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MCP-1 and CCR2 gene polymorphisms in Czech patients with allergic disorders

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Summary

Several lines of evidence suggest that chemokines play an important role in asthma and allergy. We analysed polymorphisms at –2518A/G and –2076A/T of MCP-1 and V64I of CCR2 gene in healthy subjects ($n = 306$) and allergic patients ($n = 332$). Allele and genotype frequencies did not differ significantly between groups. Nevertheless, MCP-1 variants were associated with allergen sensitization. The results suggest that MCP-1, but not CCR2 gene variants, may participate in the pathogenesis of allergic phenotypes at least in the Caucasian population.

Introduction

Asthma, allergic rhinitis and atopic eczema/dermatitis syndrome belong to allergic diseases. Their development is known to be the result of interactions between genetic background of the affected subject and environmental factors (Sengler *et al*., 2002). Increasing evidence suggests that MCP-1 (monocyte chemoattractant protein-1, also known as CCL2) and its haematopoietic cell receptor CC chemokine receptor 2 (CCR2) are involved in inflammatory disorders of the lungs (Chung, 2005). MCP-1 appears to play a significant role in asthma pathogenesis because of its ability to attract eosinophils and monocytes, activate basophils and mast cells, inducing leucotriene C4 release into the airway, which induces airway hyperresponsiveness (Campbell *et al*., 1999). MCP-1 can also drive undifferentiated T-lymphocytes towards interleukin-14 (IL-4)-producing Th2 cells (Karpus *et al*., 1997) and thus be important in allergic inflammation. Furthermore, increased MCP-1 expression has been demonstrated in the bronchial epithelium of asthmatics (Sousa *et al*., 1994). The coding gene for MCP-1 is located on chromosome 17q11.2, and gene for its receptor CCR2 lies on chromosome 3p21.3, regions for which linkage to asthma or atopy has been demonstrated (Bu *et al*., 2006; Steinke *et al*., 2008). A single nucleotide polymorphism of the MCP-1 gene in the gene regulatory region was found to be related to the expression of MCP-1 (Rovin *et al*., 1999). In addition, a substitution mutation (replacement of valine by isoleucine in the transmembrane region) has been described in the CCR2 gene (CCR2–64I). Given the importance of the chemokines and their receptors in the pathogenesis of allergic diseases, the aim of our study was to test whether allergic diseases or possibly related phenotypes are associated with selected polymorphisms in the MCP-1 and CCR2 genes in the Czech population.

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Materials and methods

Subjects

This case–control study comprised 638 unrelated Czech subjects living in the South Moravia region. A total of 332 patients with clinically manifested allergic diseases (176 men and 156 women, aged 33.6 ± 12.9 years (mean ± SD) — only asthma (34), only rhinitis (40), asthma and rhinitis (135), asthma and dermatitis (18), rhinitis and dermatitis (9) and combination of all three manifestations (96)) were studied. Allergic asthma diagnosis was based on the criteria of GINA 2002 (Global Initiative for Asthma) which include the following symptoms: non-productive cough, wheezing, breathlessness, reversibility airways obstruction, and bronchial hyperactivity and diagnostic test for IgE-mediated allergy. Diagnosis of allergic rhinitis was based on coordination between a typical history of allergic symptoms which include watery secretion from nose, nasal obstruction, nasal mucosa itching and sneezing, that are reversible spontaneously or after therapy (according to the criteria of Allergic Rhinitis and its Impact on Asthma Initiative, Bousquet *et al*., 2001) and diagnostic test for IgE-mediated allergy. The diagnosis of atopic dermatitis was defined according to the diagnostic criteria firstly described by Hanifin & Rajka (1980).

Atopy was defined as the presence of positive skin test reaction (≥ 3 mm greater than reaction to saline) to 1 or more of the common allergens (house dust mite, common mixed grass and tree pollens, mixed moulds, cat and dog dander, together with histamine and normal saline as a positive and a negative control, respectively) and/or raised specific serum IgE levels (> 0.35 kU L^{-1} by AlaSTAT test,

Table 1. MCP-1 and CCR2 polymorphisms and their relationship to the disease status, skin prick tests to selected allergens and/or body mass index

a

MCP-1 –2518A/G (rs 1024611)	Allele A, forward: 5′-GTGGGAGGCAGACAGCTA-3′ Allele G, forward: 5′-GTGGGAGGCAGACAGCTG-3′ Constant reverse: 5′-TGAGTGTTCACATAGGCTTC-3′
MCP-1 –2076A/T (rs 1024610)	Allele A, forward: 5′-CATGGTAAAGGATGCACTAACA-3′ Allele T, forward: 5′-CATGGTAAAGGATGCACTAACT-3′ Constant reverse: 5′-GTCTCAGTCCTCTGCTCA-3′

b

Locus	Allele	Genotype	Patients (%) $n = 332$	Controls (%) $n = 306$	*P* value	OR (95% CI)
MCP-1 –2518	A/G	AA	56.0	57.5	NS	1.00
		AG	39.2	34.3		0.85 (0.61–1.19)
		GG	4.8	8.2		1.65 (0.85–3.20)
MCP-1 –2076	A/T	AA	68.9	64.4	NS	1.00
		AT	29.2	32.0		1.14 (0.81–1.60)
		TT	3.9	3.6		0.95 (0.42–2.18)
CCR2 Ile64Val	A/G	Ile/Ile (AA)	1.3	2.0	NS	1.60 (0.44–5.73)
		Ile/Val (AG)	23.5	21.2		0.89 (0.61–1.29)
		Val/Val (GG)	75.3	76.8		1.00

c

	MCP-1 -2518				
Prick test — late spring mixture	AA	AG	GG	*P* value	OR (95% CI)
% total (negative/positive tests)	53.1 (66.7/38.2)	41.7 (28.3/56.4)	5.2 (5.0/5.4)	0.0020	3.24 (1.51–6.95)

d

	CCR2Val64Ile				
	Ile/Ile	Ile/Val	Val/Val	*P* value	OR (95% CI)
Body mass index	24.9 ± 2.5	23.3 ± 5.0	21.8 ± 3.9	0.0069	1.70 (1.12–3.52)

(a) Sequence-specific primers for both MCP-1 promoter polymorphisms.
(b) Genotype frequencies of MCP-1 and CCR2 polymorphisms in both groups (χ^2 test for genotype and Fisher exact test for allele frequencies were used; NS, non-significant).
(c) Results of the genetic data analysis in relation to the selected prick test (*P* value shows results of comparisons of numbers of subjects with negative (< 3 mm) versus positive (> 3 mm) prick test by Fisher exact test (odds ratio (OR) was calculated for AG + GG versus AA genotypes).
(d) Body mass index association with CCR2 polymorphism (by Kruskal–Wallis ANOVA, OR was calculated for Ile/Ile+Ile/Val versus Val/Val genotypes).
CI, confidence interval.

DPC Biermann, Bad Nauheim, Germany; produced in response to one or more of the common allergens including *Dermatophagoides farinae*, grass pollens, animal danders and moulds) and/or raised total serum IgE levels above normal values (> 100 IU mL^{-1} in non-smoking adults measured by the nephelometric test, Dade-Behring, Mannheim, Germany), as described previously (Holla *et al.*, 2002).

The control group consisted of 319 healthy subjects ($n = 306$, 161 men and 145 women, aged 41.5 ± 15.1 years) who met the following criteria: (i) no prior or current physician diagnosis of allergic diseases (rhinitis and/or asthma and/or dermatitis); (ii) no history of wheezing, shortness of breath and other symptoms of allergic diseases such as nasal and skin symptoms; (iii) no use of antiallergic medications; and (iv) absence of first-degree relatives with a history of allergic diseases.

The study was approved by the Committee for Ethics of the Faculty of Medicine, Masaryk University, and informed consent was obtained from all participants, in line with the Helsinki declaration before inclusion in the study.

Genotyping

After DNA extraction, the MCP-1 –2518A/G and –2076A/T alleles were typed using polymerase chain reaction with sequence specific primers (PCR-SSCP, Table 1a), and Val64Ile (G190A) polymorphism of the CCR-2 was investigated by a PCR-RFLP method as described earlier (Munerato *et al.*, 2003).

Statistical analysis

We calculated allele frequencies and tested agreement with Hardy–Weinberg equilibrium using a χ^2 goodness-of-fit test. We then compared differences in the allele frequencies and genotype distribution of the polymorphisms between case and control subjects using Fisher exact and χ^2 test. Where appropriate, Bonferroni correction was applied to adjust the α level according to the number of independent comparisons to an overall value of 0.05. Adjusted *P*-values are denoted as P_{corr}; $P_{corr} < 0.05$ was considered significant. The Kruskal–Wallis ANOVA test was used for the evaluation of total IgE levels and body mass index (BMI). Contingency table analysis, odds ratio (OR), 95% confidence intervals and significance values were estimated with the program package STATISTICA version 6.0 (Statsoft Inc., Tulsa, OK, USA).

Results and discussion

We found no association between any of the three investigated polymorphisms in the MCP-1 and CCR2 genes and clinical manifestation of allergic diseases ($P > 0.05$ in all cases, Table 1b). In allergic subjects, there were no relationships among CCR2 variant and a number of quantitative traits investigated in our study, including total serum IgE levels, selected specific IgE levels and skin test responses to aeroallergens (data not shown). However, this polymorphism was significantly associated with body mass index (BMI, $P = 0.007$, Kruskal–Wallis ANOVA, Table 1d). In contrast, both polymorphisms in MCP-1 promoter were related to skin prick test positivity to autumnal pollens ($P = 0.04$ for –2518A/G and $P = 0.02$ for –2076A/T, data not shown) and – 2518G allele was significantly associated with sensitisation to late spring pollens (33.6% subjects with positive test versus 19.2% subjects with negative test, $P = 0.002$ by Fisher exact test, $P_{corr} < 0.05$, OR = 3.24, 95%CI: 1.51–6.95, Table 1c), but not with IgE levels or severity of asthma (data not shown).

MCP-1, one of the CC chemokines, appears to play a significant role in asthma pathogenesis because of its ability to attract eosinophils, monocytes, and activate mast cells and basophils. It can also drive T-lymphocytes towards IL-4-producing Th-2 type cells (Karpus *et al*., 1997). A biallelic A/G polymorphism in the distal regulatory region at position –2518 has been found to affect the level of MCP-1 expression in response to inflammatory stimuli (Rovin *et al*., 1999). Monocytes from individuals carrying a G allele at –2518 produce more MCP-1 after treatment with IL-1β than monocytes from AA homozygotes. The effect of the G allele appears to be dose-dependent; cells from individuals homozygous for G allele at –2518 produce more MCP-1 than cells from G/A heterozygotes (Rovin *et al*., 1999). The –2518G allele increases susceptibility to asthma, asthma severity and eosinophil levels in Caucasians (Szalai *et al*., 2001), but not in Chinese children (Yao *et al*., 2004). In our study, the same allele was associated with allergen sensitisation to pollens. However, in contrast to both previous studies that described significant associations of Val64Ile polymorphism in the CCR2 gene with allergic asthma in Koreans (Kim *et al*., 2007) and with cedar pollinosis in Japanese (Nakamura *et al*., 2007), we found no relationship of identical variant with clinically manifested allergic diseases or related phenotypes in Caucasians. In conclusion, our study supports the idea that MCP-1, but not CCR2 gene variants, may participate in the pathogenesis of particular allergic phenotypes at least in the Czech population. However, the functional relationship of ascertained associations still needs clarification. Thus, further genetic and functional studies are needed to elucidate the role of MCP-1 polymorphisms in the molecular mechanisms underlying.

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