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Glycoprotein 130 polymorphism predicts soluble glycoprotein 130 levels

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ABSTRACT

Objective. Interleukin-6 (IL-6) is a key cytokine in inflammatory diseases. It exerts its biological function via binding to a homodimer of its signal transducer glycoprotein 130 (gp130). Soluble gp130 (sgp130) is the natural inhibitor of IL-6 trans-signalling. The aim of this study was to test a possible influence of the gp130 genotype on sgp130 serum levels.

Material and methods. In two separate populations, subjects were genotyped for the gp130 polymorphism G148C. Sgp130, IL-6 and soluble interleukin-6 receptor (sIL-6R) levels were measured. The OSLO population consisted of 546 male subjects at high risk for CAD. The VIENNA population consisted of 299 male subjects with angiographically proven CAD.

Results. In the OSLO population, 124 (22.7%) subjects were hetero- or homozygote for the rare C allele. Individuals carrying the polymorphism had significantly higher levels of sgp130. In a multivariate linear regression model this association remained significant (adjusted $p = 0.001$). In the VIENNA population, 48 (16.1%) subjects were hetero- or homozygote for the rare C allele. Consistent with the former study, sgp130 levels were significantly higher in carriers of the polymorphism compared to wildtype carriers (adjusted $p = 0.038$). In the VIENNA population, sgp130 levels were significantly higher in diabetic patients. In the OSLO population, sgp130 was higher in patients with increased body mass index and in smokers ($p < 0.05$).

Conclusions. Sgp130 serum levels are significantly higher in subjects carrying the gp130 polymorphism G148C compared to wildtype carriers. This finding proposes a possible genetical influence on sgp130 levels which may alter individual coping mechanisms in inflammatory diseases.

Abbreviations: BMI, Body mass index; CAD, Coronary artery disease; COPD, Chronic obstructive pulmonary disease; CV, Coefficient of variation; DNA, Deoxyribonucleic acid; EDTA, Ethylenediaminetetraacetic acid; ELISA, Enzyme-linked immunosorbent assay; Gp130, Glycoprotein 130; hsIL-6, High sensitive IL-6; IL-6, Interleukin-6; IL-6R, Interleukin-6 receptor; PCR, Polymerase chain reaction; RFLP, Restriction fragment length polymorphism; Sgp130, Soluble glycoprotein 130; sIL-6R, Soluble interleukin-6 receptor; STAT, Signal transducers and activators of transcription; VSMC, Vascular smooth muscle cell.

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1. Background

Atherosclerosis – and thus coronary artery disease (CAD) – is known to be an inflammatory disease [1]. Interleukin-6 (IL-6) is a systemic cytokine that plays a pivotal role in the development of atherosclerotic lesions and progression. It is able to stimulate T-cell activation as well as vascular smooth muscle cell (VSMC) motility [2–4]. Furthermore it has been shown to induce the expression of matrix metalloproteinases, and thereby enhance matrix degradation and tissue remodelling [5].

In patients with myocardial infarction, IL-6 levels were shown to be significantly elevated and to be of great prognostic value for cardiovascular events as well as cardiovascular death. Even in apparently healthy subjects, increased levels could be associated with increased risk of future myocardial infarction [6].

In the "classical" signalling pathway, IL-6 acts via binding to a membrane bound IL-6 receptor (IL-6R) and a homodimer of glycoprotein 130 (gp130), a 130-kD signal transducer [7]. Gp130 is found ubiquitously, the membrane bound IL-6R is not. As an alternative, IL-6 can build a complex with a soluble form of the IL6-R (sIL6-R), thus acting on cells that do not express IL6-R. This mechanism has been named "trans-signalling" [8,9]. It has been proposed that trans-signalling is involved in pro-inflammatory actions of IL-6 [10]. Cell types that depend on trans-signalling include T-cells, VSMCs, and endothelial cells [11–14].

Soluble gp130 (sgp130) is the natural inhibitor of the IL-6 trans-signalling pathway [15,16]. It inhibits the attachment of the IL-6/sIL-6R-complex to gp130 on the cell surface. Elevated sgp130 serum concentrations were found in inflammatory diseases, such as Crohn's disease, rheumatoid arthritis or inflammatory colon cancer [17–19]. It has been shown that sgp130 modulates leukocyte trafficking and is able to suppress the severity of experimental arthritis, colitis, and colon cancer [20,21].

Recently, an association between the gp130 polymorphism G148C (rs3729960) and a decreased risk of myocardial infarction has been identified [22].

To the best of our knowledge, the influence of the gp130 polymorphism G148C on circulating levels of its soluble form has previously not been studied. Our hypothesis was that this polymorphism was associated with higher levels of sgp130.

2. Material and methods

2.1. Study design

2.1.1. OSLO population

A total of 563 men at high risk of cardiovascular disease, aged 64–76 years, were enrolled in the Diet and Omega-3 Intervention Trial on Atherosclerosis (DOIT) study (NCT 00764010, clinicaltrials.gov). Details are given elsewhere [23]. Altogether 546 patients had samples for DNA analysis. The study was approved by the local ethics committee and was consistent with the Helsinki Declaration. All patients gave their written informed consent to participate.

2.1.2. VIENNA population

Two-hundred and ninety-nine subjects with angiographically proven CAD were included. Angiography was performed by experienced cardiologists. CAD was defined as the presence of a stenosis of greater than 50% in one or more of the main coronary arteries (left anterior descending, right coronary artery, circumflex artery or left main artery). Subjects with concurrent severe illness or who suffered an acute coronary syndrome within the 3 months before enrolment were excluded. Written informed consent was obtained from all participants. This study was approved by the Medical University of Vienna ethics committee and complies with the declaration of Helsinki.

In both populations blood samples were drawn at inclusion after overnight fasting. Serum was kept frozen at −80 °C until analysis. Routine laboratory measurements were performed by use of conventional methods. Circulating levels of sIL-6R as well as IL-6 in the OSLO population and high sensitive IL-6 (hsIL-6) in the VIENNA population, respectively, were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Abingdon, Oxon, UK) following the supplied protocol.

2.2. DNA extraction and genotyping

All laboratory measurements were performed by investigators who were blinded to subject characteristics.

2.2.1. OSLO population

DNA for genotyping was extracted from EDTA whole-blood using the Magna Pure LC Instrument (Roche Diagnostics, Mannheim, Germany) and tested for quantity and purity on the NanoDrop spectrophotometer, ND-1000 (Saveen Werner, Sweden).

Allelic discrimination of the G148C polymorphism (rs3729960) was performed by the Applied Biosystems 7900HT Real-Time PCR system (Applied Biosystems, Foster City, CA, USA), using allele specific primers and probes including in the Taqman SNP Genotyping Assay (TaqMan MGB probes; FAM and VIC dye labelled) and the TaqMan Genotyping Master Mix (Applied Biosystems). A final reaction volume of 25 μl was used and 1 μl DNA was added. In SNP analysis, 5% of the samples were repeated, with 100% concordance.

2.2.2. VIENNA population

DNA for genotyping was extracted from whole blood using the MagNA Pure LC DNA Isolation Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions.

The gp130 G148C polymorphism (rs3729960) was detected by restriction fragment length polymorphism (RFLP) analysis following polymerase chain reaction (PCR). Fragments of genomic DNA were amplified for each subject by polymerase chain reaction using primers designed with Primer 3 software [http://frodo.wi.mit.edu/] (forward: 5'TGC CTC CAG AAA AAC CTA AA-3'; reverse: 5'-CAT TCA GAT TTT AAA GTG AAG-3') and PCR SuperMix (Invitrogen. Carlsbad, CA). PCR-products were digested with SAU3AI restriction enzyme (restriction site: 5' GATC 3', New England BioLabs, Ipswich, MA, USA). Products were separated by electrophoresis on a 4% agarose gel. Polymorphisms were detected by ethidium bromide staining.