Association of Gln222Arg polymorphism in the deoxyribonuclease I (DNase I) gene with myocardial infarction in Japanese patients

Teruhiko Kumamoto¹, Yasuyuki Kawai¹, Kenichiro Arakawa¹, Norihiro Morikawa¹, Jun Kuribara², Hiroshi Tada², Koichi Taniguchi², Ryozo Tatami³, Isamu Miyamori¹, Yoshihiko Kominato⁴, Koichiro Kishi⁴, and Toshihiro Yasuda⁵*

¹Third Department of Internal Medicine, Faculty of Medical Sciences, University of Fukui, Fukui, Japan; ²Division of Cardiology, Gunma Prefecture Cardiovascular Center, Gunma, Japan; ³Division of Cardiology, Maldaru Kyosai Hospital, Kyoto, Japan; ⁴Department of Legal Medicine, Gunma University Graduate School of Medicine, Gunma, Japan; and ⁵Division of Medical Genetics and Biochemistry, Faculty of Medical Sciences, University of Fukui, Matsuoka, Fukui 910-1193, Japan

Received 16 December 2005; revised 6 June 2006; accepted 29 June 2006; online publish-ahead-of-print 28 July 2006

See page 2031 for the editorial comment on this article (doi:10.1093/eurheartj/ehl177)

KEYWORDS
Myocardial infarction; Deoxyribonuclease I; Polymorphism; Genetics

Introduction

Myocardial infarction (MI) is a complex multifactorial and polygenic disorder in which multiple environmental and genetic factors are simultaneously involved. Plaque disruption frequently causes the thrombotic complications of atherosclerosis that underlie the onset of acute MI (AMI).¹,² Potential mechanisms responsible for plaque disruption have been extensively examined; several laboratories have obtained morphological and biological evidence for apoptosis in vascular smooth muscle cells (VSMC) present in advanced atherosclerotic plaques and atheroma.³⁻⁵ It is plausible that apoptotic cell death may contribute to increasing the risk of plaque disruption, thus leading to MI.⁶,⁷ In contrast, epidemiological and clinical-genetic studies have revealed several genetic variants that affect susceptibility to MI.⁸⁻¹⁰ However, there has been no reported association of polymorphism in genes related to apoptosis with the incidence of MI.

Deoxyribonuclease I (DNase I, EC 3.1.21.1) is an endonuclease that preferentially attacks double-stranded DNA in a Ca²⁺-dependent manner to produce oligonucleotides with 5’-phospho and 3’-hydroxy termini.¹⁰ DNase I has been postulated to be one of the candidate nucleases that are responsible for internucleosomal DNA degradation during apoptosis.¹¹,¹² Recently, we demonstrated that serum DNase I activity could be used as a novel diagnostic marker for the early detection of AMI and myocardial ischaemia; abrupt elevation of serum DNase I activity levels occurs within ~3 h of the onset of symptoms in patients with AMI, permitting the diagnosis of AMI before accurate creatine

* Corresponding author. Tel: +81 776 61 8287; fax: +81 776 61 8149. E-mail address: tyasuda@fmsrsa.fukui-med.ac.jp

© The European Society of Cardiology 2006. All rights reserved. For Permissions, please e-mail: journals.permissions@oxfordjournals.org
kinase isoenzyme MB (CK-MB) and troponin T results become available. These findings suggested to us that DNase I might play some role in the pathogenesis of AMI. Previously, we demonstrated that human DNase I is genetically polymorphic and controlled by six codominant alleles at chromosome 16p13.3. Three common phenotypes, 1 and 2 (homoygote) and 1–2 (heteroygote), determined by two common alleles, DNASE1*1 and *2, have been found in Japanese and Caucasian populations. The molecular difference between DNASE1*1 and *2 is due to an A-G transition occurring at position 2317 in exon 8, regarded as a mature enzyme (Gln222Arg polymorphism).

We hypothesized that this DNase I polymorphism could be a genetic risk factor for MI. To test this hypothesis, we evaluated Gln222Arg polymorphism of the human DNase I gene in patients with MI and stable angina pectoris (AP).

Methods

Subjects

The study populations for phenotype/genotype analysis of DNase I polymorphism were composed of 730 Japanese inpatients, living in the same regions, who were admitted to our institutions between September 2002 and April 2005 because they were experiencing chest pain of possible coronary origin. All of these patients underwent coronary angiography (CAG) and left ventriculography (LVG). Patients (n = 119) with valvular disease, arrhythmia, cardiomyopathy, unstable AP, or other conditions, or those who did not provide informed consent, were excluded from the study. The remaining patients (n = 611) were divided into two groups: 311 patients with MI (first MI group) and 300 patients with AP (AP group). History of MI was verified by an episode of persistent ST-elevation or depression revealed by electrocardiography at the time of diagnosis and significant increase in the levels of serum cardiac markers such as CK-MB and troponin T up to three times the upper normal limit during the post-MI follow-up. In the MI group, patients with AMI (n = 233) were included. The diagnosis of AMI was established by the European Society of Cardiology/American College of Cardiology Committee criteria. The diagnosis was confirmed based on the presence of left ventricular wall motion abnormality on LVG and accompanying stenosis or occlusion of the coronary arteries by CAG. Only MI patients with an episode of persistent ST-segment shift at the time of diagnosis were enrolled as the first MI group in this study. All patients with AP had significant stenosis (>50%) in at least one major epicardial coronary artery and no history of MI. The second MI group included 155 Japanese patients who were admitted to our institutes between May 2005 and March 2006. The patients were selected and enrolled as the second MI group in this study based on the same clinical criteria as the first MI group. In-hospital mortality rates were 2.3 and 0.3% in the groups of patients with AMI and AP, respectively. The study protocol conformed to the Declaration of Helsinki and was approved by the Human Ethics Committee of our institutions; each subject included in the study gave written informed consent before participation.

Sample collection

Blood samples were collected from all the patients after an overnight fast. Serum samples were separated from each blood sample by centrifugation and stored at −80°C until use. All patients who agreed to participate in the study were evaluated by study cardiologists on the basis of detailed questionnaires that provided information about coronary risk factors such as smoking history and the presence of diabetes mellitus or hypertension. Smokers were defined as current smokers or those who had ceased smoking; non-smokers were defined as subjects with no history of smoking. Blood pressure was measured twice with a standard mercury sphygmomanometer with the patient in a sitting position after a 5 min rest, and then averaged. Hypertension was defined by a systolic blood pressure of at least 140 mmHg, a diastolic blood pressure of at least 90 mmHg, and/or use of antihypertensive medication. Plasma levels of total cholesterol were determined by an enzymatic procedure. Hyperlipidaemia was considered present when the fasting total plasma cholesterol level was ≥2.40 mg/mL and/or when the patient was currently using cholesterol-lowering drugs. Diabetes mellitus was defined as a fasting blood glucose level of at least 1.26 mg/mL (6.99 mmol/L) and/or the use of antidiabetic medication. Obesity was defined by a body mass index (weight in kilograms divided by the square of the height in metres) of at least 26. The baseline characteristics of patients in the MI groups and AP group enrolled in this study are shown in Table 1. Sex (male) was significantly more frequent in the first and second MI groups (72.0 and 72.9%, respectively) than in the AP group (57.0%). However, all other conventional risk factors examined, such as diabetes mellitus, hypertension, hyperlipidaemia, obesity, and smoking status, exhibited no difference among them.

DNase I phenotyping

Isoelectric focusing analysis for DNase I phenotyping from the serum samples was performed according to the method described in previous reports. Briefly, gels measuring 0.5 mm (thickness) × 90 mm (width) × 120 mm (length) were prepared using the following materials: 1.4 mL monomer solution [19.4% (w/v) acrylamide; 0.6% (w/v) N,N'-methylenebisacrylamide], 2.3 mL sucrose-glycerol 0.6% (w/v) solutions. The samples were then added to the gel mixture, and the gels were polymerized for 1 hour at room temperature. The gels were then placed in a buffer consisting of 20 mM Tris–HCl, pH 8.9, 100 mM NaCl, 10% (v/v) glycerol, and 0.01% (w/v) sodium dodecyl sulfate, and electrophoresis was performed at 20 mA for 3 hours.

Table 1  Baseline characteristics in the MI groups and AP group

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>AP group</th>
<th>MI group</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Second group</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total populations, n</td>
<td>300</td>
<td>311</td>
<td>155</td>
<td>0.380</td>
<td></td>
</tr>
<tr>
<td>Age&lt;sup&gt;b&lt;/sup&gt;, years (mean ± SD)</td>
<td>66.9 ± 11.3</td>
<td>66.7 ± 11.3</td>
<td>0.835</td>
<td>66.5 ± 9.2</td>
<td>0.380</td>
</tr>
<tr>
<td>Gender male, %</td>
<td>171 (57.0)</td>
<td>224 (72.0)</td>
<td>&lt;0.001</td>
<td>113 (72.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>107 (35.7)</td>
<td>122 (39.2)</td>
<td>0.403</td>
<td>56 (36.1)</td>
<td>0.995</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>181 (60.3)</td>
<td>176 (56.6)</td>
<td>0.367</td>
<td>94 (60.6)</td>
<td>0.971</td>
</tr>
<tr>
<td>Hyperlipidaemia, %</td>
<td>145 (48.3)</td>
<td>149 (47.9)</td>
<td>0.936</td>
<td>74 (47.7)</td>
<td>0.984</td>
</tr>
<tr>
<td>Obesity, %</td>
<td>89 (29.7)</td>
<td>101 (32.4)</td>
<td>0.485</td>
<td>49 (31.6)</td>
<td>0.749</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td>123 (41.0)</td>
<td>152 (48.9)</td>
<td>0.052</td>
<td>63 (40.6)</td>
<td>0.978</td>
</tr>
</tbody>
</table>

<sup>a</sup>Compared with AP group.

<sup>b</sup>For the patients with MI, the value refers to the age at onset of MI.
solution [20% (w/v)-10% (v/v)], 1 mL distilled water, 280 μL Amphioline 3.5-5 (Amersham Pharmacia Biotech, Uppsala, Sweden), 5 μL N,N,N′,N′-tetramethylethylenediamine and 40 μL 1.2% (w/v) ammonium persulphate. Samples for phenotyping were treated with an equal volume of 5 U/mL Clostridium perfringens neuraminidase overnight at 4°C before electrophoresis. The gel was run at V max 1000 V, I max 10 mA, and P max 3 W for 4 h under cooling at 12°C using a Multiphor apparatus (Amersham Pharmacia Biotech). After electrophoresis, the isozyme patterns of DNASE1 were visualized by activity staining using a dried agarose film overlay method.

**Analysis of the properties of the DNase I type-1 and type-2 enzymes**

Each DNA fragment containing the entire coding sequence of human DNase I cDNA, derived from DNASE1*1 and *2, was separately prepared from total RNA derived from the pancreas of homozygote subjects with phenotypes 1 and 2 by reverse transcription–PCR amplification, as described previously.

The amplified fragments were ligated into the pcDNA3.1(+) vector (Invitrogen, CA, USA) to construct each of the expression vectors. COS-7 cells were transiently transfected with each vector by the lipofection method according to a previously described method.

A mixture containing 2 μg of the relevant expression vector and 0.6 μg of the pSV-β-galactosidase vector (Promega, Madison, WI, USA; for estimation of transfection efficiency) was introduced into the cells. Two days later, the medium and cells were recovered for analysis. DNase I activity in the medium was determined by the single radial enzyme diffusion (SRED) method and the cell lysates were assayed for β-β-galactosidase. All transfections were performed in triplicate with at least five different plasmid preparations. Each enzyme (0.1 U) secreted into the medium was incubated with 0.025% (w/v) trypsin (Invitrogen) or 0.005% (w/v) chymotrypsin (Type I-S, Sigma, St Louis, MO, USA) at 37°C, and then its residual activities were measured.

**Statistical analysis**

Discrete variables are expressed as counts or percentages and were compared between two groups with χ² test or Fisher’s exact test, as appropriate. Continuous variables are expressed as mean ± standard deviation (SD) and were compared between two groups by means of the unpaired, Student’s t-test. Allele frequencies were calculated from the phenotypes observed in each group. Hardy-Weinberg equilibrium was assessed from the values by χ² test. Odds ratios with a 95% CI were calculated as a measurement of the association of the DNase I phenotype with the incidence of MI, with the effects of DNASE1*2 allele assumed to be additive, dominant, or recessive. We tested for independent association between DNase I polymorphism and the risk of MI in a multiple logistic regression, which included age, gender, hypertension, hyperlipidaemia, smoking, diabetes mellitus, and obesity as potentially confounding factors.

Model-fit analysis was evaluated using Pearson’s χ² goodness of fit test (P = 0.226). Adjustment for multiple testing in the allele frequency of DNase I polymorphism, which was the primary endpoint in the present study, was performed using the Bonferroni procedure. Other secondary analyses that were of only supplementary significance were not adjusted. All statistical tests were two-sided. Data analysis was performed with Stat View software, version 5.0 (SAS, Cary, NC, USA). Differences at P < 0.05 were considered significant.

### Results

**Association of DNase I polymorphism with susceptibility to MI**

To evaluate the association of DNase I polymorphism with the incidence of MI, isoelectric focusing analysis was performed with serum species from each patient in the MI and AP groups. Three common phenotypes, 1, 1–2, and 2, were observed in both the groups, whereas no novel phenotype specific for MI was found. To confirm the accuracy of phenotyping, we randomly selected 50 subjects in each group and subjected them to genotyping analysis of the corresponding SNP A2317G using their genomic DNA. Genotyping was performed by the mismatched-PCR-restriction fragment length polymorphism method described previously.

In each instance, the phenotype determined by isoelectric focusing analysis was perfectly identical to the results of genotyping. The phenotype distributions of DNase I polymorphism in the MI and AP groups are summarized in Table 2. These data were consistent with the distribution predicted by the Hardy-Weinberg equilibrium. The overall distribution of phenotypes in the first MI group differed significantly from that in the AP group (P < 0.001). In particular, the prevalence of DNase I phenotype 2 was high in the MI group in comparison with the latter group. Frequency of the DNASE1*2 allele was significantly higher in the first MI group than in the AP group (0.543 vs. 0.428, P < 0.001). In order to replicate these findings, patients in the second MI group were subjected to phenotyping of DNase I polymorphism. There was no significant difference on the phenotype distribution.

<table>
<thead>
<tr>
<th>Table 2 Phenotype distribution of DNase I polymorphism in the two MI groups, AP group, and healthy control groupa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MI group</strong></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Total populations, n Phenotype, n (%)</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>1–2</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td><strong>Allele frequency (%)</strong></td>
</tr>
<tr>
<td><strong>DNASE1*2</strong></td>
</tr>
</tbody>
</table>

aTaken from Yasuda et al.26
between the first and the second MI groups ($P=0.799$); in the same manner as the first MI group, frequency of the $DNASE1^*2$ allele was significantly higher in this group than in the AP group ($0.568$ vs. $0.428$, $P<0.001$) as shown in Table 2, confirming a role of DNase I gene in predisposition to MI. Furthermore, when the healthy Japanese populations reported previously without a history of CHD were used for comparison in this case–control study, the $DNASE1^*2$ allele was significantly more frequent in both the first and the second MI groups than in the healthy control group ($0.543$ vs. $0.439$, $P<0.001$ and $0.568$ vs. $0.439$, $P<0.001$, respectively). The odds ratio between each of the two MI groups and AP group were presented in Table 3. Analysis of the recessive, additive, and dominant effects of the $DNASE1^*2$ allele showed that the frequency of each was significantly higher in both the MI groups than in the AP group. These observations suggest that the $DNASE1^*2$ allele might be involved in the risk of MI. The results of multiple logistic regression analysis between the MI and AP groups are summarized in Table 4. The odds ratio of $DNASE1^*2$ between these groups was $1.51$ ($95\%$ CI $1.04$–$2.18$). Multiple logistic regression analysis revealed that the $DNASE1^*2$ allele tended to be associated with the occurrence of MI, being independent of other cardiovascular risk factors.

Differences in properties between the DNase I type-1 and type-2 enzymes

In order to examine the differences in properties between the DNase I type-1 and type-2 enzymes derived from $DNASE1^*1$ and $^*2$, respectively, we constructed the corresponding expression vectors and compared the enzyme activity secreted into the medium from transfected COS-7 cells. The activity from cells expressing the type-2 enzyme was significantly lower than that from cells expressing the type-1 enzyme by a factor of $0.41$ (Figure 1A). In contrast, however, the type-1 enzyme was more sensitive than the type-2 enzyme to proteolysis by proteases such as trypsin and chymotrypsin (Figure 1B). Thus, the corresponding amino acid transition from Gln to Arg at position 222 in the DNase I protein appeared to reduce both the specific activity secreted into the medium from transfected COS-7 cells and the sensitivity to proteolysis of the DNase I enzyme.

Discussion

Major findings

Our previous observation that serum DNase I activity increases transiently and abruptly after the onset of AMI permitted us to hypothesize that the DNase I gene may be one of the genes associated with susceptibility to MI. The present study provides the first evidence of an association between polymorphism of the DNase I gene and the incidence of MI. As the risk estimate did not change even after adjustment for clinically relevant cardiovascular risk factors, it is plausible that the $DNASE1^*2$ allele might be associated with MI, being independent of other conventional factors.

Study populations

In this study, subjects for comparison were recruited from inpatients with both AP and no diagnostic evidence of MI. Phenotype/allele distributions of DNase I polymorphism in the AP group did not deviate from those in the control group consisting of the healthy Japanese subjects reported previously, living in the same regions as the two patients groups, who had no CHD.26 No significant difference in the frequency of the $DNASE1^*2$ allele was observed between the AP group and the control group ($0.428$ vs. $0.439$, $P=0.381$). Furthermore, all the patients enrolled in this study came from the same regions in Japan to avoid spurious association; we have already confirmed that there is a general uniformity for DNase I polymorphism in the Japanese populations across Japan.27 Although many studies have examined the relationship between polymorphism and MI, the results of most of them remain controversial with no consensus about their implication, partly because of complicating environmental factors including clinical background.9 In order to overcome these problems, the clinical backgrounds of patients group used for comparison should have as high similarity as possible to those of the MI group. In this context, clinical characteristics inherent to the AP group exhibited high similarities to those of the MI groups, as shown in Table 1. Although the proportion of men in both the MI groups was significantly different from that in the AP group in this study, gender has been found to have no effect on an allele distribution of DNase I polymorphism.15 Thus, it seems plausible to conclude that the patient population we chose as the AP group was appropriate as a control group in order to clarify whether DNase I may be involved in increasing the risk of plaque disruption, thus leading to MI. Also, enrolment of a healthy Japanese

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Odds ratios between the AP group and two MI groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratios (95% CI)</td>
</tr>
<tr>
<td></td>
<td>First MI group</td>
</tr>
<tr>
<td>2 vs. 1 and 1–2</td>
<td>2.37 (1.61–3.49)</td>
</tr>
<tr>
<td>2 and 1–2 vs. 1</td>
<td>1.46 (1.02–2.10)</td>
</tr>
<tr>
<td>2 vs. 1</td>
<td>2.55 (1.61–4.03)</td>
</tr>
<tr>
<td>1–2 vs. 1</td>
<td>1.12 (0.76–1.64)</td>
</tr>
<tr>
<td>$DNASE1^*2$ vs. $DNASE1^*1$</td>
<td>1.59 (1.27–1.99)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Multiple logistic regression analysis for variables differing between the MI group and the AP group enrolled in this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>Odds ratio$^b$</td>
</tr>
<tr>
<td>Allele $DNASE1^*2$</td>
<td>1.51</td>
</tr>
<tr>
<td>Age$^c$</td>
<td>1.00</td>
</tr>
<tr>
<td>Gender</td>
<td>1.79</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.18</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.92</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>1.01</td>
</tr>
<tr>
<td>Obesity</td>
<td>1.19</td>
</tr>
<tr>
<td>Smoking status</td>
<td>1.10</td>
</tr>
</tbody>
</table>

$^a$First MI group.
$^b$Odds ratios and 95% CIs were calculated by multiple logistic regression analysis.
$^c$Age was included as a continuous variable in the analysis.
Figure 1 Differences in the properties of the DNase I type-1 and type-2 enzymes. (A) Relative enzymatic activity and (B) sensitivity to proteolysis by trypsin. Each expression vector containing an insert of the entire coding region of DNase I type-1 or type-2 mRNA was transfected into COS-7 cells and then the DNase I activity secreted into the medium from the transfected cells was examined. (A) Levels of the activity derived from type-1 DNase I were expressed as a ratio relative to those of the type-2 enzyme. Values are expressed as mean ± SD (bars) of the results from five independent experiments. The activity level of the type-1 enzyme was significantly higher than that of the type-2 enzyme (P < 0.001). (B) The type-1 (solid line) or type-2 (broken line) DNase I enzyme was incubated with 0.025% (w/v) trypsin at 37°C for the durations indicated; the residual DNase I activities were then determined by the SRED method. The sensitivity of each enzyme to proteolysis by chymotrypsin was similar to that by trypsin.

Possible involvement of DNase I in the aetiology of MI

MI with ST-segment shift (ST-shift MI) is in most cases due to coronary thrombosis, caused by plaque disruption, which frequently occurs in atherosclerotic plaque known as vulnerable plaque, but does not usually take place at the most stenotic sites. The onset of ST-shift MI is well known to be closely related to plaque disruption and not to gradual plaque progression.1,7,8 Vulnerable plaque is characterized by a thin fibrous cap, the presence of many inflammatory cells, a large lipid pool, and few VSMC. In this context, the death of VSMC, in part because of apoptosis, may also contribute to depletion of collagen in the fibrous cap and increase the risk of plaque disruption.6,7,30 Analysis of markers for apoptosis indicates that many T lymphocytes may undergo apoptosis after they infiltrate atherosclerotic lesions.31 Several lines of evidence implicate the FasL/Fas-caspase death pathway in the induction of apoptosis in atherosclerotic plaque, leading to plaque instability.32 In contrast, Oliveri et al.33,34 have recently reported that DNase I behaves as a transcription factor that modulates Fas expression, leading to induction of apoptosis. These findings, together with others suggesting that DNase I or DNase I-like endonucleases may be responsible for internucleosomal DNA degradation during apoptosis,11,12 have focused attention on the potential physiological roles of DNase I in apoptosis occurring in advanced atherosclerotic plaque and atheroma. As all the MI patients enrolled in this study exhibited persistent ST-elevation or depression at the time of diagnosis, due mainly to plaque rupture, one of the aetiological differences between the patient group with ST-shift MI and that with AP recruited for this study was the distinct occurrence of plaque disruption, leading to the thrombotic complications of atherosclerosis. From comparison of DNase I polymorphism between these two patient groups, we demonstrated that carrying the DNASE1*2 allele increases the risk of ST-shift MI but not of AP, thus suggesting a pivotal role of DNase I in the pathophysiology of plaque disruption. On the other hand, it has been demonstrated that ischaemia of the myocardium/reperfusion injury may initiate apoptosis.5,7 Therefore, it seems plausible that individuals carrying the DNASE1*2 allele may be less capable of managing myocardial ischaemia, resulting in association of DNase I polymorphism with the incidence of MI. The results of the present investigation will facilitate further in vitro studies to clarify the contribution of DNase I to plaque disruption leading to the incidence of AMI or managing myocardial ischaemia.

A full genome-wide scan for CHD has reportedly provided strong evidence for linkage between CHD and a locus on chromosome 16p13.3.35 We have previously assigned the gene for DNase I to band 16p13.3.36 Irrespective of the association between DNase I polymorphism and the incidence of MI suggested by the present study, we cannot exclude the possibility that DNase I may be linked with other genes responsible for CHD. However, we found an appreciable difference in properties between the type-1 and -2 DNase I enzymes derived from DNASE1*1 and *2, respectively, indicating that Gln222Arg polymorphism results in functional alterations of the DNase I enzyme, as shown in Figure 1, leading to the generation of DNase I enzymes that have different properties in vivo. Although there is still no direct experimental evidence for how levels of DNase I activity and sensitivity to proteolysis affect apoptosis in atherosclerotic plaque or managing myocardial ischaemia, it seems plausible that these functional alterations in the enzyme may contribute to MI susceptibility.

Limitations of this study

Some limitations of the present study should be considered. Although two MI groups were separately phenotyped to elucidate the association of DNase I polymorphism with the incidence of ST-shift MI, the study was limited by the relatively small sample size. This may have led to weak statistical significance and wide CIs when estimating odds ratios.
In a previous population-genetic survey, a German population was found to exhibit a phenotype distribution of DNase I polymorphism that differed from a Japanese population; the frequency of DNASE1*2 in the former population was significantly higher than that in the latter. Therefore, there is a need to examine whether the magnitude of this association is comparable in other ethnic populations. The erosion of fibrous plaque rich in SMC and proteoglycans, as well as rupture of vulnerable plaque, is known to result in acute coronary thrombus. It remains to be clarified whether the DNase I polymorphism is associated with non-thrombotic MI without an episode of ST segment shift.

Conclusions

The present study has demonstrated that Gln222Arg polymorphism in the DNase I gene is associated with MI in the Japanese; the DNASE1*2 allele in DNase I polymorphism is significantly more frequent in patients with MI than in patients with AP. The DNASE1*2 allele tended to be associated with MI, being independent of other cardiovascular risk factors. Our results may have clinical implications for the prevention of MI.

Acknowledgement

This study was supported in part by Grants-in-Aid from the Japan Society for the Promotion of Science (15209023 and 17659196 to T.Y. and 16209023 to K.K.).

Conflict of interest: none declared.

References

Clinical vignette

Rapid resolution of large right atrial mass after anticoagulant therapy

Lukasz Chrzanowski*, Piotr Lipiec, and Jaroslaw D. Kasprzak

Department of Rapid Cardiac Diagnostics, II Chair of Cardiology, Medical University of Lodz, 1/5 Kniaziewicza St., 91-347 Lodz, Poland

* Corresponding author. Tel: +48 42 6539909/42 2516207; fax: +48 42 6539909. E-mail address: chrzanowski@ptkardio.pl

A 75-year-old male with liver cirrhosis and hepatic tumour on ultrasound was referred to echocardiographic laboratory because of the symptoms of right heart failure. The patient’s general condition has deteriorated gradually and lower limb oedema and ascites have been present for a few weeks. The echocardiographic study revealed a large right atrial mass (of approximately \(52 \times 30 \text{mm}^2\) dimension), protruding from dilated inferior vena cava (IVC) and producing minimal obstruction of IVC inflow. No other cardiac pathology was present. The plasma D-dimer level was normal. Tentative anticoagulation with acenocoumarol was initiated, but after 2 months it was discontinued due to concomitant thrombocytopenia. The patient’s general condition and symptoms, however, improved substantially. Unexpectedly, the follow-up echocardiographic study demonstrated no right atrial mass, providing strong evidence for thrombotic composition of the lesion and excluding its metastatic origin (Panels A–F). The report demonstrates the difficulties in differential diagnosis of intracardiac masses and the spectacular response of a large lesion to medical therapy.

Panel A. Echocardiographic apical four-chamber view showing a large right atrial mass.

Panel B. Irregular shape of right atrial lesion seen in modified parasternal long axis view.

Panel C. Echocardiographic image from subcostal view showing dilated IVC (arrows) with intraluminal mass protruding into the right atrium.

Panel D. Colour Doppler image from subcostal view showing turbulent IVC inflow into the right atrium.

Panel E. Mild IVC obstruction demonstrated by continuous Doppler echocardiography from subcostal view.

Panel F. Normal heart at follow-up echocardiographic study.