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Duffy antigen receptor genetic variant and the association with Interleukin 8 levels

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ABSTRACT

The aim of this study is to identify loci associated with circulating levels of Interleukin 8 (IL8). We investigated the associations of 121,445 single nucleotide polymorphisms (SNPs) from the Illumina 200 K CardioMetabochip with IL8 levels in 1077 controls from the Stockholm Heart Epidemiology Program (SHEEP) study, using linear regression under an additive model of inheritance.

Five SNPs (**rs12075A/G**, **rs13179413C/T**, **rs6907989T/A**, **rs9352745A/C**, **rs1779553T/C**) reached the predefined threshold of genome-wide significance ($p < 1.0 \times 10^{-5}$) and were tested for *in silico* replication in three independent populations, derived from the PIVUS, MDC-CC and SCARF studies. IL8 was measured in serum (SHEEP, PIVUS) and plasma (MDC-CC, SCARF). The strongest association was found with the SNP rs12075 A/G, Asp42Gly ($p = 1.6 \times 10^{-6}$), mapping to the Duffy antigen receptor for chemokines (*DARC*) gene on chromosome 1. The minor allele G was associated with 15.6% and 10.4% reduction in serum IL8 per copy of the allele in SHEEP and PIVUS studies respectively. No association was observed between rs12075 and plasma IL8.

Conclusion: rs12075 was associated with serum levels but not with plasma levels of IL8. It is likely that serum IL8 represents the combination of levels of circulating plasma IL8 and additional chemokine liberated from the erythrocyte *DARC* reservoir due to clotting. These findings highlight the importance of understanding IL8 as a biomarker in cardiometabolic diseases.

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1. Introduction

Genetic variants regulating circulating Interleukin 8 (IL8) levels are largely unknown [1]. A secondary analysis performed in a genome-wide association study (GWAS) of the genetic basis of circulating monocyte chemoattractant protein-1 (MCP-1), reported an association between rs12075 in the Duffy antigen receptor for chemokines (*DARC*) gene and serum IL8 concentration [2]. Recently, in a large population-based case control study, we have analyzed the association of genetic variants in the *IL8* and *IL8* receptors (*IL8R*) genes with levels of serum IL8 and risk of myocardial infarction

Abbreviations: IL8, Interleukin 8; DARC, Duffy antigen receptor for chemokines; SNPs, single nucleotide polymorphisms; SHEEP, Stockholm Heart Epidemiology Program; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; MDC-CC, Malmö Diet and Cancer study-cardiovascular cohort; SCARF, Stockholm Coronary Artery Risk Factor; MCP-1, Monocyte chemoattractant protein 1; IL8R, Interleukin 8 receptors; CVD, cardiovascular disease; MAF, minor allele frequency; IQTR, interquantile range.

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(MI) [3]. We have observed that a variant in the promoter region of *IL8* is associated with a modest increase in the MI risk in men. However, the genetic variants in *IL8* and *IL8R* genes did not regulate IL8 serum levels.

The identification of the genes involved in the regulation of IL8 levels is of importance to comprehend the mechanisms underlying IL8 synthesis and release in physiological conditions as well as in cardiometabolic disorders; yet few studies have measured circulating IL8 at a population based level [4,5].

The primary aim of this study was to identify the genetic determinants of levels of circulating IL8 in the control samples from Stockholm Heart Epidemiology Program (SHEEP) using single nucleotide polymorphisms (SNPs) contained on the Illumina 200 K CardioMetabochip. Findings made in the discovery study were replicated in three independent materials. To the best of our knowledge, this study represents the largest investigation of the genetic determinants of IL8 performed so far.

2. Materials and methods

2.1. Study populations

The SHEEP study, a population based case-control, was used as discovery study. A detailed description of SHEEP is given elsewhere [6,7]. In brief, the study base comprised of all Swedish citizens living in the Stockholm County who were 45–70 years of age at inclusion and were not diagnosed with MI previously. First time incident MI cases ($n = 1213$) were identified during a 2-year period (1992–1993) for men and during a 3-year period (1992–1994) for women. Controls ($n = 1561$) were randomly recruited from the study base continuously over time within 2 days of the case occurrence (density sampling) and matched to cases on age (5-years interval), sex and hospital catchment area using computerized registers of the population of Stockholm. Five controls were sampled simultaneously to be able to replace potential non-respondent controls. Occasionally, because of late response of the initial control, both the first and the alternative controls were considered resulting in the inclusion of more controls than cases. Postal questionnaires covering a wide range of exposures areas were distributed to the participants. Clinical investigations were performed at least three months after MI of cases and their matched controls, including blood samplings under fasting conditions with collection of whole blood for DNA extraction, serum and plasma. The participation rate for blood drawing in cases was about 77% and in controls about 67%. For the present study, only controls matched with the non-fatal MI cases with available IL8 and genotyping data were analyzed ($n = 1077$).

The study was approved by the Regional Ethical Review Board at Karolinska Institutet, Stockholm, Sweden. All participants gave their informed oral consent to be enrolled in the study. At the time the study was conducted (1992) no forms for the written consent were in current use. Cases were informed about the study at the time they were discharged from the hospital while controls were invited via mail.

We replicated our findings in three independent studies, the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS), the Malmö Diet and Cancer (MDC) study-cardiovascular cohort (MDC-CC) and the Stockholm Coronary Artery Risk Factor (SCARF) study.

PIVUS is described in detail elsewhere [8] and at http://www.medsci.uu.se/pivus/pivus.htm. Briefly, all 70-year-old men and women living in Uppsala, Sweden between 2001 and 2004 were eligible for the study. Of 2025 individuals invited for the study, 1016 agreed to participate. Participants with available serum IL8 and genotype data were included in the present investigation ($N = 931$). All the participants gave written informed consent and the Ethics Committee of Uppsala University, Uppsala, Sweden, approved the study protocol.

The MDC-CC comprises 6103 men and women from the population-based MDC cohort, who were invited to take part in a substudy of the epidemiology of cardiovascular disease (CVD). Baseline examinations were performed between 1991 and 1994 [9]. Plasma IL8 and genotype data were available in 700 individuals and in close to 800 individuals with metabolic syndrome (we have merged the data for those with repeated measurements $N = 1218$). All participants provided written informed consent and the study was approved by the Regional Ethical Review Board in Lund, Sweden.

SCARF is a case-control study from the northern part of Stockholm (recruitment in 1996–2000) comprising 387 MI survivors below the age of 60 and 387 control individuals matched for age, sex and area of residence. Three months after the index event, whole blood for DNA extraction and EDTA plasma samples were collected under fasting conditions. Details about the SCARF study have been reported elsewhere [10]. Only controls with plasma IL8 and genotypes data ($N = 350$) were considered in the analyses.

The study was approved by the Regional Ethical Review Board at Karolinska Institutet, Stockholm, Sweden. All participants gave their informed oral consent to be enrolled in the study.

2.2. IL8 measurements

SHEEP study. IL8 was measured in serum ($n = 1077$) and in a subset ($n = 669$) of available plasma samples by an electrochemiluminescence immunoassay established by Meso Scale Discovery Multi-Array® (MSD) technology. Samples were analyzed in 96-well plates using the MSD® 6000 instrument, following the manufacturer's instructions. Serum concentrations were derived from the standard curve and expressed as picograms per milliliter (pg/mL).

PIVUS study. The levels of IL8 in serum were measured using Evidence® array biochip analyzer (Randox laboratories Ltd., Crumlin, UK) [11].

MDC-CC study. IL8 levels in the MDC-CC were measured in heparin plasma by an electrochemiluminescence immunoassay (Human Ultra-Sensitive Kit) using a SECTOR Imager 6000 instrument (MesoScale Discovery, Gaithersburg, MD, USA).

SCARF study. IL8 levels were measured in EDTA plasma samples using Evidence® array biochip analyzer (Randox laboratories Ltd., Crumlin, UK) [11].

2.3. Genotyping analysis and SNPs selection

Genomic DNA from SHEEP study participants was genotyped using the Illumina 200 K CardioMetabochip, a custom-made genotyping array that captures DNA variation at regions identified by meta-analyses of GWAS for diseases and traits relevant to metabolic and atherosclerotic/cardiovascular diseases.

The chip is a custom genotyping array of about 200,000 genetic variants, identified by chromosomal location and/or SNP identification number [12].

In the SHEEP study, individual-level exclusion criteria were genetic variants/SNPs with the following characteristics: minor allele frequency (MAF) < 0.01 ($n = 61{,}153$ SNPs), highly significant deviation from Hardy–Weinberg equilibrium ($p < 1 \times 10^{-5}$, $n = 1180$ variants), variants missingness (>5% missing information, $n = 3475$ SNPs) due to more than 5% missing information and genotype call rates <0.95. After quality control procedures, 121,445 autosomal SNPs were included in the association analysis.

A major challenge in genome wide association studies is to derive the multiple testing threshold when hypothesis tests are conducted using a large number of SNPs. In the present study, an *a priori* p value $<1 \times 10^{-5}$ was set to reflect the association with

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