Clinica Chimica Acta xxx (2008) xxx–xxx

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Letter to the Editor

Association of chronic stable angina pectoris with MCP-1-2518 A/G single nucleotide polymorphism in the Slovak population

Dear Editor,

Inflammation and genetic factors are both prominent mechanisms in the pathogenesis of atherosclerosis and atherothrombosis — one of the leading causes of coronary artery disease (CAD). A number of population studies have explored the association of CAD with gene polymorphisms of different inflammatory molecules [1]. In this regard, monocyte chemoattractant protein-1 (MCP-1, also known as CCL2) and its receptor CCR2 have been proposed as candidate genes for investigating the genetic markers in CAD [1–4]. In this study we, therefore, explored the relationship between a functional MCP-1 gene polymorphism and chronic stable angina pectoris as a representative example of CAD.

Our case control study comprised 151 patients with chronic stable angina pectoris (AP) (62 males / 89 females, mean age 61±9.7 / 64±7.6 years). The population control group comprised 449 unrelated healthy subjects recruited from persons of Slovak origin without symptoms of cardiac or other inflammatory pathologies (284 males / 165 females, mean age 50±10.7 years / 48±9.6 years).

Angina pectoris was defined as chronic intermittent ischemia presenting with typical chest pain, ECG ST segment deviation (exercise test, Holter or ECG at rest) and response to ~~antiangose~~ therapy. A complete personal and medical history was taken by qualified physicians. The parameters analysed were whole blood count, lipid profile, glucose, total antioxidant status, homocysteine and vitamin B status, inflammatory markers and several oxidative stress parameters. Subjects in whom AP was diagnosed according to the above criteria were considered patients, apparently healthy subjects were considered controls. Cardiovascular symptomatology in the control subjects was also evaluated according to the standard Rose questionnaire [5] and in 55% of control subjects by exercise electrocardiography.

Patients and control subjects were unrelated and of Caucasian origin. The study was approved by the local Ethics Committee (School of Medicine, Comenius University, Bratislava) and all subjects signed an informed consent.

After DNA extraction by a standard salting out procedure [6], the MCP-1 wild-type (A) and mutant (G) alleles were typed using polymerase chain reaction with sequence specific primers (PCR-SSP) [7,8]. 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 ATG; constant reverse: 5′TGA GTG TTC ACA TAG GCT TC. MCP-1-2518 genotypes were assessed according to the presence/absence of PCR amplicons specific to the particular alleles in a standard 2% agarose gel stained with ethidium-bromide.

The genotype, allele frequencies, as well as carriage rate (phenotype frequency) in the patient and control populations were compared using a standard 2×2 χ^2 test using SIGTEST with Woolf–Haldane correction. The chi-squared statistic, 95% confidential interval and the relative risk (odds ratio — OR) were also calculated. The population samples were tested for conformity to the Hardy–Weinberg equilibrium (HWE).

The healthy control group was within the HWE with regard to distribution of the MCP-1-2518 A/G genotypes ($P>0.05$). The group of AP patients deviated from HWE owing to higher frequency of GG genotype and lower frequency of AG heterozygotes ($P<0.001$).

Mutant GG genotype was over-represented in AP patients (15.2%) in comparison to the controls (5.8%) ($P=0.003$) [Table 1]. The patients were then further divided into subgroups according to the first

Table 1
Distribution of MCP-1-2518 A/G genotype frequencies in Slovak patients with chronic stable AP and in Slovak healthy control population

Study group	Genotypes			Statistics (significant values in bold)			
Patients with:	AA	AG	GG	*P*=	95% C.I.	OR/Etiologic fraction	χ^2
AP ($n=151$)	58.3% ($n=88$)	26.5% ($n=40$)	15.2% ($n=23$)	**0.003**	1.414 – 4.746	2.590/12.8%	9.489
Early AP ($n=72$)	63.9% ($n=46$)	20.8% ($n=15$)	15.3% ($n=11$)	**0.022**	1.129 – 5.155	2.412/11.2%	5.168
Late AP ($n=61$)	50.8% ($n=31$)	36.1% ($n=22$)	13.1% ($n=8$)	**0.025**	1.118 – 6.199	2.632/12.5%	4.904
Controls ($n=449$)	57.5% ($n=258$)	36.7% ($n=165$)	5.8% ($n=26$)				
AP in men ($n=62$)	58.0% ($n=36$)	22.6% ($n=14$)	19.4% ($n=12$)	**0.002**	1.568 – 7.119	3.341/17.5%	9.764
AP in women ($n=89$)	58.4% ($n=52$)	29.3% ($n=26$)	~~12.4%~~ ($n=11$)	**0.046**	1.006 – 4.537	2.137/9.2%	3.906
Early AP in men ($n=30$)	56.7% ($n=17$)	20.0% ($n=6$)	23.3% ($n=7$)	**0.003**	1.626 – 10.747	4.181/22.1%	8.817
Early AP-women ($n=42$)	69.0% ($n=29$)	21.4% ($n=9$)	9.5% ($n=4$)	0.526	0.511 – 4.336	1.488/3.3%	0.531
Late AP in men ($n=24$)	54.2% ($n=13$)	29.2% ($n=7$)	16.7% ($n=4$)	**0.040**	1.041 – 10.160	3.252/15.9%	4.115
Late AP in women ($n=37$)	48.6% ($n=18$)	40.5% ($n=15$)	10.8% ($n=4$)	0.122	0.786 – 7.164	2.373/10.0%	2.349
Controls in men ($n=284$)	52.8% ($n=150$)	40.5% ($n=115$)	6.7% ($n=19$)	0.157	0.779 – ~~4.50~~	~~81.874~~/5.6%	1.969
Controls in women ($n=165$)	65.6% ($n=108$)	30.3% ($n=50$)	4.2% ($n=7$)				

Legend: AP, chronic stable angina pectoris; C.I., confidence interval; OR, odds ratio.

0009-8981/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.cca.2008.03.001

appearance of AP symptoms (early AP: ≤50 years; late AP: >50 years) and gender. In comparison with healthy population, higher rate of GG homozygous genotype was characteristic both for patients with early (15.3%) (P=0.022) as well as late AP (13.1%) (P=0.025) and was present in both male (23.3%) (P=0.003) and female (16.7%) (P=0.040) AP patients. The significance was higher for men than women. There were no significant differences either in allele frequencies or carriage rates between patient or control groups.

Though data on the contribution of the MCP-1-2518 A/G single nucleotide polymorphism (SNP) to the pathogenesis of coronary atherosclerosis are heterogeneous [2,4,8], the current view is that this SNP is associated with severe CAD [2], myocardial infarction [9] and occult ischemia in a high-risk asymptomatic population [4]. The functional relevance of the MCP-1 SNP was revealed by its association with MCP-1 plasma levels [10].

In aggregate, our results are in line with these observations. The frequency of mutant GG genotype in Slovak patients with AP was significantly higher than in control subjects and it was also more frequent in men than women. We conclude that chronic stable angina pectoris in Slovak patients is linked to MCP-1-2518 (A/G) single nucleotide polymorphism and that inflammatory processes have important roles in the aetiology of this disease.

Acknowledgements

This work was supported by the grant VEGA 1/0528/03 and also in part by Czech and Slovak Ministries of Schools and Education, projects Kontakt 1/SK-CZ-02806, MSM6198959205 and ME-856.

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13 February 2008

Please cite this article as: Bucova M, et al, Association of chronic stable angina pectoris with MCP-1-2518 A/G single nucleotide polymorphism in the Slovak population, Clin Chim Acta (2008), doi:10.1016/j.cca.2008.03.001