Polymorphism in the MHC2TA gene is associated with features of the metabolic syndrome and cardiovascular mortality.

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INTRODUCTION
Cardiovascular disease is the leading cause of death in Sweden and in most Western countries. Almost 50% of deceased individuals died due to cardiovascular causes in Sweden in 2005 (Swedish National Board of Health and Welfare http://www.socialstyrelsen.se). Both genetic and environmental factors modify the risk for cardiovascular diseases including myocardial infarction (MI) [1], smoking, dyslipidemia, diabetes, obesity and hypertension, are present in up to 90% of patients with MI [2,3]. Family history of MI has been shown to be a strong independent risk factor for coronary heart disease [4-7].

Microalbuminuria is an established risk marker for cardiovascular morbidity and mortality both in diabetic [8] and non-diabetic patients [9]. Inflammation is believed to play a major role in the pathogenesis of both microalbuminuria [10] and MI [11]. Insulin resistance has been proposed as a common denominator for these conditions, and has also been related to subclinical chronic inflammation [12].

A -168 A→G polymorphism in the MHC class II transactivator gene (MHC2TA) was recently found to be associated with MI, rheumatoid arthritis and multiple sclerosis [13]. The -168 A→G polymorphism was associated with lower expression of MHC2TA after stimulation of leukocytes with interferon-γ in humans and differences in expression of MHC class II molecules in different rat strains. Because of the role of MHC class II molecules in recognition of antigen molecules, genes like MHC2TA that can influence expression of MHC class II, are also candidate genes for autoimmune diseases [13].

To address this issue, we searched for any association between MHC2TA -168 A→G polymorphism and cardiovascular morbidity and mortality as well as their predictors, microalbuminuria and the metabolic syndrome (MetS).

MATERIALS AND METHODS
Study Subjects
Patients were selected from three large populations in Finland and Sweden; the Botnia study, the Malmö Diet and Cancer Study (MDC) and the Diabetes Registry in Southern Sweden (DR). The protocols were approved by local Ethics committees, and informed consent was obtained from all subjects.

The Botnia Study
The Botnia Study was initiated in 1990 and represents a large population-based type 2 diabetes (T2D) cohort from north-western Finland (the Botnia region). The study is conducted in collaboration with the Units of Endocrinology and Metabolism and Cardiology, Department of Medicine, Oulu University Hospital, Oulu, Finland. The study design has been described earlier [14-16]. The study population consists of 3,065 T2D patients from the Local Swedish Diabetes registry.

The Malmö Diet and Cancer Study
The Malmö Diet and Cancer Study (MDC) was initiated in 1990 and comprises an MI case-control sample, and 3,245 control subjects participating in the Malmo¨ Diet and Cancer study. A total of 2,122 patients with MI and 2,345 control subjects.

The Diabetes Registry in Southern Sweden (DR)
The Diabetes Registry in Southern Sweden is a nationwide register for diabetic patients and it was initiated in 1990. Since then, the registry has included approximately 50% of all diabetic patients in southern Sweden. Among these diabetic patients, 1,800 have been enrolled in the Southern Sweden Heart Study which is a case-control study for MI. As the majority of diabetic patients in southern Sweden are included in the registry, this study population is representative for MI in the region.

Background. Recently, a -168A→G polymorphism in the MHC class II transactivator gene (MHC2TA) was shown to be associated with increased susceptibility to myocardial infarction (MI). Aim. To confirm the association between the MHC2TA -168A→G polymorphism and MI and to study its putative role for microalbuminuria, the metabolic syndrome (MetS) and cardiovascular mortality.

Materials and Methods. Using an allelic discrimination method we genotyped 11,064 individuals from three studies: 1) 4,432 individuals from the Botnia type 2 diabetes (T2D) study, 2) 1,222 patients with MI and 2,345 control subjects participating in the Malmo¨ Diet and Cancer study and comprising an MI case-control sample, and 3) 3,065 T2D patients from the Local Swedish Diabetes registry. Results. No association between the -168A→G polymorphism in MHC2TA and MI was observed. However, in the Botnia cohort the AG/GG genotypes were associated with cardiovascular mortality after MI (1.78 [1.09–2.92], p = 0.02). In addition, the AG/GG genotypes were more common in subjects with MetS (40.1% vs. 36.9%, p = 0.03) and in non-diabetic subjects with microalbuminuria (45.4% vs. 36.5%, p = 0.003) compared to control subjects. Conclusions. A polymorphism in MHC2TA was associated with cardiovascular mortality and predictors of cardiovascular mortality, microalbuminuria and MetS.


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family study in Finland and Sweden, aiming at identification of genes increasing susceptibility to T2D, MetS and associated complications. Details of the study cohort, sampling strategy as well as anthropometric and metabolic measurements have been described earlier [14,15]. At the baseline examination, a structured questionnaire was completed by specially trained nurses, covering information on life style (i.e., smoking status), personal diseases (e.g., MI, hypertension, diabetes mellitus) and family relations.

Statistical methods
Data are presented as mean ± SD or as median [25%-75%] percentile. Chi-square tests were used to analyze differences between allele- and genotype frequencies. To test differences between group means, the Student’s two-tailed t-test was used for normally distributed values and Mann-Whitney U-test for non-normally distributed measurements. In order to identify factors associated with MI and microalbuminuria, a multiple logistic regression analysis with forward selection was performed. Because of the nature of Botnia cohort as a family collection, the analyses were adjusted for the family variables. For the mortality analyses, clinical variables together with MHC2TA genotypes were entered into a forward stepwise Cox regression model adjusted for sex, age and family relations.

All data were analyzed with a NCSS 2004 (NCSS statistical software, Kaysville, UT, USA). A p-value of <0.05 was considered statistically significant. Power analysis was made using Genetic Power Calculator [20].

RESULTS
The clinical characteristics of the study groups are given in Table 1. The genotype and allele frequencies of the MHC2TA polymorphism were similar in patients with or without MI, regardless of the study population and T2D status (Table 2). No association with T2D was observed, neither in the Finnish (Botnia), nor in the Swedish (MDC or DR) cohorts.

No correlation between the MHC2TA-168 AG/GG genotypes and cardiovascular mortality was found in the Botnia Study population (HR 0.96 [0.93–1.02], p = 0.47). As the MHC2TA polymorphism was earlier shown to be associated with MI, we performed a subgroup analysis of individuals with previous MI. The AG/GG genotype was protective against cardiovascular death in the whole group (HR 0.38 [0.21–0.69], p = 0.002) (Table 3, Figure 1). We also tested the AG/GG genotypes were associated with increased risk of cardiovascular death compared with AA genotype carriers (HR 1.76 [1.09–2.82], p = 0.02) (Table 3, Figure 1). We also tested the MHC2TA AG/GG genotype against the AA or AG genotypes and found that the GG genotype was protective against cardiovascular death in the whole group (HR 0.31 [0.16–0.62], p = 0.005), but not in patients with previous MI (HR 0.45 [0.27–0.78], p = 0.004).

The MHC2TA AG/GG genotypes were more frequently found among patients with MetS (30.1 vs. 36.9%, p = 0.039) as well as among non-diabetic individuals with microalbuminuria in the Botnia cohort (30.0% vs. 36.0%, p = 0.003). In contrast, the AG/GG genotypes were not associated with microalbuminuria among T2D patients, neither in the Botnia, nor the DR cohort (Table 2). Correspondingly, logistic regression analysis with age, waist-hip ratio, fasting insulin, systolic- and diastolic blood pressure as well as smoking status, hypertension, diabetes mellitus and other cardiovascular diseases was performed.

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pressure, smoking, gender and MHC2TA AG/GG genotypes as independent factors, revealed the AG/GG genotypes as risk factors for microalbuminuria in non-diabetic subjects (OR 2.07)

Table 1. Clinical characteristics of the study subjects.

<table>
<thead>
<tr>
<th></th>
<th>Botnia</th>
<th>MDC</th>
<th>DR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-diabetic</td>
<td>T2D</td>
<td>p</td>
</tr>
<tr>
<td>Male/Female</td>
<td>1307/1566</td>
<td>731/828</td>
<td>0.37</td>
</tr>
<tr>
<td>Age (Yrs.)</td>
<td>54±13</td>
<td>67±12</td>
<td>&lt;1×10⁻⁶</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>26.2±4.1</td>
<td>28.1±4.7</td>
<td>&lt;1×10⁻⁶</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>135±20</td>
<td>150±22</td>
<td>&lt;1×10⁻⁶</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>81±10</td>
<td>83±11</td>
<td>&lt;1×10⁻⁶</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.6±0.5</td>
<td>7.5±1.7</td>
<td>&lt;1×10⁻⁶</td>
</tr>
<tr>
<td>Age at onset of Diabetes (Yrs.)</td>
<td>-</td>
<td>59±14</td>
<td>-</td>
</tr>
<tr>
<td>Smoking (%)*</td>
<td>38.0</td>
<td>36.7</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Numbers are mean±SD or percent. *Both previous and current smoking. P-values refer to comparison between non-diabetic and T2D patients.

doi:10.1371/journal.pone.0000064.t001

Table 2. The genotype frequencies of the MHC2TA -168 A→G polymorphism in different study populations according to history of previous MI and microalbuminuria status.

<table>
<thead>
<tr>
<th></th>
<th>MI</th>
<th>p</th>
<th>Microalbuminuria (MALB)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA/AG/GG</td>
<td></td>
<td>AA/AG/GG</td>
<td></td>
</tr>
<tr>
<td>Botnia</td>
<td>Non-diabetic</td>
<td>(N=112)</td>
<td>(N=2686)</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>T2D</td>
<td>(N=184)</td>
<td>(N=1326)</td>
<td>0.90</td>
</tr>
<tr>
<td>MDC</td>
<td>Non-diabetic</td>
<td>(N=1071)</td>
<td>(N=2312)</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>T2D</td>
<td>(N=151)</td>
<td>(N=123)</td>
<td>0.24</td>
</tr>
<tr>
<td>Diabetes registry</td>
<td>T2D</td>
<td>(N=316)</td>
<td>(N=1974)</td>
<td>0.44</td>
</tr>
</tbody>
</table>

The numbers are allele frequencies (%) and number of patients. P-values refer to comparison between genotype frequencies of the –168A risk genotypes (AG or GG) among individuals with or without MI and with or without microalbuminuria, respectively.

doi:10.1371/journal.pone.0000064.t002

Table 3. Predictors of cardiovascular mortality among all individuals and patients with previous MI from the Botnia study.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Previous MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Sex</td>
<td>1.34 (1.00–1.80)</td>
<td>1.20 (0.84–2.25)</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.42 (1.04–1.96)</td>
<td>1.33 (0.76–2.25)</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>1.11 (0.91–1.22)</td>
<td>1.10 (0.87–1.39)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>0.57 (0.38–0.85)</td>
<td>0.76 (0.37–2.32)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>1.00 (0.99–1.01)</td>
<td>1.00 (0.96–1.01)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>1.00 (0.99–1.01)</td>
<td>0.99 (0.97–1.02)</td>
</tr>
<tr>
<td>MHC2TA (AG or GG)</td>
<td>0.96 (0.75–1.22)</td>
<td>1.76 (0.97–3.02)</td>
</tr>
</tbody>
</table>

Numbers are hazard ratio (HR) and 95% Confidence limits. HRs were mutually adjusted.

*HR per mmHg

doi:10.1371/journal.pone.0000064.t003

Figure 1. Cardiovascular mortality in the Botnia cohort in patients with previous MI according to MHC2TA -168 A→G genotypes. Kaplan Meier survival curves illustrating a higher risk for CV mortality (HR 1.76 [1.09–2.82], p=0.02) in AG/GG genotype carriers with previous History of MI. doi:10.1371/journal.pone.0000064.g001
The statistical power to detect differences in risk of MI among patients with G allele, HOMA –index, and higher blood pressure (data not shown) was restricted to non-diabetic subjects. This could reflect the fact that in diabetic subjects other factors including hyperglycaemia, smoking, heart failure and renal atherosclerosis, and has been suggested to reflect a state of low-grade systemic inflammation. Thus, the HMIC2TA -168 A→G polymorphism influenced both outcome and prediction of cardiovascular disease. Among patients with previous MI, carriers of the AG or GG genotypes had increased risk of cardiovascular death compared with AA genotype carriers (Table 1 and Figure 1). It is known that inflammation plays a key role in development of atherosclerosis [11] and activated T-lymphocytes are already present in the atherosclerotic plaque, as well as in the immediate site of plaque rupture or superficial erosions in patients who have died due to MI or unstable angina [23]. Our results suggest that the G allele [and in particular the AG G polymorphism] could be a risk factor for cardiovascular mortality after MI, although the mechanism remains unclear. Swanson et al. suggested that the G allele could cause reduced induction of MHC-class II genes thus leading to less efficient presentation of antigens to regulatory T cells [13]. However, the previous association analysis compared AG and GG genotype carriers to AA genotype carriers and showed that in particular the AG (and not GG) genotype carriers were at higher risk [13]. In contrast, the expression analysis compared a pool of AA, AG, and GG genotype carriers to GG genotype carriers, thus not challenging the possibility of a difference between the more common AA and AG genotypes [13]. Interestingly, we observed that in fact the GG carriers had a lower risk for death due to cardiovascular events compared to AA or AG genotype carriers (HR: 0.38 [0.16–0.92]). It is therefore unclear whether the risk really is associated with less induction of the MHC II genes in response to inflammation stimuli. To clarify this issue, expression levels of all genotypes would therefore be of interest, especially comparisons between the AA and MI genotypes. The key finding of the present study was an association between the HMIC2TA -168 A→G polymorphism and cardiovascular mortality as well as with predictors thereof, microalbuminuria [22]. The association with microalbuminuria was, however, restricted to non-diabetic subjects. This could reflect the fact that in diabetic subjects other factors including hyperglycaemia may influence the day-to-day variation in albumin excretion. The non-diabetic patients with microalbuminuria had several features of M San including higher waist to hip ratio, higher HOMA index, and higher blood pressure (data not shown) compared to individuals without microalbuminuria. Accordingly, the HMIC2TA -168 A→G polymorphism was also associated with MetS. Therefore the HMIC2TA -168 A→G polymorphism influenced both outcome and prediction of cardiovascular disease. Among patients with previous MI, carriers of the AG or GG genotypes had increased risk of cardiovascular death compared with AA genotype carriers (Table 1 and Figure 1).

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