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ORIGINAL ARTICLE

MUTATION ANALYSIS OF ABCC9 GENE IN JAPANESE PATIENTS WITH CORONARY SPASTIC ANGINA

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Abstract Coronary artery spasm plays an important role in the etiology of coronary spastic angina (CSA) and other acute coronary syndromes. Mice with a targeted disruption of the ATP-binding cassette transporter C9–ABCC9 gene were developed as an animal model of CSA. Thus, the ABCC9 may be involved in the regulation of coronary artery vasomotility. The aim of this study was to investigate whether mutation in the coding region of the ABCC9 gene is detected in Japanese patients with CSA. The study included 9 Japanese patients with CSA (6 men and 3 women with a mean age of 51 ± 13 years). Genomic DNA was extracted from the whole blood, and Mutation analysis of the coding region of ABCC9 was performed by direct sequencing. In one CSA patient, we found a single base substitution (G to A) at nucleotide position 126 in exon 21 of the coding region, which was heterozygous and did not cause amino acid substitution (T878T, silent mutation). In the remaining 8 patients, no base substitution was detected in the coding region of the ABCC9 gene. The results indicate that the mutation of the ABCC9 gene may not be involved in the genetic pathogenesis of CSA in humans.

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Key words: vasospasm; ABCC9; mutation.

原 著

日本人冠攣縮性狭心症患者における ABCC9 遺伝子変異の有無の解析

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抄録 冠攣縮は異型狭心症や急性冠症候群の病因に重要な役割を果たしている．ABCC9 遺伝子のノックアウトマウスは冠攣縮性狭心症の動物モデルとして報告され，ABCC9 は冠動脈の収縮拡張の調整に関与している可能性がある．今回，我々は日本人の冠攣縮性狭心症患者において，ABCC9 遺伝子のコード領域の変異の有無について検討した．9 例の患者(男 6 例 女 3 例 平均年齢51 ± 13歳)の血液より DNA を抽出し，ABCC9 遺伝子のコード領域の遺伝子変異を直接シーケンス法により解析した．その結果，一例でこれまで報告されていない遺伝子変異(エクソン21領域126番目のG→Aの変異)が検出された．この変異はヘテロであり，アミノ酸置換を伴わなかった(T878T サイレント変異)．他の 8 例では，ABCC9 遺伝子のコード領域で塩基置換は検出されなかった．ABCC9 遺伝子の変異は冠攣縮性狭心症の成因に必ずしも関与しないことが示された．

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キーワード：冠攣縮性狭心症；ABCC9；遺伝子変異.

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INTRODUCTION

Coronary artery spasm plays an important role in the pathogenesis of Prinzmetal variant angina[1, 2] and the other acute coronary syndromes[3, 4]. We and other investigators have shown that the basal vasomotor tone of the entire coronary artery system of Japanese patients with variant angina is enhanced[5–7]. In addition, the coronary artery constrictor response to diverse constrictor stimuli is enhanced[8], and occlusive constriction is readily induced when the coronary artery is exposed to such a stimulus.

ATP-sensitive potassium (K_{ATP}) channels are involved in the response to cardiac stress, ischemic preconditioning, vascular smooth muscle tone, skeletal muscle glucose uptake, neuronal excitability, transmitter release, and insulin secretion from pancreatic β-cells[9]. K_{ATP} channels are hetero-octameric complexes which consist of four pore-forming inwardly rectifying K^+ channel (Kir6.x) and four members of the ATP-binding cassette transporter C9 (ABCC9)[10, 11]. The former serves as an inwardly rectifying potassium channel pore, while ABCC9 is an ATPase-harboring ATP-binding cassette protein endowing sensitivity to potassium channel opener drugs[12]. The presence of vasospasm in ABCC9-deficient mice had underscored the physiological importance of the ABCC9 subunit in the overall regulation of vascular tone[13]. ABCC9-deficient mice exhibited a tonic increase in vasomotor tone and coronary episodic vasospasm that were probably related to a shift in the balance of vasodilatory and vasoconstrictive cues[13].

These findings raise the possibility that a loss of function of KATP channels may play a pivotal role in the pathogenesis of coronary spasm, but there is no proof for the direct contribution to the enhanced coronary vasomotility in patients with CSA. Recently, it was reported that genetic variations in the coding sequence of ABCC9 gene could be associated with precocious myocardial infarction (MI before the age of 60 years) in humans[14], suggesting that it was possible that there may be a close relationship between precocious MI and CSA in genetic variations of the coding sequence of ABCC9 gene. The aim of this study was to investigate whether mutation in the coding region of the ABCC9 gene is detected in Japanese patients with CSA and whether it causes abnormality in the primary structure of ABCC9.

MATERIALS AND METHODS

Study patients.

The ethics committee of our institution approved the study protocol. Written informed consent was obtained from all patients before the study. This study population included 9 Japanese patients with CSA (6 men and 3 women with a mean age of 51±13 years). The coronary arteriographic study was performed in all patients. Coronary spasm, defined as total or subtotal occlusion or severe vasoconstriction of the coronary artery associated with chest pain and ischemic ECG change, was induced with intracoronary injection of acetylcholine in 6 CSA patients. In the remaining 3 patients, ST segment elevation was recorded on the electrocardiogram during a spontaneous attack. After intracoronary injection of isosorbide dinitrate, the coronary arteriograms revealed normal or almost normal coronary arteries with diameter stenosis ≦50% of the lumen diameter in all patients.

Extraction of genomic DNA and direct sequencing of ABCC9 gene.

Genomic DNA was extracted from the whole blood using the QIAamp DNA blood kit (Qiagen, Valencia, CA, USA). The entire coding sequence of ABCC9 was amplified by using 39 couples of primers (Table 1). The tag single nucleotide polymorphisms genotyped to the entire coding sequence of ABCC9 were shown in Fig. 1. Each PCR reaction contained

Table 1 Primers used for direct sequencing of the ABCC9 gene.

Fragment		Sequence	Amplicon size
Exon1	Sense	5'- CCCTCTATTCAAGGCTTTAA	430 bp
	Antisense	5'- ATTTCACCTACGGAAGATCA	
Exon2	Sense	5'- CCCCTGCCTTAAAGCTTATC	334 bp
	Antisense	5'- GCACATTTATGGGCACAAGTT	
Exon3	Sense	5'- CGCAGAACGATGTCTTTCAA	413 bp
	Antisense	5'- TTCTACTCCCCACACACTCTGA	
Exon4	Sense	5'- ACCAAGTAAAACCATGTGGAAA	409 bp
	Antisense	5'- TCAAATACATGTGTTCATCCTTGTC	
Exon5	Sense	5'- AAGAATATTTCAGAAAGGGATGG	450 bp
	Antisense	5'- TCATGCCAACAGGACCATTCT	
Exon6	Sense	5'- GCTATGCTGAAAGGCACACA	450 bp
	Antisense	5'- AAAAAGCCTATTTGAACAACTCA	
Exon7	Sense	5'- GGGATTTTATGGATTTACCTGGTT	402 bp
	Antisense	5'- CAAAACTGAAGCTACCGCTATTC	
Exon8	Sense	5'- TGGCTGATTTAAGAAGATGATCC	452 bp
	Antisense	5'- TTTCTGAGTCCCAGCTTCCT	
Exon9	Sense	5'- ACCCTAATACCGTTGACTTCCA	404 bp
	Antisense	5'- TGCTGCTGAAGAACTGAAGTG	
Exon10	Sense	5'- GTCTCCTCAATCCCTGTCCA	395 bp
	Antisense	5'- GCTATTGGACAGCACGTGTTTA	
Exon11	Sense	5'- TGGGAACTCATAAAACTGCACA	362 bp
	Antisense	5'- GCAATGGAGACTGCCATAGAG	
Exon12	Sense	5'- ACAATGGACATACTGGCATAGC	366 bp
	Antisense	5'- GGTTGTCTCCTGGCAGTGAT	
Exon13	Sense	5'- CACAGGCATCCTACTCACCA	355 bp
	Antisense	5'- GCAAGTCGTGATTTTTCTTCAC	
Exon14	Sense	5'- CAAGAGGCATGTGAATGAGG	398 bp
	Antisense	5'- AAAGGCACAATTTGGGACAC	
Exon15	Sense	5'- GCCTGGCCTACAAATTATTA	211 bp
	Antisense	5'- AACTATGGTTACGGTCATGA	
Exon16	Sense	5'- TCAAAACTGGGCCATTGATT	338 bp
	Antisense	5'- TGTGCTTATTTCTGCGTGGT	
Exon17	Sense	5'- ACCATTTGGGAAATGTGCTC	356 bp
	Antisense	5'- AAGGAGCCACTTTGTTTGGT	
Exon18	Sense	5'- AAGGTTGGTGGTCTTTAAATTTTTC	346 bp
	Antisense	5'- GGTTCCAGAAACCATTGTTCA	
Exon19	Sense	5'- GAGCAGAGGCTGGCATATAA	371 bp
	Antisense	5'- AGGACTCCCATCCTTGGTTT	
Exon20	Sense	5'- TGGCCAGTAGACAGTTTCCA	347 bp
	Antisense	5'- CCATCTTTCCTTGAGTTACTTGAC	
Exon21	Sense	5'- GGGGATGATTTGCCCTTAGT	355 bp
	Antisense	5'- AAAAGCCTTGATTGGCAAAA	
Exon22	Sense	5'- ACGCATGCTGACTGGGTAAG	436 bp
	Antisense	5'- TGCAAAGATACAATTGCTTTGG	
Exon23	Sense	5'- ACAGAGCGCTTGAGATTGGT	319 bp
	Antisense	5'- CCCTGGCAAAGTGGCTTAT	
Exon24	Sense	5'- AAGGTTTGCCCTACTAAAAGCA	479 bp
	Antisense	5'- TGGCATTTGGGATATAAGCAT	
Exon25	Sense	5'- CCGTCTAAATGCATGCAAAA	432 bp
	Antisense	5'- GCATTGCTAATGGGCATCTT	
Exon26	Sense	5'- TCCTTGAGCAAATGAATAGGG	324 bp
	Antisense	5'- GATACAAATACAAATGGGCCTTTA	
Exon27	Sense	5'- GGGAAGTGAGTGAAGAATTGC	397 bp
	Antisense	5'- GATCTGTTGTTGGCAGAACC	
Exon28	Sense	5'- TGTTAATTGAGTGCTAGGGGAAA	341 bp

	Antisense	5'- CACAATGGGGAGATCAAACA	
Exon29	Sense	5'- CATTCCATGCCATCAGTTTG	330 bp
	Antisense	5'- TGTGAAGCTAGTGCCGAATC	
Exon30	Sense	5'- TAAGAACTTGGGAGTAACCTTT	389 bp
	Antisense	5'- ATGGATACATACGCAGAACAT	
Exon31	Sense	5'- CGCATGACATTCTCCTTGGT	363 bp
	Antisense	5'- AAACAACAATGTGCCCAACA	
Exon32	Sense	5'- AACTGCCACATAGTTTGGTTCA	367 bp
	Antisense	5'- GGCTGGGAAGTATGAAGAGC	
Exon33	Sense	5'- GTGCCCAGCCTGTTTTTATC	408 bp
	Antisense	5'- TGGGATATCTGCCTTGAACC	
Exon34	Sense	5'- TGGAAAAGTGGGTCTCCTGA	382 bp
	Antisense	5'- GGCTTTTCCAATCCAAATTGT	
Exon35	Sense	5'- ACTGCTCTGGGCACTGTTCT	441 bp
	Antisense	5'- GGTTCACCCTCTTGTACCC	
Exon36	Sense	5'- GTCCTTGCACCAGCACTAAG	355 bp
	Antisense	5'- GGATTCAGAACCCAATCAGG	
Exon37	Sense	5'- GCTCACAAGGGATTCAGTGG	349 bp
	Antisense	5'- GCAGACAGTTTGGAAAAGAACA	
Exon38	Sense	5'- TGCAAGTGGATTCTTGCTCA	354 bp
	Antisense	5'- CCCTTCCCTTTATTCACAGC	
Exon39	Sense	5'- CCCTACATCTTGTCACTCACCA	351 bp
	Antisense	5'- GCCACTTTACAGAGGTCAAGC	

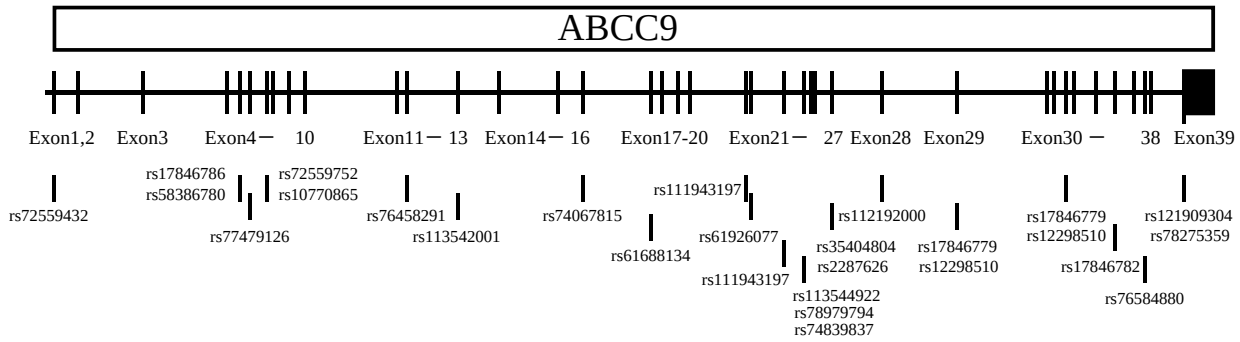

Figure 1 Locations of the tag single nucleotide polymorphisms genotyped to the entire coding sequence of ABCC9

the following: 10X Hotstart Taq buffer, 2.5 mM dNTP mixture, 10 μM primers, 2.5 U/μl Labo Pass Hotstart Taq DNA polymerase, and 40-80 μg genomic DNA in a total reaction volume of 30 μl. The thermal cycling parameters for PCR reaction were as follows: 1 cycle of denaturation (95°C for 15 min), 30 cycles of denaturation (95°C for 30 sec), annealing (55°C for 30 sec) and extension (74°C for 30 sec) followed by a final extension at 74°C for 15 min on the i Cycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). All PCR products were purified using Rapid PCR purification system (Edge Biosystems, Gaithersburg, MD, USA). Both strands of each amplicon were sequenced using a fluorescent dye terminator reaction (BigDye Terminator v1.1 cycle sequencing kit, Applied Biosystems, Foster City, CA, USA) on the ABI Prism 3730 genetic analyzer (Applied Biosystems, Foster City, CA,USA). The sequences of the coding region of the ABCC9 gene obtained from CSA patients were compared with that of the ABCC9 gene previously reported (NM_005691.2/ NM_020297.2/NM_020298.2).

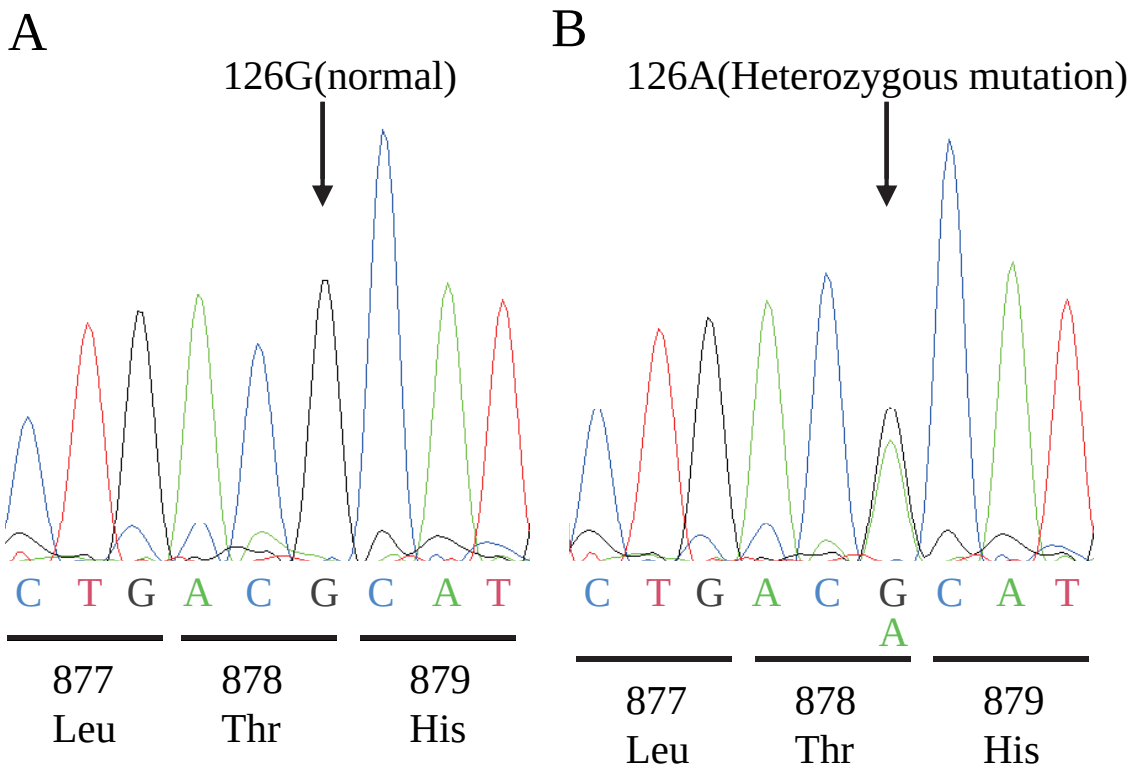

Figure 2 Sequence pattern from codons 877 to 879 of the human ABCC9 gene.
Normal (A) and heterozygous (B) conditions at nucleotide position 125 in exon 39. Val, Valine; Ala, Alanine; Arg, Arginine; Thr, Threonine; and His, Histidine.

RESULTS

Genomic DNA obtained from 9 CSA patients was analyzed. This analysis detected one previously described coding single nucleotide polymorphisms (reported in dbSNP as rs10770865). As shown in Fig. 2, single base substitution (G to A) at nucleotide position 126 in exon 21 of the coding region was detected in one female CSA patient. This substitution was heterozygous and did not cause amino acid substitution (Thr878Thr, silent mutation). In the remaining 8 patients, no base substitution was detected in the coding region of the ABCC9 gene.

DISCUSSION

K_{ATP} channel consists of four Kir6.x and four members of ABCC9[10, 11]. Kir6.1 or SUR2 knockout mice cause coronary vasospasm[13, 15]. No mutation in the coding region of the Kir6.1 gene was detected in 18 Italian patients with impaired coronary vasomotility[16]. Previously, we could not find any amino acid substitution in the primary structure of Kir6.1 in Japanese patients with CSA[17]. These findings suggest that the mutation of Kir6.1 gene may not be involved in the genetic pathogenesis of CSA in humans. In this study, we found a heterozygous single base substitution in exon 21 of the ABCC9 coding region in one Japanese patient with CSA. However this mutation did not cause any amino acid substitution in the primary structure of ABCC9. These findings suggest that mutation of ABCC9 gene may not be involved in the genetic pathogenesis of CSA in humans.

In ABCC9 knockout mice, smooth muscle

restoration of coronary artery K_{ATP} channels was ineffective in reducing spasm and the consequent atrioventricular heart block and sudden death that accompanies this spasm[18]. The mice overexpressing dominant negative Kir6.x pore-forming subunits in cardiac myocytes had no phenotype of coronary spasm, which had an increased mortality after the age of 4-5 months[19]. Also, in the mice overexpressing dominant negative Kir6.x pore-forming subunits in the endothelium, coronary spasm was not observed, but basal coronary perfusion pressure was elevated[20]. Thus it may be noted that both subunits are coexpressed in neuronal cell types [21, 22]. Also, there are diverse phenotypes in the specific molecule mutant-based spasm models, and there may be no close relationship between the enhanced coronary vasomotility and sudden cardiac death or elevated basal coronary perfusion pressure.

In conclusion, the present study showed that any mutation affecting any amino acid substitution of ABCC9 was not detected in 9 Japanese patients with CSA. Thus mutation of ABCC9 gene may not be involved in the genetic pathogenesis of CSA in humans. Further studies on a large population would be required to clarify the the role of ABCC9 gene in the generation of CSA.

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