	2.381/11.1%	6.176
IHD - women (n = 148)	56.8% (n = 84)	33.8% (n = 50)	9.5% (n = 14)	0.137	0.843 - 3.323	1.674/5.7%	2.168
MI in men (n = 57)	52.6% (n = 30)	31.6% (n = 18)	15.8% (n = 9)	<b>0.009</b>	1.324 - 6.973	3.038/15.4%	6.874
MI in women (n = 35)	62.9% (n = 22)	31.4% (n = 11)	5.7% (n = 2)	0.904	0.276 - 4.250	1.084/0.9%	0.013
Early MI (men) (n = 26)	46.2% (n = 12)	30.8% (n = 8)	23.1% (n = 6)	<b>0.002</b>	1.813 - 14.189	5.072/26.6%	9.573
Early MI (women) (n=12)	50.0% (n = 6)	41.7% (n = 5)	8.3% (n = 1)	0.614	0.365 - 13.891	2.251/5.7%	0.764
Controls in men (n = 284)	52.8% (n = 150)	40.5% (n = 115)	6.7% (n = 19)	0.157	0.779 - 4.508	1.874/5.6%	1.969
Controls in women (n=165)	65.6% (n = 108)	30.3% (n = 50)	4.2% (n = 7)				

Legend: IHD – ischemic heart disease, MI – myocardial infarction, 95 % C.I. – 95% confidence interval, OR – odds ratio,  $\chi^2$  – the value of chi square test

presence of documented typical angina pectoris (ECG or Holter) or documented silent ischemia (ECG, Holter ECG or exercise test) or pathological finding on coronary arteries during selective coronarography. Complete personal and medical history was taken by qualified physicians. Fasting blood sample collection without arm compression was performed after 5 minutes in the sitting position. Patients were asked to abstain from coffee, alcohol, smoking and vigorous physical activity 12 hours before collection. Analysed parameters included whole blood count, complete lipid profile, glucose, total antioxidant status, homocysteine and vitamin B status, inflammatory markers and several oxidative stress parameters.

Subjects in whom ischemic heart disease (according to mentioned criteria) was diagnosed were considered as patients, apparently healthy subjects were considered as controls. Another part of control group was recruited from the participants of the

bone marrow donor registry. Cardiovascular symptomatology in this part of control subjects was evaluated according to standard of Rose's questionnaire (15) and additionally, in a substantial part of control subjects (55 %) by exercise electrocardiography.

Together, our case control study comprised 270 patients with IHD (122 males/148 females, mean age  $62 \pm 10.1/64 \pm 7.6$  years), out of them 92 with MI (57 males/35 females, mean age  $60 \pm 9.9/64 \pm 6.5$  years). The control group comprised of 449 unrelated healthy subjects recruited from persons of Slovak origin not suffering from IHD (284 males/165 females, mean age  $50 \pm 10.7$  years/ $48 \pm 9.6$  years).

All patients and control 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.

**Tab. 3. Distribution of MCP-1 -2518 (A/G) allele frequencies in Slovak patients with IHD, MI and in Slovak healthy control population.**

Study group Number of alleles	Alleles		Statistics (significant values are signed bold)				
	A	G	*P =	95% C.I.	OR	Etiologic fraction	$\chi^2$
IHD (n = 540)	71.9% (n = 388)	28.1% (n = 152)	0.089	0.966 – 1.566	1.230	5.3%	2.819
MI (n = 184)	72.3% (n = 133)	27.7% (n = 51)	0.297	0.847 – 1.725	1.209	4.7%	1.090
Controls (n = 898)	75.8% (n = 681)	24.2% (n = 217)					
Early MI (n = 76)	64.5% (n = 49)	35.5% (n = 27)	<b>0.025</b>	1.066 – 2.843	1.741	15.0%	4.904
Late MI (n = 108)	77.8% (n = 84)	22.2% (n = 24)	0.695	0.565 – 1.461	0.908	—	0.157

Legend: IHD – ischemic heart disease, MI – myocardial infarction, 95% C.I. – 95% confidence interval, OR – odds ratio,  $\chi^2$  – the value of chi square test.

### Genotyping

Genomic DNA was extracted using a standard salting out procedure (16). MCP-1 wild-type (A) and mutant (G) alleles were typed by polymerase chain reaction using sequence specific primers (PCR-SSP). Two reaction format with specific reactions either to A or G allele of the MCP-1 -2518 SNP was used and an internal control was adopted from Phototyping (17). The sequences of specific primers were: allele A, forward: 5'GTG GGA GGC AGA CAG CTA; allele G, forward: 5'GTG GGA GGC AGA CAG CTG; constant reverse: 5'TGA GTG TTC ACA TAG GCT TC. The PCR mixture was according to Phototyping (17). The cycling protocol was described earlier (18). MCP-1 -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

Comparisons were made between the genotype, allele frequencies, as well as carriage rate (phenotype frequency) in the disease and control populations. The carriage rate gives the number of individuals carrying one (or two) copies of a particular allele on one or both (maternal and paternal) chromosomes. The data sets were compared using a standard  $2 \times 2 \chi^2$  test by SIGTEST, a computer-based program that uses Woolf-Haldane correction in cases of small numbers. This program has a facility for the calculation of chi-square statistic, the significance value, 95 % confidential interval, and the relative risk (odds ratio – OR). A p-value <0.05 was considered to be significant. The populations were tested for conformity to the Hardy-Weinberg equilibrium using a  $2 \times 2 \chi^2$  test between observed and expected numbers.

### Results

The healthy control group was in Hardy-Weinberg equilibrium (HWE) with regard to the distribution of the MCP-1 -2518 (A/G) genotypes ( $p > 0.05$ ). However, IHD patients ( $p = 0.02$ ) and

patients with MI ( $p = 0.04$ ) were deviated from H-W equilibrium due to the higher frequency of GG genotype and lower frequency of AG heterozygotes.

The genotype and allele frequencies, as well as the carriage rate (phenotype frequency) in the disease and control populations, were determined. Frequencies of the mutant GG genotype in Slovak patients with IHD and MI significantly differed from the frequency in the control subjects (Tab. 1). There were 29 GG homozygous individuals among our 270 IHD patients (10.7 %,  $p = 0.019$ ), 11 GG homozygous individuals among 92 MI patients (12.0 %,  $p = 0.046$ ) and 26 GG homozygous individuals (5.8 %) among the control group of 449 healthy subjects.

The patient groups were further subdivided into subgroups according to the first MI episode (early MI:  $\leq 50$  years; late MI:  $> 50$  years) (Tab. 1), according to their sex (Tab. 2). The early appearance of MI episodes (18.4 %,  $p = 0.004$ ) is associated with higher rate of homozygous GG genotypes (Tab. 1). We found a higher rate of homozygous mutant G alleles both in IHD male patients (12.3 %,  $p = 0.013$ ) and MI male patients (15.8 %,  $p = 0.009$ ). Both the total incidence and the early occurrence of MI were associated with male (15.8 %,  $p = 0.009$ ; 23.1 %,  $p = 0.002$ ) (Tab. 2).

Frequencies of alleles in Slovak patients with IHD and MI are shown in Tables 3 and 4. We found significant differences in the frequencies of alleles against control group in IHD male patients (30.3 %,  $p = 0.046$ ), in early MI male patients (38.5 %,  $p = 0.019$ ). There were no differences in carriage rate between any group of patients and the control group (data not shown).

### Discussion

A number of population studies have explored the association of CAD with gene polymorphisms of different inflammatory molecules (7, 9). Chemokine, monocyte chemoattractant protein-1 (MCP-1 or CCL2), and its receptor CCR2 belong to key factors in chronic inflammatory diseases including atherosclerosis (8, 19). MCP-1/CCL2 is a potent chemoattractant for monocytes, T cells and NK cells. MCP-1 induces the transmi-

**Tab. 4. Distribution of MCP-1 -2518 (A/G) allele frequencies in Slovak patients with IHD, MI and in Slovak control population according to sex.**

Study group Number of alleles	Alleles		Statistics (significant results are signed bold)					$\chi^2$
	A	G	P =	95% C.I.	OR	Etiologic fraction		
IHD in men (n = 244)	69.7% (n = 170)	30.3% (n = 74)	<b>0.046</b>	1.002 – 1.871	1.369	8.1	3.893	
IHD in women (n = 296)	73.6% (n = 218)	26.4% (n = 78)	0.555	0.834 – 1.519	1.126	2.9%	0.600	
MI in men (n = 114)	68.4% (n = 78)	31.6% (n = 36)	0.076	0.956 – 2.220	1.457	9.8%	3.065	
MI in women (n = 70)	78.6% (n = 55)	21.4% (n = 15)	0.659	0.488 – 1.568	0.875	—	0.201	
Early MI in men (n = 52)	61.5% (n = 32)	38.5% (n = 20)	0.019	1.114 – 3.507	1.976	18.9%	5.421	
Early MI in women (n = 24)	70.8% (n = 17)	29.2% (n = 7)	0.513	0.563 – 3.202	1.343	6.6%	0.442	
Late MI in men (n = 62)	74.2% (n = 46)	25.8% (n = 16)	0.722	0.621 – 1.990	1.112	2.2%	0.127	
Late MI in women (n = 50)	76.0% (n = 38)	24% (n = 12)	0.958	0.528 – 1.961	1.017	0.2%	0.003	
Controls in men (n = 568)	73.1% (n = 415)	26.9% (n = 153)	<b>0.011</b>	1.098 – 2.121	1.526	9.4%	6.348	
Controls in women (n = 330)	80.6% (n = 266)	19.4% (n = 64)						

Legend: IHD – ischemic heart disease, MI – myocardial infarction, 95% C.I. – 95% confidence interval, OR – odds ratio,  $\chi^2$  – the value of chi square test

gration of CCR2+ monocytes from the circulation, promotes their differentiation to lipid-laden macrophages (20–21) and contributes to the proliferation of arterial smooth muscle cells (22) which, along the macrophages, constitute the key cellular components of atherosclerotic plaques. This chemokine plays a dual role in myocardial ischaemia. In addition to several negative roles in the process of atherosclerosis, thrombotic occlusion of a coronary artery and in the process of reperfusion, this chemokine protects myocytes from hypoxia-induced cell death and has also positive effect in myocardial infarct healing (6, 23–24).

Cardiovascular disease is a heritable condition (25) and is associated with inflammatory polymorphisms (26–28). Polymorphism of the receptor for MCP-1 – CCR2 has been implicated as susceptibility factor for myocardial infarction by several independent investigators (12–14). In our previous work we found an association of MCP-1 -2518 G polymorphism with chronic stable angina pectoris (4). An association of CCR2 polymorphisms with the number of closed coronary artery vessels in coronary artery disease was also found (29). Deletion of MCP-1/CCL2 or CCR2 resulted in a large (50–80 %) reduction in atherosclerotic plaque size (19, 30–31). However, the data on contribution of the MCP-1 polymorphisms to the pathogenesis of coronary atherosclerosis are not uniform (18, 32–33).

Szalai et al (10) showed an association of MCP-1 -2518 G homozygous form with severe CAD. The MCP-1 -2578 G allele was also significantly associated with higher prevalence of myocardial infarction in a dominant genetic model (33). An association of MCP-1 polymorphism with occult ischemia in a high risk asymptomatic population was also detected (34).

We found that frequencies of the MCP-1 -2518 mutant GG genotype in Slovak patients with IHD and MI were significantly higher than in the control subjects (Tab. 1). While the rate of GG homozygous individuals among the control group was 5.8 %, in the group of IHD patients and MI patients was twofold – higher in men than in women (Tab. 2). After subdividing patients according to the first MI episode we found more than three times higher rate of MCP-1 -2518 GG homozygous genotype in patients with early MI (Tab. 1).

Our results confirm that IHD and MI in Slovak patients are linked to MCP-1 -2518 (A/G) single nucleotide polymorphism.

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