p122 Protein Enhances Intracellular Calcium Increase to Acetylcholine
Its Possible Role in the Pathogenesis of Coronary Spastic Angina

Reiichi Murakami, Tomohiro Osanai, Hirofumi Tomita, Satoko Sasaki, Atsushi Maruyama, Ken Itoh, Yoshimi Homma, Ken Okumura

Objective—To investigate the role of p122 in enhanced vasomotility by examining p122 expression in the cultured skin fibroblasts obtained from patients with and without coronary spasm, intracellular Ca2+ [Ca2+]i concentration at baseline and after stimulation with acetylcholine in the cells transfected with p122, and promoter in genomic DNA.

Methods and Results—Phospholipase C-δ1 activity is enhanced in patients with coronary artery spasm, and a p122 protein was recently cloned to potentiate phospholipase C-δ1 activity. p122 protein and gene expression levels in patients with coronary spasm (n=11) were enhanced compared with levels in control subjects (n=9) (P<0.01 for both). [Ca2+]i at baseline and the peak increase in [Ca2+]i in response to acetylcholine were both 2 times higher in cells transfected with p122 than in those without p122. Conversely, knockdown of p122 resulted in diminished [Ca2+]i response. In the p122 promoter analysis, the −228G/A and −1466C/T variants revealed the increase in luciferase activity. Although the −1466C/T variant was similar between 144 patients with coronary spasm and 148 controls, the −228G/A variant was more frequent in male patients than in male controls (P<0.05).

Conclusion—The p122 protein is upregulated in patients with coronary spasm, causing increased [Ca2+]i to acetylcholine and thereby seems to be related to enhanced coronary vasomotility. (Arterioscler Thromb Vasc Biol. 2010;30:00-00.)

Key Words: calcium ion ■ coronary spasm ■ p122 ■ PLC-δ1 ■ vasoconstriction ■ calcium ■ coronary vasospasm ■ vascular biology ■ phospholipase C ■ p122 protein

Coronary artery spasm plays an important role in the pathogenesis of variant angina and other forms of ischemic heart disease. Considering that esophageal motility is also enhanced in patients with angina pectoris due to coronary spasm (ie, coronary spastic angina [CSA]), the presence of a generalized disorder of smooth muscle contraction is strongly suggested. Phospholipase C (PLC) hydrolyzes phosphatidyl inositol 4,5-bisphosphate (PIP2) and produces inositol 1,4,5-trisphosphate (IP3) and diacylglycerol. The inositol phosphatidyl inositol 4,5-bisphosphate (PIP2) and produces店

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Table 1. Clinical Profiles of the Study Patients*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients With CSA (n=11)</th>
<th>Control Subjects (n=9)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>58±5</td>
<td>52±6</td>
<td>0.45</td>
</tr>
<tr>
<td>Male sex†</td>
<td>8 (73)</td>
<td>7 (78)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Smoking‡</td>
<td>7 (64)</td>
<td>4 (44)</td>
<td>0.65</td>
</tr>
<tr>
<td>Hypertension‡</td>
<td>4 (36)</td>
<td>5 (56)</td>
<td>0.65</td>
</tr>
<tr>
<td>CCB‡</td>
<td>3</td>
<td>4</td>
<td>NA</td>
</tr>
<tr>
<td>ACE-I‡</td>
<td>0</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td>ARB‡</td>
<td>0</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td>Nitrate‡</td>
<td>2</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>5.6±0.3</td>
<td>6.0±0.4</td>
<td>0.30</td>
</tr>
<tr>
<td>HbA1c</td>
<td>8.2</td>
<td>8.0</td>
<td>0.97</td>
</tr>
<tr>
<td>Sulfonamideur‡</td>
<td>1</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td>Dyslipidemia (LDL-C)</td>
<td>139±15</td>
<td>134±11</td>
<td>0.78</td>
</tr>
<tr>
<td>Statin‡</td>
<td>2</td>
<td>0</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Data are given as mean±SD unless otherwise indicated.
†Data are given as number (percentage).
‡Data are given as number affected.

ACE-I indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CCB, Ca2+ channel blocker; Hb, hemoglobin; LDL-C, low-density lipoprotein cholesterol; NA, not applicable.

The primary skin fibroblasts were obtained from control subjects and patients with CSA at cardiac catheterization. They were prepared by an explant method, as previously described,10 to determine the protein and mRNA expression of p122. Fibroblasts from the second to fourth passages were used for the study.

For the p122 promoter assay study, we enrolled 114 patients with CSA (91 men and 53 women; mean±SEM age, 61±11 years) and 148 control subjects without hypertension or any history suggestive of angina pectoris (62 men and 86 women; mean±SEM age, 52±8 years). Coronary spasm was demonstrated during coronary angiographic study in all patients with CSA.

Protein Expression of p122 by Western Blot Analysis

The fibroblasts were harvested, pelleted, and resuspended in radioimmunoprecipitation assay lysis buffer. Equal amounts of protein, 20 μg per lane, were applied to sodium dodecyl sulfate–polyacrylamide gel electrophoresis wells and transferred electrophoretically to a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Hercules, Calif). After incubating with the primary antibody for p122 and GPDH (Santa Cruz Biotechnology, Calif), the protein bands were detected by the enhanced chemiluminescence plus detection system (Amersham Pharmacia Biotech, NJ).

Gene Expression of p122

Two-step RT-PCR was performed according to the protocol supplied with a commercially available kit (TaqMan Gold RT-PCR kit; Applied Biosystems). Oligonucleotide primers and a TaqMan probe for p122 were designed using Primer Express, version 1.5 (Applied Biosystems). The sense and antisense primers were 5'-CGG CTA CTA CTT CTA CTA CTA CTA-3' and 5'-GTT GCC GAA AGA GTA GCC GCT AT-3', respectively. The TaqMan probe was 5'-TCT GCC CCA GGA ACT TGG CAG C-3'. To assess p122 mRNA stability, the skin fibroblasts were exposed to actinomycin D, 5 μg/mL.

Measurement of Intracellular Ca2+ in HEK 293 Cells and Vascular Smooth Muscle Cells

HEK 293 cells and A7r5 cells (American Type of Cell Culture) were cultured in DMEM supplemented with 10% FBS. Human coronary artery smooth muscle cells (hCASMCs) (Clonetics) were cultured in smGM2 medium (human epidermal growth factor, 0.5 ng/mL; insulin, 5 μg/mL; human fibroblast growth factor, 2 ng/mL; and 5% FBS), and the cells from the fourth to seventh passages were used for the study.

For the p122 expression study, we enrolled 11 patients with CSA (8 men and 3 women; mean±SEM age, 58±5 years) and 9 control subjects without any history suggestive of angina pectoris who were undergoing cardiac catheterization (7 men and 2 women; mean±SEM age, 52±6 years). The clinical profiles of the patients with CSA and the control subjects are shown in Table 1. Coronary spasm, defined as total or subtotal coronary artery occlusion associated with chest pain and ischemic electrocardiographic change, was induced by intracoronary injection of ACh in all patients with CSA. After intracoronary injection of isosorbide dinitrate, the coronary arteriograms revealed healthy or almost healthy coronary arteries with a stenosis diameter of less than 50% of the lumen diameter.

The expression of the p122 protein and PLC-1 activity was examined by Western blot analysis. After loading with 5-μmol/L fura-2-acetoxyethyl ester, intracellular Ca2+ [Ca2+]i in response to ACh at 10-6 and/or 10-5 mol/L, was measured at excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm, as previously described.16 ACh was used because it is widely used for the induction of coronary spasm in Japan.10,17

Measurement of PLC Activity

The PLC assay system included the following components: N2-hydroxyethylpiperezine-N’-2-ethanesulfonic acid, 50 mmol/L; calcium chloride, 0.1 mmol/L; sodium cholate, 9 mmol/L; tritium-PiP2, 40 000 counts per minute; and the cell protein, 20 μg. The reaction was discontinued with a combination of chloroform, methanol, and hydrogen chloride, followed by 1N hydrogen chloride containing...
Determination of Initiation of p122 Transcription (SMART–Rapid Amplification of cDNA Ends)
SMART–Rapid Amplification of cDNA Ends (RACE) technology (BD Biosciences, San Jose, Calif) was used to determine the initiation site of p122 transcription. Total RNA isolated from the skin fibroblasts was reverse transcribed, and a complete cDNA with the addition of a BD SMART sequence at the 5′ end, was generated according to the manufacturer’s instructions. RACE-ready p122 cDNA was amplified using universal primer mix (BD Biosciences) and gene-specific primer (5′-TGC TGA GGC TGC GGA CGG AAG-3′), as recommended by the manufacturers. The promoter region of p122 that spans from nt −1599 to 114 relative to the translation start site, was generated according to the manufacturer’s instructions. The promoter region of p122 was amplified by PCR using 5 sets of primers. Each PCR product was amplified by a kit (BigDye Terminator Cycle Sequencing Kit) and then sequenced by a genetic analyzer (ABI PRISM 310).

DNA Isolation and Analysis of Genomic DNA for the p122 Promoter
Genomic DNA was isolated from the whole blood using a kit (QIAamp DNA Blood Mini Kit; Qiagen, Valencia, Calif). The promoter region of p122 was amplified by PCR using 5 sets of primers. The promoter product was amplified by a kit (BigDye Terminator Cycle Sequencing Kit) and then sequenced by a genetic analyzer (ABI PRISM 310).

Luciferase Assay for p122 Promoter
The p122 promoter-luciferase reporter construct, Picagene basic vector (Toyobo, Osaka, Japan), was obtained by PCR amplification (LA PCR, Takara) of human p122 promoter between −1599 and 114 relative to the translation start site. The mutated p122 promoter-luciferase reporter constructs, Picagene basic vector −1466C-T and −228G-A, were obtained by direct mutagenesis of single-nucleotide polymorphism sites in Picagene basic vector. The mutations were confirmed by sequencing. The National Institutes of Health 3T3 cells cultured in complete media were transfected with p122 promoter vectors containing or lacking the nucleotide conversions of −1466C-T and −228G-A, using Effectene Transfection Reagent (Qiagen). After incubation for 48 hours, the cell lysates were assayed with the Dual-Luciferase Reporter Assay System (Promega), according to the manufacturer’s instructions.

Analysis for p122 Promoter Variants at −228 and −1466
The −228G-A variant of the p122 promoter was analyzed by mutant allele-specific amplification. The promoter region of p122 that spans from nt −250 to 86 was amplified by PCR using 2 sets of primers (sense, 5′-AAC CGA GGG AAC CGA GGG GGC G-3′; to detect the −228A variant, and 5′-AAC CGA GGG AAC CGA GGC GGC A-3′; to detect the −228G variant; and antisense, 5′-AGT TGG CGG AGA AGT CCG TGA G-3′ to detect both variants). For both variants, a 336-bp amplicon was detected as the PCR product. The −1466C-T variant was analyzed by restriction fragment length polymorphism with endonuclease Smal (TaKaRa; Ohtsu, Shiga, Japan). The promoter region of p122 from nt −1599 to −978 was amplified by PCR. Smal I digests the wild-type 621-bp amplicon containing −1466C into 2 fragments (488 and 133 bp), and it does not digest the variant-type 621-bp amplicon containing −1466T.

Data Analysis
All data are expressed as mean±SEM. Categorical variables were compared by χ² analysis. An unpaired t test for comparison of 2 variables and a 1-way ANOVA for multiple comparisons, followed by a Bonferroni test, were used for statistical analysis. Differences were considered significant when P<0.05.

Results
Protein and Gene Expression of p122 in Cultured Fibroblasts
To assess the protein expression of p122, subconfluent skin fibroblasts, in which PLC-δ1 864G/A variant was not detected by single-strand conformation polymorphism analysis, were scraped after 24-hour serum starvation. In these conditions, the protein expression of p122 was constant and reproducible from the second to the fourth passages. As shown in Figure 1A, the ratio of p122 to GAPDH protein was reproducible from the second to the fourth passages. As shown in Figure 1A, the ratio of p122 to GAPDH protein was reproducible from the second to the fourth passages. As shown in Figure 1A, the ratio of p122 to GAPDH protein was reproducible from the second to the fourth passages.
coronary spasm, the level of the ratio of p122 to GAPDH was 3.9, which was the highest among the patients with CSA. In the same culture condition, p122 gene expression was increased in patients with CSA compared with control subjects by 37±23% (P<0.01) (Figure 1B), suggesting that upregulation of p122 protein expression is partially caused by enhanced gene expression. Figure 1C illustrates the representative amplification curves for p122 and GAPDH mRNA after the blockade of mRNA synthesis by actinomycin D. p122 mRNA was decreased at 5 and 10 hours in a time-dependent manner, and the ratio of p122 to GAPDH mRNA in patients with CSA was similar to that in control subjects (Figure 1D), suggesting that the p122 increase in patients with CSA is independent of mRNA stability but dependent on the transcription and/or translation level.

**Effects of p122, p122 siRNA, or PLC-δ1 siRNA Transfection on the Response of [Ca^{2+}]_i to ACh**

In the HEK 293 cells transfected with plasmid DNA of p122, a single immunoreactive compound was confirmed at 122 kDa (Figure 2A), and the ratio of p122 to GAPDH protein was increased by approximately 2 compared with the cells transfected with empty vector instead of p122, being comparable to the degree of its increased expression in patients with CSA. HEK 293 cells per se showed no [Ca^{2+}]_i response to ACh, whereas those expressing muscarine M1 receptor and PLC-δ1 showed a constant [Ca^{2+}]_i response to ACh. Figure 2B illustrates the representative waveforms of [Ca^{2+}]_i in response to ACh. ACh at both 10^{-6} and 10^{-5} mol/L caused a rapid transient increase in [Ca^{2+}]_i, followed by a lower but sustained phase of the increase. In HEK 293 cells transfected with p122, the transient increase in [Ca^{2+}]_i was augmented and the sustained phase was prolonged. As shown in Figure 2C, the [Ca^{2+}]_i level at baseline was 23±1 nmol/L in the cells without p122 and 39±2 nmol/L in cells with p122 (P<0.01). The peak increase in [Ca^{2+}]_i from baseline after ACh at 10^{-5} mol/L was higher than that after ACh at 10^{-6} mol/L in both types of cells (P<0.05 for both) (Figure 2D). The peak increase in [Ca^{2+}]_i from baseline after ACh was significantly greater in the cells transfected with p122 than in those without p122 (68±6 versus 33±4 nmol/L at 10^{-6} mol/L ACh and 128±11 versus 67±8 nmol/L at 10^{-5} mol/L ACh, respectively; P<0.01 for both).

As shown in Figure 3A, [Ca^{2+}]_i at baseline was decreased from 68±6 to 44±3 nmol/L in the knockdown of p122 with siRNA in hCASMCs (n=6, P<0.05). The peak increase in [Ca^{2+}]_i from baseline after ACh at 10^{-5} mol/L was decreased (n=6, P<0.05). By the knockdown of p122, PLC-δ1 protein expression was unchanged, but PLC activity was decreased.

As shown in Figure 3B, [Ca^{2+}]_i at baseline was increased from 76±12 to 123±35 nmol/L by the overexpression of p122 in A7r5 cells (n=6, P<0.05). The peak in [Ca^{2+}]_i from baseline after ACh at 10^{-5} mol/L was increased (n=6, P<0.05), and PLC activity was also enhanced by p122 overexpression (n=6, P<0.05). Furthermore, in this condition, knockdown of PLC-δ1 with siRNA cancelled the elevated baseline [Ca^{2+}]_i level and abolished the increase in [Ca^{2+}]_i, in response to ACh and enhancement of PLC activity.

**Sequencing of Genomic DNA for the p122 Promoter**

As shown in Figure 4A, we analyzed the promoter sequence (between nt −1599 and 114) of the p122 genomic DNA obtained from the whole blood of 6 patients with CSA and 6
control subjects. Then, we compared the sequence of the p122 genomic DNA obtained from the patients with CSA with that of the p122 genomic DNA previously reported (GenBank accession No. AF514295). Eight different nucleotides were found at position 1466 (C to T), 1319 (T to C), 833 (T to A), 572 (T to G), 276 (G to A), 228 (G to A), 144 (G to C), and 14 (T to C).

**p122 Promoter Assay**
To investigate the contribution of genomic DNA variants to gene expression, a luciferase assay was performed using mutated p122 promoter constructs. We conventionally named the p122 promoter obtained from a control subject containing neither 1466T nor 228A variant as wild type. As shown in Figure 4B, luciferase activities in the cells transfected with 1466T and 228A variant promoter constructs were both significantly increased compared with those with wild-type promoter construct by 1.34 ± 0.27 times (P < 0.03) and 1.57 ± 0.34 times (P < 0.001), respectively.

**Incidence of −228G-A Variant in Genomic DNA**
To examine the variance at nt −228, we amplified the corresponding promoter region by PCR using genomic DNA of 144 patients with CSA and 148 control subjects and examined the presence of this variant by the mutant allele-specific amplification method. Figure 4A (left and center panels) demonstrates 2 representative patterns of the PCR products, corresponding to the 228G/G normal homozygote.

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**Figure 3.** A, The effects of knockdown of p122 with siRNA in human coronary artery smooth muscle cells. Representative waveforms of [Ca²⁺]i, shown in the upper panel, and representative bands of protein expression of each parameter are shown in the middle panel. [Ca²⁺]i at baseline, peak increase in [Ca²⁺]i, from baseline after 10⁻⁵ mol/L ACh, and PLC activity in control and knockdown cells are shown in the lower panel. B, The effects of p122 overexpression with and without PLC δ1 knockdown in A7r5 (rat aortic smooth muscle) cells. Representative waveforms of [Ca²⁺]i, are shown in the upper panel, and representative bands of protein expression of each parameter are shown in the middle panel. [Ca²⁺]i at baseline, peak increase in [Ca²⁺]i, from baseline after 10⁻⁵ mol/L ACh, and PLC activity in control cells and p122 overexpression with and without PLC δ1 knockdown are shown in the lower panel. *P < 0.05 vs each control (C).

**Figure 4.** A, Sequence analysis of the p122 promoter region on genomic DNA. Eight different nucleotide sites were found by using 5 sets of primers. B, Effect of genomic DNA variants on p122 promoter activity. Luciferase activity of the wild-type reporter construct was arbitrarily set as 1, and the mean ± SEM values of relative luciferase activity from 3 independent experiments are shown, each performed in duplicate. *P = 0.03 and **P < 0.001 (n = 6). Ex1 indicates exon 1; Luc, luciferase gene.
and the −228A/A homozygote. As shown in Figure 4B, the incidence of the −228A/A homozygote or −228G/A heterozygote variant was greater in male patients with CSA than in male control subjects (9 of 91 patients with CSA versus 1 of 62 control subjects; P<0.05). However, by the analysis of the T-allele influence to the risk of CSA, no significant effects were found in the additive (G versus A allele), dominant (GG versus AA and GA alleles), and recessive (GG and AG versus AA alleles) models (Table 2).

**Incidence of −1466C-T Variant in Genomic DNA**

As shown in Figure 4A (right panel), 2 nucleotide patterns of the −1466C/C normal homozygote and the −1466C/T heterozygote were demonstrated. The −1466C/C normal homozygote and the −1466C/T heterozygote were present in 120 (83.3%) and 24 (16.7%), respectively, of the 144 patients with CSA. The −1466C/C normal homozygote and the −1466C/T heterozygote were present in 125 (88.0%) and 17 (12.0%), respectively, of the 142 control subjects. The incidence of the −1466C/T heterozygote tended to be more frequent in patients with CSA than in control subjects (P=0.08), but it did not reach statistical significance.

**Discussion**

The major findings of this study were as follows. Protein expression of p122 was enhanced by approximately 3 times in patients with CSA, being associated with the enhanced expression of p122. The 8 variants of the nucleotide were detected in the p122 promoter, and the −228G/A and −1466C/T variants revealed the increase in promoter activity. Thus, p122 protein is upregulated in patients with CSA, and its enhancement may be involved in the increased coronary vasomotility via the increased Ca<sup>2+</sup> mobilization induced by PLC<sub>1</sub>. This suggests a critical role of p122 protein in the genesis of coronary spasm.

**Role of p122 in Enhanced Vasomotility**

The novel regulatory protein p122 plays 2 important roles in signaling pathways. One of the dual functions is the ability to enhance the PIP<sub>2</sub>-hydrolyzing activity of PLC<sub>1</sub> and the other is a GAP activity specific for P<sub>1</sub>. Regarding the GAP activity, it was recently reported that p122 is involved in the reorganization of the actin cytoskeleton and focal adhesions. However, the role of p122 as a positive regulator for PLC<sub>1</sub> in coronary spasm still remains unclear. In the present study, we showed that the protein expression of p122 in the cultured skin fibroblasts obtained from patients with CSA was upregulated by 3 times compared with control. All protein levels of p122 in patients with CSA exceeded the highest level in control subjects and were greater than the +1 σ of the mean. Therefore, it is likely that patients with CSA could be distinguished from control subjects by the protein level of p122. The gene expression of p122 was increased by 37% in patients with CSA compared with the control, and p122 mRNA stability was similar between patients with CSA and control subjects, suggesting that upregulation of p122 protein expression is not fully, but is significantly, dependent on enhanced gene expression. Homma and Emori showed that recombinant PLC-δ1 catalyzes the hydrolysis of PIP<sub>2</sub> in a Ca<sup>2+</sup>-dependent manner; in the presence of p122, its activity is 5- to 10-fold increased in the range of physiological Ca<sup>2+</sup> concentration. Thus, it is conceivable that upregulation of p122 protein observed in patients with CSA is responsible for the high activity of PLC-δ1.

By using 3 kinds of cultured cells (HEK 293 cells transfected with muscarine M1 receptor, PLC<sub>1</sub>, and p122; hCASMCs with p122 siRNA; and A7r5 cells with muscarine M1 receptor and p122 or PLC-δ1 siRNA), we tested the hypothesis that p122 upregulation, such as that observed in patients with CSA, is associated with increased [Ca<sup>2+</sup>], response to ACh. In these cells, p122 was upregulated approximately 2 times compared with the cells without transfection with p122, and it was comparable to the degree of upregulated p122 protein in patients with CSA. We used ACh because it is widely used as a provocation test for coronary spasm in the clinical setting in Japan. It binds the muscarine M<sub>1</sub> receptor linked to the Gq–PLC-β pathway. The results showed that the peak increase in [Ca<sup>2+</sup>]<sub>i</sub> in response to ACh was augmented and the sustained phase was prolonged in the cells transfected with p122. In addition, [Ca<sup>2+</sup>]<sub>i</sub> at baseline was elevated in the cells transfected with p122. These results are consistent with the previous findings of the 864G-A variant of PLC<sub>1</sub>, in which the conformational change is associated with upregulation of PLC<sub>1</sub> activity. These characteristics seem to explain the pathogenesis of CSA in which the basal vascular tone and the vasoconstrictor response to the diverse stimuli were both enhanced. It binds the muscarine M<sub>1</sub>-linked PLC-β pathway.

The p122 protein has another function, a GAP activity for PLC<sub>1</sub>, in which the conformational change is associated with upregulation of PLC<sub>1</sub> activity. These characteristics seem to explain the pathogenesis of CSA in which the basal vascular tone and the vasoconstrictor response to the diverse stimuli were both enhanced. Previous studies have shown that the expression of p122 is associated with the development of coronary spasm like the PLC-δ1 activation. On the other hand, Ca<sup>2+</sup> mobilization induced by PLC-δ1 activation may upregulate p and kinase in the vascular smooth muscle. Thus, the role of p122 in the regulation of p is complicated, and the relation of p122 to the genesis of coronary spasm via p activity remains to be determined.

**Possible Mechanisms for Enhanced Protein Expression of p122**

Some variants that upregulate mRNA expression may be involved in upregulation of p122 protein. Therefore, we first
clarified the translation start site of the p122/PLC-δ1 RACE procedure and then sequenced the p122 genomic DNA coding the promoter from 1599 through 92 by the genetic analyzer. According to the reported sequence, we found 2 sites of single allele mutation at 228G-A and 1466C-T, both of which resulted in increased p122 promoter activity. We analyzed the incidence of these variants in the relatively small, but strictly defined, population in whom coronary spasm was confirmed by total or subtotal coronary artery occlusion after intracoronary injection of ACh. The result showed that the incidence of 228G-A was more frequent in male patients with CSA than in male control subjects, suggesting that this variant is a possible candidate responsible for upregulation of p122 protein in CSA. The mechanism of enhancement of p122 promoter activity by the 228G-A variant may be related to transcription factor SP because this variance causes loss of binding to SP1 in its region. Upregulation of p122 protein was observed in the skin fibroblasts, but the variant of p122 promoter should be present in the overall tissues, including the coronary arteries. Therefore, upregulation of p122 might play an important role in the pathogenesis of CSA in humans. It is obvious that the p122 protein in patients with CSA is upregulated. However, other factors or a combination of factors may contribute to or play a more important role in the mechanism for the mRNA or protein upregulation of p122. Further studies are required.

**Study Limitations**

Upregulation of p122 was shown in the skin fibroblasts obtained from patients with CSA. Further studies are required to confirm whether this abnormality is present in the coronary artery, although it is impossible to obtain the coronary artery smooth muscle cells from the patients with CSA. In addition, we showed a possible role of p122 in the pathogenesis of CSA in the Japanese. It remains unclear whether this is observed in the overall population.

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**Disclosures**

None.

**References**


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