

## The CCR2 promoter polymorphism T-960A, but not the serum MCP-1 level, is associated with endothelial function in prediabetic individuals

Kilian Rittig<sup>a,1</sup>, Andreas Peter<sup>a,1</sup>, Katrin M. Baltz<sup>b</sup>, Otto Tschritter<sup>a</sup>, Cora Weigert<sup>a</sup>, Francesco Andreozzi<sup>c</sup>, Francesco Perticone<sup>c</sup>, Dorothea I. Siegel-Axel<sup>d</sup>, Norbert Stefan<sup>a</sup>, Andreas Fritsche<sup>a</sup>, Helmut R. Salih<sup>b</sup>, Erwin Schleicher<sup>a</sup>, Fausto Machicao<sup>a</sup>, Giorgio Sesti<sup>c</sup>, Hans-Ulrich Häring<sup>a</sup>, Bernd M. Balletshofer<sup>a,\*</sup>

<sup>a</sup> Department of Endocrinology, Diabetes, Vascular Medicine, Nephrology and Clinical Chemistry, University of Tübingen, Otfried-Müller Straße 10, 72076 Tübingen, Germany

<sup>b</sup> Department of Hematology and Oncology, University of Tübingen, Germany

<sup>c</sup> Department of Experimental and Clinical Medicine, University Magna Graecia, Viale Europa, Località Germaneto, Catanzaro, Italy

<sup>d</sup> Department of Cardiology, University of Tübingen, Germany

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### Abstract

Monocyte-chemoattractant-protein (MCP)-1 and its receptor CCR2 have been shown to play a pivotal role in vascular inflammation and atherosclerotic plaque formation. However, it is currently unclear whether MCP-1/CCR2 triggered inflammation affects nitric oxide (NO)-bioavailability, hence influencing vascular function, a sign of early atherosclerosis. Therefore, we sought to investigate the association between serum levels of MCP-1 and NO-bioavailability, expressed as flow mediated dilation (FMD) *in vivo*, and the impact of CCR2 gene variations on FMD. We studied a German population of 242 prediabetic individuals (144 women, 98 men; mean age  $45 \pm 0.8$  years) via FMD by high-resolution ultrasound (13 MHz). In order to replicate our findings, a second, independent population ( $n = 115$ ; 44 women, 77 men; mean age  $48 \pm 1.0$  years) (total = 357 individuals) from Italy was studied. Vascular function in the Italian population was studied via intra-arterial application of acetylcholine. MCP-1 serum-levels were assessed by ELISA and CCR2 polymorphisms were determined by sequencing. MCP-1 serum levels showed no association with FMD ( $p = 0.90$ ), whereas the CCR2 promoter polymorphism was associated with elevated FMD (T/T:  $5.6 \pm 0.3\%$ ; T/A:  $6.7 \pm 0.4\%$ ; A/A:  $8.3 \pm 0.8\%$ ;  $p = 0.01$ ) after adjusting for possible confounders. These results were confirmed in the independent Italian population (A/A:  $97.1 \pm 20.3$  vs. T/T:  $60.5 \pm 5.6\%$  forearm blood-flow increase;  $p < 0.05$ ). When testing for the functional relevance of the T-960A (rs3918359) polymorphism, we found that the A/A-genotype was associated with moderately increased protein binding in EMSA, increased promoter activity in luciferase assays and reduced transendothelial monocyte migration. In conclusion, MCP-1 serum levels do not reflect endothelial function *in vivo* in prediabetic individuals. However, the functionally relevant CCR2 promoter polymorphism T-960A (rs3918359) is associated with elevated vascular function. This might be due to reduced subendothelial inflammation, mediated by reduced transendothelial monocyte-migration ability.

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**Keywords:** Endothelium; Vasodilation; MCP1/CCR2; Inflammation; Nitric oxide; Genetics

**Abbreviations:** Ach, acetylcholine; BP, blood pressure; bp, base pairs; CCR2, chemokine receptor 2; cm, centimeter; diast., diastolic; dl, decilitre; EMSA, electro mobility shift assay; FACS, fluorescence activated cell sorting; F, female; Fig., figure; FMD, flow mediated dilation; GTN dil., glyceroltrinitrate dilation; h, hour; hsCRP, high sensitive C-reactive protein; ISI, insulin-sensitivity-index; m, male; MCP-1, monocyte chemoattractant protein-1; mg, milligram; mmHg, millimeter mercury; mmol, millimol; PBMC, peripheral blood mononuclear cells; s, second; syst., systolic.

\* Corresponding author. Tel.: +49 7071 29 82711; fax: +49 7071 29 4454.

E-mail address: bernd.balletshofer@med.uni-tuebingen.de (B.M. Balletshofer).

<sup>1</sup> These authors contributed equally.