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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 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, dyslipidaemia, 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.

Materials and Methods
Using an allelic discrimination method we genotyped 11,064 individuals from three study populations: 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 Malmö 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 -168 A→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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Competing Interests: Professor Leif Groop has been a member of the Advisory Board of the Bristol-Myers Squibb AB.
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 about lifestyle factors, medical history of MI and stroke and data on smoking habits. Both previous and current smokers were recorded as smokers. Diagnosis of MI was always established in the hospital.

Microalbuminuria was defined as urinary albumin excretion rate >20 μg/min in an overnight urine collection. Total and cardiovascular mortality were assessed with a median follow up time of 7.9 years and the mortality data was obtained from the central death-certificate registry in Finland. Cardiovascular mortality was classified using the 9th revision of the International Classification of Diseases (cardiovascular diagnosis codes 390–459) before 1997 and the 10th revision (codes 100–199) thereafter. Causes of death were classified as cardiovascular death (coronary heart disease), cerebrovascular death (including both thrombotic stroke and cerebral haemorrhage) or other cardiovascular (including pulmonary embolism, abdominal aortic aneurysm, hypertensive complications, general atherosclerosis and peripheral artery disease with gangrene) or 2) other causes of death (neoplasms, violent or other). MetS was defined according to the National Cholesterol Education Program (NCEP) [16].

In total, 4,432 individuals were genotyped for the MHC2TA -168 A→G polymorphism including 2,264 individuals without diabetes mellitus and 1,557 with T2D. Data on MI was available in 97% and on microalbuminuria in 64% of the subjects. Data on cardiovascular mortality was available for all patients.

The MI case-control population from the Malmö diet and cancer study (MDC) The Malmö Diet and Cancer study population (MDC) [17] includes 29,096 randomly selected men (born 1923–1945) and women (born 1923–1950) living in the city of Malmö (population 230,000) in Sweden. A baseline examination was carried out between 1991 and 1996 encompassing a comprehensive assessment of lifestyle factors, heredity, medical history as well as previous and current diseases. On December 31st, 2000 the study population was checked against the Swedish National Board of Health and Welfare’s National Patient Registry and Cause of Death Registry. MI cases (first MI) were identified in the Swedish Patient Registry or in the Swedish Cause of Death Registry using ICD-9 codes 410 and 412 in the Swedish Patient Registry and 410–414 and E21–E25 in the Swedish Cause of Death Registry.

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 [0.75–1.22], p = 0.74) (Table 3). As the MHC2TA polymorphism was earlier shown to be associated with MI, we performed a subgroup analysis of individuals with previous history of MI. In fact, among these patients the MHC2TA AG/GG genotype was associated with increased risk of cardiovascular death compared with AA genotype carriers (HR 1.16 [1.09–1.22], p = 0.002) (Table 3, Figure 1). We also tested the MHC2TA GG genotype against the AA or AG genotypes and found that the GG genotype was protective against cardiovascular death in the whole group (HR 0.30 [0.16–0.92], p = 0.05), but not in patients with previous MI (HR 0.45 [0.49–4.16], p = 0.40).

The MHC2TA AG/GG genotypes were more frequently found among patients with MetS (40.1 vs. 36.9%, p = 0.036) as well as among non-diabetic individuals with microalbuminuria in the Botnia cohort (50.9 vs. 36.0%, p = 0.003) (Table 2). 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, 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>Botnia</th>
<th>MDC</th>
<th>DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic</td>
<td>T2D</td>
<td>p</td>
</tr>
<tr>
<td>Male/Female</td>
<td>1307/1566</td>
<td>731/828</td>
</tr>
<tr>
<td>Age (Yrs.)</td>
<td>54/13</td>
<td>67/12</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2±4.1</td>
<td>28.1±4.7</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>133±20</td>
<td>150±22</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>81±10</td>
<td>83±11</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.5±0.5</td>
<td>7.5±1.7</td>
</tr>
<tr>
<td>Age at onset of Diabetes (Yrs.)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Smoking (%)*</td>
<td>38.0</td>
<td>36.7</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>MI</th>
<th>Microalbuminuria (MALB)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA/AG/GG</td>
<td>AA/AG/GG</td>
</tr>
<tr>
<td>Botnia</td>
<td>(N = 112)</td>
<td>(N = 2686)</td>
</tr>
<tr>
<td>T2D</td>
<td>(N = 184)</td>
<td>(N = 1326)</td>
</tr>
<tr>
<td>MDC</td>
<td>(N = 1071)</td>
<td>(N = 2312)</td>
</tr>
<tr>
<td>Diabetes registry</td>
<td>(N = 316)</td>
<td>(N = 1974)</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>All patients</th>
<th>Previous MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Sex</td>
<td>1.34 (1.00–1.80)</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.42 (1.04–1.96)</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>1.11 (1.01–1.22)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>0.57 (0.38–0.85)</td>
</tr>
<tr>
<td>SBP (mmHg)*</td>
<td>1.00 (0.99–1.01)</td>
</tr>
<tr>
<td>DBP (mmHg)*</td>
<td>1.00 (0.99–1.01)</td>
</tr>
<tr>
<td>T2D</td>
<td>2.07 (2.22–2.97)</td>
</tr>
<tr>
<td>MHC2TA (AG or GG)</td>
<td>0.96 (0.75–1.22)</td>
</tr>
</tbody>
</table>

Numbers are hazard ratios (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
with previous MI, carriers of the AG or GG genotypes had reduced risk of cardiovascular death compared with AA genotype carriers (Table 3 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 genotype) could be a risk factor for cardiovascular mortality after MI, although the mechanism remains unclear. Swanberg et al. suggested that the G allele could cause reduced induction of HIF-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 genotype carriers [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 HIF II genes in response to inflammation stimuli. To clarify this issue, expression levels of all genotypes would therefore be of interest, especially comparison between the AA and AG genotypes.

Thus, the MHC2TA -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.

DISCUSSION

The key finding of the present study was an association between the MHC2TA -168 A→G polymorphism and cardiovascular mortality as well as MI in patients with MetS. These findings support the earlier report of association between this polymorphism and MI [12]. However, in contrast to the earlier study [13], the -168 A→G was not associated with MI in our study. One possible explanation could be differences in definition of MI. Our study consisted of population based material, whereas the information of MI was collected retrospectively, whereas the study population of Swanberg et al. was recruited from all patients below 60 yrs that were admitted to the hospital for acute MI.

Microalbuminuria is a risk marker for cardiovascular disease [9] and has been suggested to reflect a state of low-grade systemic inflammation [21]. Several factors like high blood pressure, hyperglycaemia, smoking, heart failure and renal atherosclerosis, all of which themselves are associated with increased inflammatory activity, are known to play a role in the development of 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 MetS including higher waist to hip ratio, higher HOMA-index, and higher blood pressure (data not shown) compared to individuals without microalbuminuria. Accordingly, the MHC2TA -168A→G polymorphism was also associated with MetS.

Thus, the MHC2TA -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 3 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 genotype) could be a risk factor for cardiovascular mortality after MI, although the mechanism remains unclear. Swanberg et al. suggested that the G allele could cause reduced induction of HIF-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 genotype carriers [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 HIF II genes in response to inflammation stimuli. To clarify this issue, expression levels of all genotypes would therefore be of interest, especially comparison between the AA and AG genotypes.

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Author Contributions

Conceived and designed the experiments: OM MO. Performed the experiments: MO. Analyzed the data: PA EL. Contributed reagents/materials/analysis tools: LG GB OM CA MO. Wrote the paper: LG GB EL OM CA MO.

REFERENCES