With aging, cardiac hypertrophy or left ventricle diastolic dysfunction due to volume or pressure overload is an independent risk factor for morbidity and mortality. Mechanotransduction, the conversion of a mechanical stimulus into a cellular response, is an important phenomenon in which each cycle of contraction and relaxation leads to dynamic deformation.

1) Stretch induces muscle growth in mechanical overload and hypertension-induced hypertrophy, and it is conceivable that polymorphisms in the genes responsible for transducing mechanical stretch in myocytes influence the myocardial stretch response and represent a basis for cardiac disease, including the development of cardiac diastolic dysfunction.

3,4) Previous studies have demonstrated that a variety of cellular transduction elements, including humoral factors, channels, and second messengers, mediate the response to mechanical stretch, and more recent studies suggest that the cytoskeletal structure and related Z-disc proteins may also play a role.

6,7) Indeed, the physiologic importance of these elements is supported by the fact that mutation of these proteins is associated with human dilated cardiomyopathy.8-10) Recent studies have reported that the novel Z-disc related protein, myospryn, is expressed in striated muscle cells, where it co-localizes with the sarcomeric α-actinin in mice, and we have demonstrated the possible relationship between the polymorphisms and alterations in cardiac function in hypertensive patients and provided the novel therapeutic target for cardiac adaptation in response to pressure overload as an anti-aging therapy.

**Abstract**

**BACKGROUND:** With aging, left ventricle diastolic dysfunction due to pressure overload is an independent risk factor for morbidity and mortality. Mechanisms by which alterations in stretch-induced mechanotransduction contribute to left ventricular diastolic dysfunction remain unclear. Recently, since novel Z-disc related protein, myospryn, expressed in striated muscle cells, has been reported, we examined the relationship between myospryn polymorphisms and alterations in cardiac function with patients in larger population.

**METHODS:** A total of 743 patients with high blood pressure (defined as systolic blood pressure >140 mmHg and/or systolic blood pressure >90 mmHg or taking antihypertensive medication) were enrolled in this study. Two-dimensional ultrasound echocardiography, electrocardiography, blood pressure, serum glucose, cholesterol, creatinine, uric acid, and myospryn K2906N polymorphism.

**RESULTS:** The myospryn K2906N polymorphism was significantly associated with a marker of left ventricular diastolic cardiac dysfunction, A/E, which represents the ratio of the peak velocity of the early diastolic filling wave (E wave) to the atrial filling (A wave).

**CONCLUSIONS:** These data demonstrated that a polymorphism of myospryn was associated with left ventricular diastolic dysfunction in hypertensive patients and provided the novel therapeutic target for cardiac adaptation in response to pressure overload as an anti-aging therapy.

**KEYWORDS:** LV diastolic dysfunction, polymorphism, cytoskeleton, pressure overload

**Introduction**

With aging, cardiac hypertrophy or left ventricle diastolic dysfunction due to volume or pressure overload is an independent risk factor for morbidity and mortality. Mechanotransduction, the conversion of a mechanical stimulus into a cellular response, is an important phenomenon in which each cycle of contraction and relaxation leads to dynamic deformation. Stretch induces muscle growth in mechanical overload and hypertension-induced hypertrophy, and it is conceivable that polymorphisms in the genes responsible for transducing mechanical stretch in myocytes influence the myocardial stretch response and represent a basis for cardiac disease, including the development of cardiac diastolic dysfunction. Previous studies have demonstrated that a variety of cellular transduction elements, including humoral factors, channels, and second messengers, mediate the response to mechanical stretch, and more recent studies suggest that the cytoskeletal structure and related Z-disc proteins may also play a role. Indeed, the physiologic importance of these elements is supported by the fact that mutation of these proteins is associated with human dilated cardiomyopathy. Recent studies have reported that the novel Z-disc related protein, myospryn, is expressed in striated muscle cells, where it co-localizes with the sarcomeric α-actinin in mice, and we have demonstrated the possible relationship between the polymorphisms and cardiac hypertrophy in small scale study. Therefore, the goal of the present study was to characterize the relationship between myospryn polymorphisms and alterations in cardiac function in patients with hypertension in larger population.
Myospryn and LV diastolic dysfunction

Methods

Patients

A total of 743 patients with high blood pressure (defined as a systolic blood pressure of >140 mmHg and/or a systolic blood pressure of >90 mmHg by repeated measurements or when medication was taken for treatment of hypertension) from the National Cardiovascular Center were enrolled in this study. Blood samples were collected from all of the subjects after overnight fasting, and systolic (SBP) and diastolic (DBP) blood pressure were measured at rest in a sitting position. Fasting plasma glucose level (FPG) and plasma levels of total cholesterol (TC), neutral lipids (TG) and high-density lipoprotein cholesterol (HDL) were determined by standard methods. We calculated low-density lipoprotein cholesterol (LDL) by the Friedwald formula, and non-high-density lipoprotein cholesterol (non-HDL) by subtracting HDL from TC. Informed consent was obtained from all patients, and all procedures were carried out in accordance with institutional and national ethical guidelines for human study. The study protocol was approved by the Ethical Review Committee of the National Cardiovascular Center and the Osaka University Graduate School of Medicine.

Ultrasound echocardiographic examination

Comprehensive two-dimensional echocardiography was performed using a cardiac ultrasound unit (Sonos 5500; Hewlett Packard, Andover, MA, USA). Measurements included interventricular septal thickness (IVSTD), posterior wall thickness (PWTd), LV diameter at end-diastole (LVDd) and LV diameter at end-systole (LVDs). Fractional shortening was calculated as (LVDd-LVDs)/LVDd. LV mass was estimated using the formula validated by Devereux and Reichek: LV mass (g)=1.04×[(IVSTD+PWTD+LVDd)3–LVDd3]–13.6. LV mass was normalized against body surface area and expressed as LV mass index. To assess LV diastolic function, the diastolic filling of LV (LV inflow) was examined using Doppler echocardiography. The LV diastolic filling pattern was obtained with the sample volume at the tips of the mitral valve in the apical four-chamber view and recorded at the end-expiratory phase during quiet breathing. The peak velocity of the early diastolic filling wave (E wave) and the peak velocity of atrial filling (A wave) were recorded, and the E to A ratio (E/A) was calculated. The deceleration time was measured as the time between the top of the E wave and the point where the descending part of the E wave or its asymptote crossed the zero line.

SNP selection and genotyping

Twenty-seven SNPs of the myospryn gene were selected from NCBI’s SNP database. In the analysis of the SNP frequency by direct sequence method or Taqman PCR, genomic DNA was extracted from the peripheral blood lymphocytes of 50 Japanese volunteers. Thirteen PCR primer sets used in the direct sequence were shown in Supplementary Table. All PCR products were sequenced using a BigDye terminator v3.1 and an ABI Prism 3700 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA). TaqMan assay was performed on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems) using the manufacturer’s protocols. Probe sets for the TaqMan assay were obtained from Applied Biosystems.

Statistical and haplotype analysis

Values are expressed as the mean ± SE. All statistical analyses were performed using the JMP 5.1 statistical software package (SAS institute, Cary, NC) or Stat-View 5.0 software (SAS Institute, Inc., NC). Data were compared using the unpaired Student’s t test for comparisons between two groups and the ANOVA followed by Dunnett test for multiple comparisons. Haplotype frequencies were estimated with EM algorithm, using the web-based programs SNP HAP (UP http://www.genemapping.cn/zh.htm UP). The linkage disequilibrium were calculated using spreadsheet software (Excel).

Results

Human myospryn polymorphism

From the Hapmap database (http://www.hapmap.org/index.html.en), in the locus of human myospryn (chromosome 5; position 79.02 Mb – 79.13 Mb) we focused on one haplotype block with a tagSNP (rs6859595) in which 17 SNPs were in linkage disequilibrium (rs4704584, rs12654905, rs12655355, rs16877109, rs1366270, rs1366271, rs16877124, rs6893869, rs16877135, rs1428224, rs16877141, rs16877147, rs16877150, rs6895595, rs2278239, rs3828611, rs7709188), as shown in LD triangle plot (Figure 1). Although 27 coding SNPs were found in the coding region of the human myospryn gene, this tag SNP covered 12 SNPs in linkage disequilibrium among these coding SNPs. Thus, in subsequent experiments we focused on the K2906N myospryn polymorphism.

Seven hundred forty three hypertensive patients were enrolled in the present study. The minor allele frequency of the K2906N polymorphism was 0.48, and the allele frequency of the V1006A polymorphism was 0.49. A significant association was observed with the K2906N polymorphism and LV diastolic dysfunction in hypertensive patients. The minor allele of the K2906N polymorphism was significantly associated with a marker of left ventricular diastolic cardiac dysfunction, A/E which represents the ratio of the peak velocity of the early diastolic filling wave (E wave) to the atrial filling (A wave). In contrast, this polymorphism was not associated with LV mass index, body mass index (BMI), or blood pressure. In analysis using a dominant model, the K2906N polymorphism was also significantly associated with a marker of left ventricular diastolic cardiac dysfunction, A/E (Figure 2a). Multivariate analysis demonstrated an association between the K2906N polymorphism (AA+AC vs. CC) and A/E (>1.5) that was independent of age, gender, duration of hypertension, systolic and diastolic blood pressure, or BMI (Figure 2b). These results suggest that the K2906N polymorphism is clinically associated with left ventricular diastolic dysfunction in hypertensive patients.

Discussion

Previous studies have demonstrated mutations in many sarcomeric protein-encoding genes can result in hypertrophic cardiomyopathy, an inherited predisposition towards increased ventricular wall thickening and a corresponding decrease in size of the ventricular cavity. The present study demonstrated that the novel sarcomeric protein, myospryn, is also associated with the development of left ventricular diastolic dysfunction.
Table 1  Clinical and echocardiographic characteristics according to K2906N allele status in hypertensive patients

<table>
<thead>
<tr>
<th>Genotype</th>
<th>AA</th>
<th>AC</th>
<th>CC</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n</td>
<td>214</td>
<td>340</td>
<td>189</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.9 ± 0.7</td>
<td>65.6 ± 0.6</td>
<td>63.6 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>56.5%</td>
<td>56.8%</td>
<td>58.2%</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.1 ± 0.2</td>
<td>24.1 ± 0.2</td>
<td>24.4 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>143.9 ± 1.2</td>
<td>145.6 ± 1.1</td>
<td>146.2 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>83.6 ± 0.9</td>
<td>84.6 ± 0.8</td>
<td>86.3 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Pulse rate (beats/min)</td>
<td>69.7 ± 0.8</td>
<td>70.7 ± 0.7</td>
<td>70.9 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Home SBP (mmHg)</td>
<td>134.9 ± 1.0</td>
<td>134.4 ± 0.8</td>
<td>135.7 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Home DBP (mmHg)</td>
<td>79.5 ± 0.7</td>
<td>80.0 ± 0.6</td>
<td>80.9 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>HT duration (%)</td>
<td>18.6 ± 0.8</td>
<td>17.9 ± 0.6</td>
<td>18.3 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>LVMI</td>
<td>127.5 ± 2.7</td>
<td>133.0 ± 2.2</td>
<td>125.0 ± 2.8</td>
<td>NS</td>
</tr>
<tr>
<td>A/E</td>
<td>1.258 ± 0.025</td>
<td>1.279 ± 0.022</td>
<td>1.181 ± 0.026</td>
<td>p=0.0151</td>
</tr>
<tr>
<td>Deceleration time (s)</td>
<td>218.2 ± 3.5</td>
<td>218.3 ± 2.9</td>
<td>214.9 ± 3.6</td>
<td>NS</td>
</tr>
<tr>
<td>SV1+RV5 (%)</td>
<td>30.4 ± 0.7</td>
<td>31.7 ± 0.6</td>
<td>30.5 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>T-chol (mg/dl)</td>
<td>202.7 ± 2.4</td>
<td>203.7 ± 2.0</td>
<td>199.7 ± 2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>142.8 ± 11.4</td>
<td>133.8 ± 4.5</td>
<td>130.7 ± 4.9</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-chol (mg/dl)</td>
<td>52.4 ± 1.1</td>
<td>51.3 ± 0.8</td>
<td>52.3 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.06 ± 0.08</td>
<td>1.11 ± 0.06</td>
<td>1.17 ± 0.12</td>
<td>NS</td>
</tr>
<tr>
<td>UA (mg/dl)</td>
<td>6.10 ± 0.11</td>
<td>6.07 ± 0.09</td>
<td>6.24 ± 0.12</td>
<td>NS</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>104.3 ± 1.4</td>
<td>103.4 ± 1.1</td>
<td>102.4 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.70 ± 0.06</td>
<td>5.69 ± 0.04</td>
<td>5.63 ± 0.06</td>
<td>NS</td>
</tr>
<tr>
<td>IRI (µU/ml)</td>
<td>6.28 ± 0.35</td>
<td>6.41 ± 0.27</td>
<td>6.56 ± 0.32</td>
<td>NS</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; HT, hypertension; LVMI, left ventricular mass index; A/E, ratio of peak velocity of early diastolic filling wave (E wave) and that of atrial filling wave (A wave); SV1+RV5, criteria of left ventricular hypertrophy in electrocardiography; T-chol, total cholesterol; UA, uric acid; FBG, fasting blood glucose; HbA1c, glycohemoglobin A1c; IRI, immunoreactive insulin

Fig. 1  LD triangle plot and genomic structures in myospryn (chromosome 5; position 79.02 Mb – 79.13 Mb). Pairwise LD indices presented in the triangle plot, and red color indicates value of LD (cut off: 0.8). Open square indicates the exons of myospryn gene in the middle panel.

Fig. 2  Association of myospryn polymorphism and left ventricular diastolic function in hypertensive patients. a) The K2906N polymorphism was significantly associated with an echocardiographic marker of diastolic cardiac dysfunction, A/E, which is the ratio of the peak velocity of the early diastolic filling wave (E wave) to the atrial filling wave (A wave). b) Multivariate analysis demonstrated an association between A/E (>1.5) and the K2906N polymorphism (AA+AC vs. CC), but not to age, gender (Sex: male), hypertension (HT) duration, systolic and diastolic blood pressure (SBP and DBP), or body mass index (BMI).
Mechanotransduction is a highly conserved process that occurs in a wide variety of different cells, including endothelial cells, fibroblasts, and cardiomyocytes. However, the presence of a stretch sensing mechanism in these cell types does not mean that they all share a common mechanism. In fact, the molecular mechanism of the mechanosensor in cardiomyocytes remains unknown. Two major paradigms have emerged: a localized model of mechanotransduction in which a cellular signal is generated in close proximity to the plasma membrane, and a decentralized model in which the forces applied at the cell surface are transmitted to other locations via the cytoskeleton.

Studies of the association between genetic elements and cardiac hypertrophy have suggested that the former model may be the dominant paradigm in the context of pressure induced-cardiac hypertrophy involving the renin-angiotensin system. However, the association with genetic elements has not been addressed to the latter tensegrity model that has been applied to describe the transmission of mechanical forces from one part of the cell to another. Indeed, in the tensegrity model of mechanotransduction, the unique cardiac cytoskeletal structure and related Z-disc proteins have a variety of functions that are not limited to simply providing mechanical stability and passive stiffness.

We recently reported the possible association between myospryn polymorphisms and cardiac hypertrophy. We confirmed that in both population AA genotype in K2906N was risk allele in left ventricle diastolic dysfunction, A/E, however the results of both studies showed the discrepancy. In the previous study K2906N polymorphism was associated with the antero-septal wall thickness of the left ventricle and A/E in a recessive model (AA vs. AC+CC), but in this study same polymorphism was significantly associated with A/E in a dominant model (AA+AC vs. CC). For this reason, we speculated the contribution of significant difference in blood pressure in each population. The average systolic/diastolic blood pressure in patients with Osaka University (previous study) or National cardiovascular center (this study) is about 160/93 mmHg or 145/84 mmHg, respectively. Thus, possible explanation may be that cardiac function can be more affected by high blood pressure or anti-hypertensive medication in patients with AC genotype in K2906N polymorphism. A limitation of the present work may be that the distribution of the whole hypertensive group is not defined in terms of hemodynamic stage of hypertension, degree of target organ damage, frequency and classes of antihypertensive medications in the patient population. Therefore, to reach the final conclusion we should further examine the detailed history of antihypertensive medications or complication. In the cardiac marker of diastolic function, A/E is one of the typical markers to assess LV diastolic function, however it is not enough because it might be sometimes pseudo-normalized to reflect severe increase in LV chamber stiffness resulting in a high E velocity and a low A velocity. We realize the needs to examine the association using the additional parameters in future.

In conclusion, the present study demonstrated that a myospryn polymorphism was associated with left ventricular diastolic dysfunction in hypertensive patients, and provided the novel therapeutic target for cardiac adaptation in response to pressure overload as an anti-aging therapy.

Acknowledgement
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