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Defect of Delta-Sarcoglycan Gene Is Responsible for Development of Dilated Cardiomyopathy of a Novel Hamster Strain, J2N-k: Calcineurin/PP2B Activity in the Heart of J2N-k Hamster

Shinya Mitsuhashi[1], Naohiro Saito[2], Keiko Watano[3,4], Keiichi Igarashi[5], Seiichi Tagami[6], Hiroshi Shima[1] and Kunimi Kikuchi[*,1]

[1]Division of Biochemical Oncology and Immunology, Institute for Genetic Medicine, Hokkaido University, Kita-15, Nishi-7, Kita-ku, Sapporo 060-0815; [2]Tokyo Research Laboratories Pharmaceutical Division KOWA Co., Ltd., Tokyo; [3]Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Hokkaido University; [4]Division of Cardiology, National Hakodate Hospital, Hokkaido; [5]Division of Cardiology, Hokkaido Central Hospital for Social Insurance, Sapporo; and [6]First Department of Medicine, School of Medicine, Hokkaido University

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It has been shown that calcineurin (CN), a serine/threonine protein phosphatase type 2B (PP2B), plays an important role in the development and diseases of cardiac muscles. However, reports on CN activity in dilated cardiomyopathy (DCM) are inconsistent, since there are few good disease models and the measurement of the amount of CN is difficult. Previously, we developed a novel line of DCM hamster, J2N-k, and its healthy control counterpart, J2N-n, by crossbreeding cardiomyopathy (CM) hamsters, Bio 14.6, and Golden hamsters followed by consecutive sib mating. In this study, we identified the DCM-causative gene in J2N-k by analysis of F2 of these two lines, and then we analyzed the change in CN gene expression in the course of the disease, and the change in CN activity using a newly developed method. We show that: (i) the DCM gene of J2N-k hamster is the δ- sarcoglycan (SG) gene, (ii) CN expression and potential CN activities (CN activity fully activated with Ca^{2+} and calmodulin) in the hearts of J2N-k and J2N-n hamsters are the same levels, (iii) transcription levels of natriuretic peptides, which are augmented by activation of Ca^{2+}/calmodulin-dependent enzyme including CN, are significantly increased in the DCM stage in J2N-k hamster. J2N-k and J2N-n hamsters will be a useful tool for studying the pathogenesis, therapy, and prevention of human DCM. Although the total amount and potential activity of CN did not change in the cell extracts, targets of CN *in vivo* were activated in cardiomyocytes of DCM, suggesting that CN activity in the cells is activated by the raising of Ca^{2+} concentration in cardiomyocytes of DCM, which is caused by the defect in the δ-SG gene. Our results reveal the complexity of CN regulation in the heart and indicate the need for additional experimentation.

Key words: calcineurin, calcium influx, dilated cardiomyopathy, delta-sarcoglycan, J2N-k hamster, protein phosphatase.

Abbreviations used: PP2B, serine/threonine protein phosphatase type 2B; CN, calcineurin; DCM, dilated cardiomyopathy; CM, cardiomyopathy; SG, sarcoglycan; CHF, congestive heart failure; δ-SG, delta-sarcoglycan; LV, left ventricule; NF-AT, nuclear factor of activated T cells; Cys, cyclosporine; CK, creatine kinase; ECG, electrocardiogram; PBS, phosphate-buffered saline (divalent cation free); CNBα1, regulatory subunit of CN; ANF, atrial natriuretic factor; BNP, b-type natriuretic peptide.

Dilated cardiomyopathy (DCM), a group of disorders characterized by cardiac dilation and pump dysfunction, has an extremely poor prognosis (*1*) and its etiology has not been fully elucidated. Genetic mutations (*2*), viral infections (*3*), and autoimmune process (*4*) are thought to play important roles in the pathogenesis. Several cytoskeletal and myofibrillar genes, such as dystrophin, sarcoglycan (SG), and cardiac α-actin, have been suggested to be causative genes for DCM (*5*, *6*). Mutations in the dystrophin gene lead to a high incidence of DCM in Duchenne and Becker muscular dystophy patients and can cause X-linked DCM (*6*).

Bio 14.6 hamsters and their sublines, such as UMX7.1 and Bio 53.58 /TO-2, has been extensively investigated as models for cardiomyopathy (CM) (*7*, *8*). A genetic linkage map localized the disease locus on hamster chromosome 9qa2.1-b1 and suggested that the delta-sarcoglycan (δ-SG) gene may be responsible for the disease (*9*). Futher experiments demonstrated that the δ-SG gene is deleted in Bio14.6 (*9*). δ-SG form on SG complex with α, β, and γ-SG, and the SG complex is thought to play an important role in the function of dystrophin. A defect in the δ-SG gene induces disappearance of the other three SG proteins in heart and skeletal muscle (*7*). In humans, the mutation of dystrophin or SG results in DCM (*10*).

[*]To whom correspondence should be addressed. Tel/Fax: +81-11-706-7541, E-mail: kikuchi@imm.hokudai.ac.jp

To understand the pathogenesis, and to develop therapy and means of prevention of a human disease, it is crucial to have available model animals, that show similar symptoms of the disease. However, no DCM model animal that shows similar symptoms to human DCM and has a proper healthy control has been reported (*7*, *8*). Previously, we developed a novel line of DCM hamster, J2N-k, and its healthy control hamster, J2N-n (*11*, *12*). In the present study, we propose these two hamsters strains as useful tools for studying human DCM. J2N-k hamsters began to show myocardial necrosis at 4–5 weeks of age, exhibited cardiac dilatation and dysfunction at about 20 weeks of age and finally died of congestive heart failure (CHF) at approximately 1 year of age (*11*, *12*). J2N-n hamster, which has very similar genetic background to J2N-k hamster, is a healthy control strain. We found that DCM in J2N-k hamster is caused by disruption of the δ-SG gene by analysis of F2 hamsters of J2N-k and J2N-n.

It has been shown that calcineurin (CN), serine/threonine protein phosphatase type 2B(PP2B), plays a definitive role in the development and differentiation of cardiac and skeletal muscles (*13*). CN is a heterodimer composed of a 61-kDa catalytic subunit and 19-kDa regulatory subunit, the former of which binds calmodulin. CN is regulated by intracellular Ca^{2+} and calmodulin, and in the heart CN is thought to be involved in hypertrophy and apoptosis of myocytes by activating transcription factors, such as the nuclear factor of activated T cells (NF-AT) and MEF2. CN was shown to be augmented in hearts that had developed hypertrophy caused by pressure overload with aortic banding and systemic hypertension (*14–17*). Overexpression of CN inhibitor protein in heart and deletion of the CN gene prevented cardiac hypertrophy caused by pressure overload, isoproterenol infusion, angiotensin II infusion, and treadmill running (*18*).

It was also reported that the amount and enzyme activity of CN were increased in hearts of humans and tropomodulin transgenic mice that had developed DCM (*19–21*). Cyclosporine (Cys) and FK506, which are inhibitors of CN, prevented cardiac dilatation in tropomodulin or β-tropomyosin transgenic mice (*20*). However, during the CHF transition of Dahl salt-sensitive rats, CN expression decreased and administration of Cys did not improve left ventricle (LV) systolic function (*17*). Tsao et al. reported reduction of CN expression in human failing hearts (*22*). Thus, the relationship between DCM and CN is contradictory.

In recent years, intensive investigation has centered around characterizing CN signaling pathways that are associated with heart failure in an attempt to design novel therapeutic strategies. Therefore, it is important to analyze the regulation of CN activity in heart failure.

We previously developed a new method for accurate measurement of CN activity (*23*). In the present study, we analyzed changes in potential activities (CN activity fully activated with Ca^{2+} and calmodulin) of CN in the course of disease using newly established J2N-k and J2N-n hamsters. Although CN activities *in vitro* were the same in J2N-k and J2N-n hamsters, the transcription levels of natriuretic peptides, which are up-regulated by Ca^{2+}/calmodulin-dependent enzymes including CN, were significantly different in the dilated cardiomyopathy stage. A defect in the δ-SG gene may result in an increase in intracellular Ca^{2+} that activates CN activity in the heart of DCM.

MATERIALS AND METHODS

Animals—J2N-k and J2N-n hamsters were bred at Kowa Pharmaceutical. CM was diagnosed by the presence of elevated serum creatine kinase (CK) and abnormalities in an electrocardiogram (ECG). Serum CK activities were measured with a CPKII TestWako kit (Wako). All animals used in the present study were male, and all were deeply anesthetized before the experiment.

Histological Examination—Blood was removed by perfusing the right ventricle with 9 mM $NaPO_4$ (pH 7.4) containing 134 mM NaCl and 16 mM KCl. Immediately, the relaxed heart and biceps femoris were removed from each animal and kept in 100 mM $NaPO_4$ (pH 7.4) containing 10% formalin for 2–4 days. The fixed heart and skeletal muscle were embedded in paraffin, sectioned at 6 μm, and stained with Masson trichrome (*24*).

ECG—Hamsters were restrained in the supine position on a board under anesthesia, and ECGs were recorded using the standard limb leads (I, II, III, aVR, aVL, and aVF) and chest leads (V_1-V_6) according to the protocol of Takeda *et al*. (*25*).

Genomic PCR—Genomic DNA was isolated from the hamster liver by the standard SDS/proteinase K method. Genomic PCR was performed according to the method of Sakamoto *et al*. (*7*) with a slight modification. Genomic DNA fragments corresponding to exon 1C were amplified by PCR with the following primer set: 5′-AGTGAAGGGACCAGGTGGAC-3′ (primer 3)/ 5′-GCATATATAGCATGGTCTTC-3′ (primer 4). The genomic deletion breakpoints near exon 1A and exon 2 are designated here as the 5′-deletion breakpoint (5′-BP) and the 3′-breakpoint (3′BP), respectively (*7*). The genomic regions corresponding to the sequences surrounding 5′-BP (Fig. 3A) or encompassing 5′-BP and 3′-BP (Fig. 3A) were amplified with the following primer sets: 5′-TTTCCTCTGAGAAGTGTGTCC-3′ (primer 1)/5′-GATAGGATTTCTCTGTATTG-3′ (primer 2) or primer 1/5′-CTCAAATGAGCTAGTGCCAGG-3′(primer 5), respectively (Fig. 3B). Using a GENEAMP PCR system 9600 (Perkin Elmer), PCR was performed under the following conditions: preheating at 94°C for 2 min, then 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. *TAKARA Taq* (TaKaRa, Shiga) was chosen as a thermostable polymerase.

Northern Blot Analyses—Total RNA from the hamster heart was prepared by the acid guanidinium thiocyanate/phenol/chloroform extraction method (*26*). Northern blot analyses procedures were essentially the same as those previously described (*26*). Total RNA was separated on an agarose gel containing 17% formaldehyde, blotted on to nitrocellulose membranes, and fixed by UV irradiation. Transcripts for δ-SG, ANP, and BNP were scanned with hamster cDNA containing all of the coding region (*7*, *27*). Transcripts for regulatory subunit of CN (CNBα1) were scanned with rat cDNA containing all of the coding region (*28*). The mRNA levels were quantified with a Fluoro image analyzer, FLA-3000G (FujiFilm, Tokyo).

Assay for CN Activity—Hamsters were killed and the hearts were immediately removed. Each heart was rinsed with ice-cold washing solution containing 150 mM

NaCl and 0.1 mM EGTA, then frozen in liquid nitrogen and kept at –80°C until use.

All of the following procedures were carried out at 4°C. The frozen samples were suspended with 4 vols. of homogenizing buffer containing 50 mM Tris-HCl, pH 8.0, 250 mM sucrose, 0.4 mM benzamidine, 2 mM EGTA, 10 μg/ml soybean trypsin inhibitor, 4 μg/ml antipain, 10 μg/ml leupeptin, 0.2 mM PMSF, 0.15% β-mercaptoethanol, 5 mM ascorbic acid, and 2.5 mM DTT, and homogenized 20 times with a Teflon homogenizer. The homogenates were centrifuged at 20,000 ×g for 10 min, and the resulting supernatants were diluted with an equal volume of glycerol and stored at –24°C until use. The protein concentration was measured by the method of Bradford using BSA as a standard (*29*). CN activity was measured as previously described (*23*). These assays were carried out in triplicate, and the mean values are presented. One unit of the enzyme is defined as the amount of enzyme required to catalyze the release of 1 μmol of phosphate per min.

RESULTS

Although Bio 14.6 hamsters and their controls, Bio F1B hamsters, have been widely used for the study of CM, their genetic backgrounds are considerably different. For instance, the body weights of Bio14.6 and Bio F1B hamsters are clearly different even before the CHF stage, 85.4 ± 6.4 and 128 ± 3.1 g at the age of 8 weeks, respectively (*8*). Nagano *et al*. cross-bred Bio 14.6 and normal Golden hamsters consecutively and established J2N (N8) hamsters (Fig. 1). To separate diseased and healthy strains, J2N-k and J2N-n hamsters were established by consecutive sib mating of J2N (N8) hamsters (Fig. 1) (*11*, *12*). Since J2N-k and J2N-n hamsters have very similar genetic backgrounds, they were considered to be a useful model for the study of DCM.

Figure 2 shows the results of histological analysis. J2N-k hamsters began to show fibrosis at the age of 5–6 weeks (data not shown). As shown in Fig. 2, A–D, the LV dimension was increased and the wall thickness decreased in J2N-k hamsters. Fibrotic change had become more severe and degeneration was most prominent in the middle layer of the myocardium. The heart weights of J2N-k and J2N-n hamsters are not significantly different before the CHF stage, 396 ± 18 and 404 ± 32 mg (mean ± SD, $n = 8$) at the age of 21 weeks, respectively. Using echocardiography, Takagi *et al*. demonstrated that LV end-diastolic and -systolic dimension had increased in 20-week-old J2N-k hamsters (*30*). These results suggest that the symptom observed in the heart of J2N-k hamsters is DCM but not cardiac hypertrophy.

Considerable fibrosis, decrease in the number of cardiomyocytes and hypertrophic changes in the remaining cardiomyocytes were observed in J2N-k hamsters (Fig. 2F), while there were no significant changes in the hearts of J2N-n hamsters (Fig. 2E). In contrast to cardiac muscle, skeletal muscles developed only slight lesions in J2N-k hamsters (Fig. 2, G and H).

The genomic deletion interval of the Bio14.6 hamster was reported to be 29.8 kb, containing exon 1B and exon 1C of δ-SG (Fig. 3A) (*7*). This deletion causes a defect in δ-SG. To try to determine the structure of the δ-SG gene,

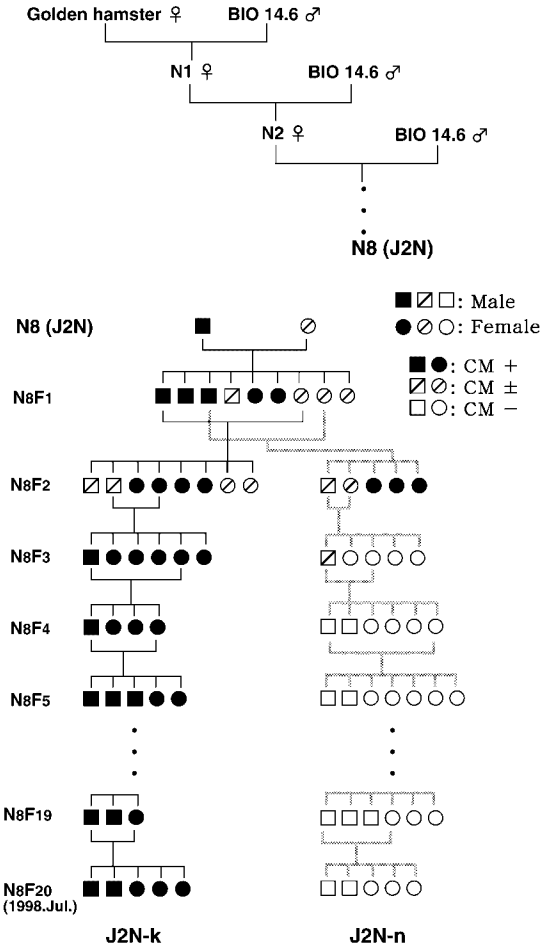

Fig. 1. **Pedigree of J2N-k, a cardiomyopathic hamster, and J2N-n, a non-cardiomyopathic hamster.**

we carried out genomic PCR (Fig. 3B). The primer set of primer 1/primer 5 amplified no genomic DNA fragments from normal hamsters (Golden and J2N-n) but a 361-bp genomic DNA fragment from J2N-k hamsters. Thus, the genomic deletion in J2N-k hamsters is located very close to the common deletion in other CM hamsters. In addition, genomic PCR with the primer sets of primer 1/primer 2 and primer 3/primer 4 detected 276-bp and 116-bp bands in normal hamsters, respectively, but no corresponding bands in J2N-k hamsters, confirming the genomic deletion interval for J2N-k hamsterts. In Northern blot analysis, 9.5-kb and 1.4-kb transcripts for δ-SG were predominant in normal hearts, but both of these were missing in the hearts of J2N-k hamsters (Fig. 3C). These results suggest that a defect in the δ-SG gene is also responsible for CM in J2N-k hamsters, and that J2N-n hamsters have normal genes.

To explore whether the mutation of δ-SG gene is responsible for cardiac disease, we cross-bred J2N-k hamsters and J2N-n hamsters (Fig. 4A). Fig. 4B and Table 1 show deletion in the δ-SG gene and serum CK activity of F2 hamsters. The δ-SG^{del}/δ-SG^{del} hamsters exhibited markedly increasing of CK activity. The δ-SG^{del}/+ hamsters and the +/+ hamsters exhibited normal CK activity. Cardiomyopathic pattern on ECG, such as pathologic Q waves in II, III and aVF, poor R wave progression

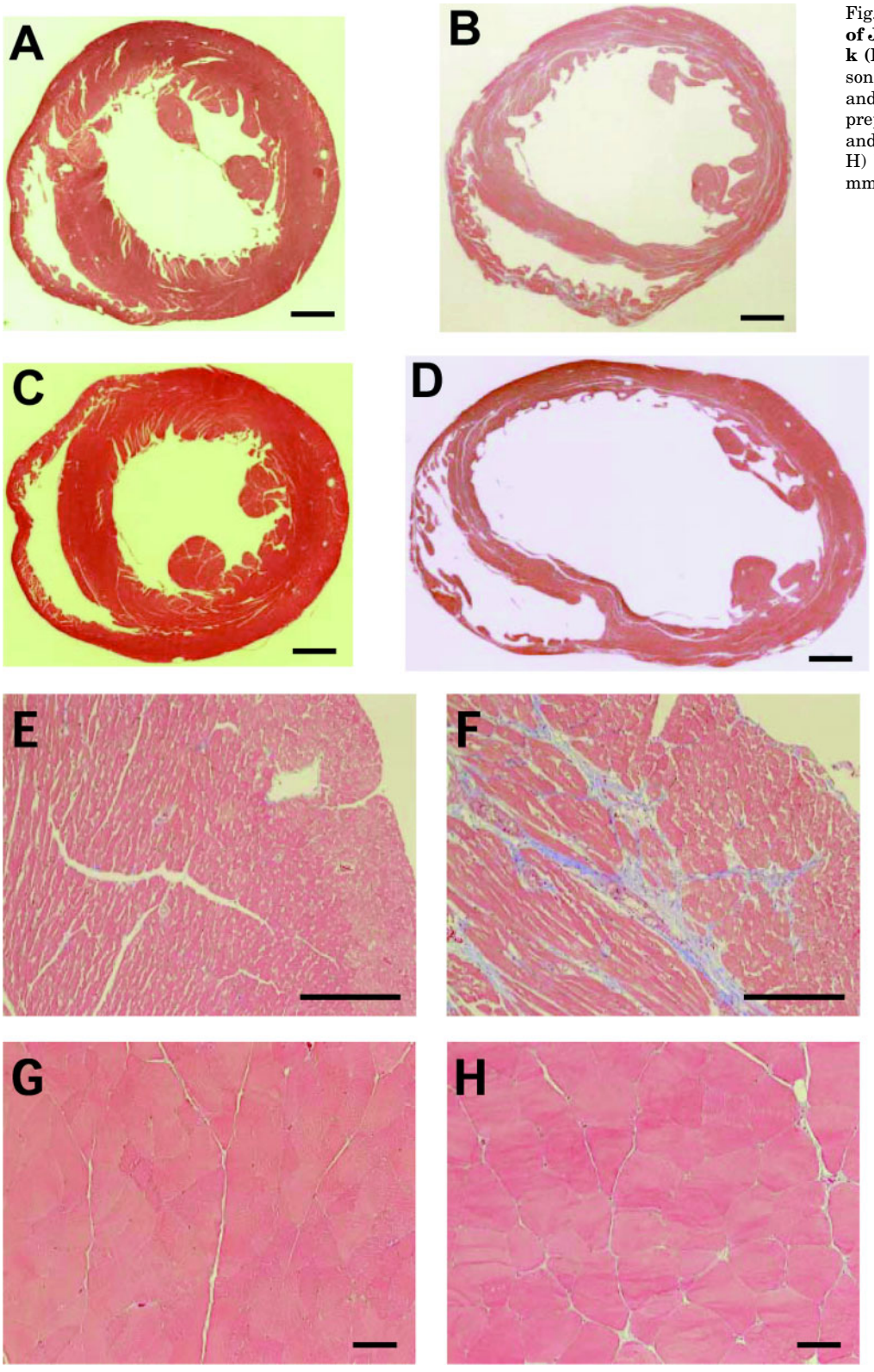

Fig. 2. **Histological examination of J2N-n (A, C, E, and G) and J2N-k (B, D, F, and H) hamsters.** Masson trichrome-stained hearts (A–F) and skeletal muscles (G and H) were prepared from 20-week-old (A, B, E, and F) and 30-week-old (C, D, G, and H) hamsters. Lengths of bars are 1 mm (A–D) and 200 μm (E–H).

in chest leads, and a more spiky P wave in I, II, aVL, aVF, and V_5-V_6 , appeared at δ-SG^{del}/δ-SG^{del} hamsters but not in other genotype hamsters (Table 1).

Fig. 5 and Table 1 show the life span of F2 hamsters. The survival curve of δ-SG^{del}/+ hamsters is similar to that of the +/+ hamsters (Fig. 5). On the other hand, the δ-SG^{del}/δ-SG^{del} phenotype was characterized by accelerated mortality. More than 95% of δ-SG^{del}/δ-SG^{del} hamsters died of CHF, presenting signs such as subcutaneous and pulmonary edema, congestive liver, atrial and ventricular

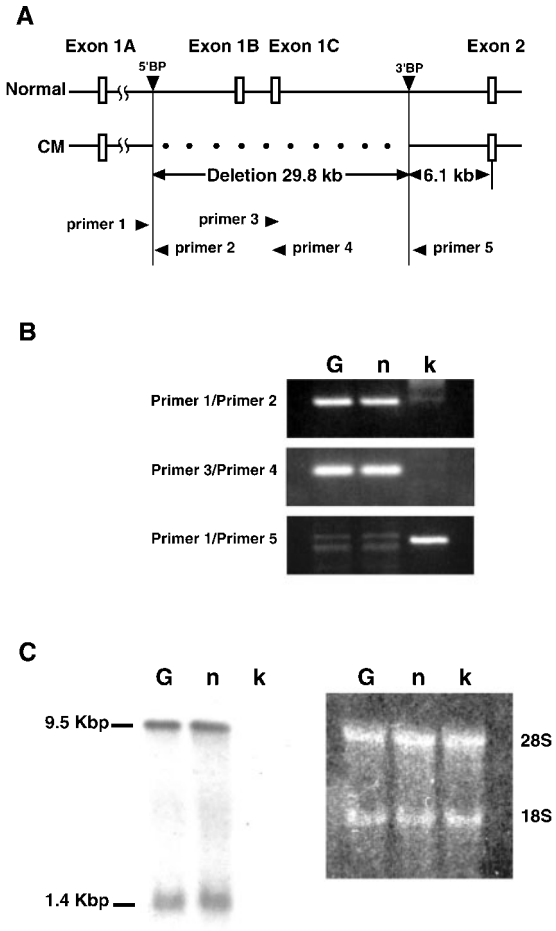

Fig. 3. **Genomic deletion of δ-SG in the J2N-k hamster.** (A) Genomic structure of the 5′ upstream region of the δ-SG gene in normal and cardiomyopathic hamsters. Exon 1A, exon 1B, exon 1C, and exon 2 are depicted as boxes. The genomic interval deleted in CM hamsters is indicated by a dotted line. 5′BP, 5′ deletion breakpoint; 3′BP, 3′ deletion breakpoint. (B) Deletion of a selective genomic region in J2N-k and J2N-n hamsters by PCR. The sizes of PCR products for primer1/primer2, primer3/primer4, and primer1/primer5 are 276, 116, and 361 bp, respectively. G, Golden; n, J2N-n; k, J2N-k. (C) Northern blot analyses of δ-SG. The transcription sizes are 9.5 kb and 1.4 kb, neither of which was detectable in the J2N-k hamster (left panel). Equal loading of samples was confirmed by ethidium bromide staining (right panel). Other conditions are as described in "MATERIALS AND METHODS."

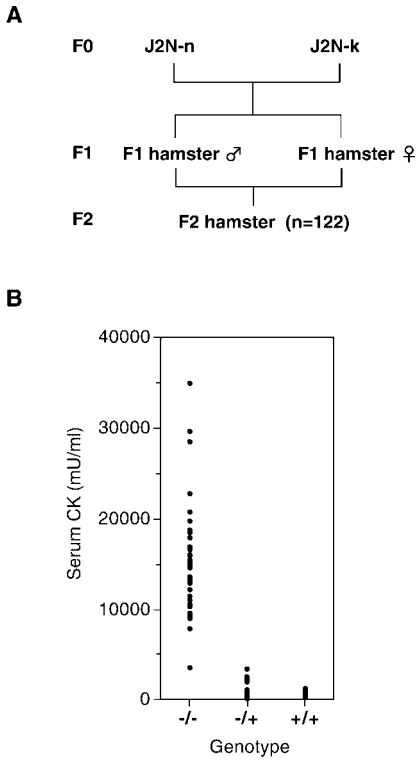

Fig. 4. **Genomic deletion of δ-SG and serum CK activity in the hamsters.** (A) Pedigree of F2 hamster. (B) Relationship between Genomic deletion of δ-SG and serum CK activity of 23-week-old F2 hamsters. Genotypes were determined by genomic PCR. δ-SG^{del}/δ-SG^{del} type (–/–, n = 40), δ-SG^{del}/+ type (–/+, n = 53), and wild type (+/+, n = 29).

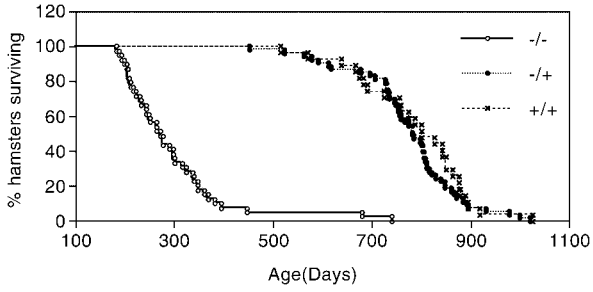

Fig. 5. **Genotype and life span in the F2 hamsters.** The life span of δ-SG^{del}/δ-SG^{del} type (–/–, n=39), δ-SG^{del}/+ type (–/+, n=53), and wild type (+/+, n=27) hamsters were monitored. All of δ-SG^{del}/δ-SG^{del} hamsters died of heart failure.

thrombus, and pleural effusion. These results suggested that the genetic defect in δ-SG is the cause of autosomal recessive DCM in J2N-k hamster.

To determine the potential CN activity (CN activity fully activated with Ca^{2+} and calmodulin) in the heart, lysate was obtained from each animal at 4–37 weeks of age. As shown in Fig. 6, neither the CN mRNA expression nor the potential activity changed during the course of the disease, although cardiac dilatation was prominent at 37 weeks of age. Even though the potential CN activity did not change in tissue extract, intracellular activity of

Table 1. **Genomic deletion of δ-SG and cardiac phenotype in F2 hamsters.**

Genomic type	δ-SG^{del}/δ-SG^{del}	δ-SG^{del}/+	+/+
Number of animals	40	53	29
CK activity (mU/ml)*	15337 ± 6161	735 ± 633	538 ± 267
Abnormal ECG rate (%)**	97.5, **100**	0, **0**	0, **0**
Life span (days)	297.7 ± 117.3	773.4 ± 110.9	787.6 ± 114.3

*Shown as mean ± SD. **Value at 23 weeks (normal typeface) or 30 weeks (bold typeface).

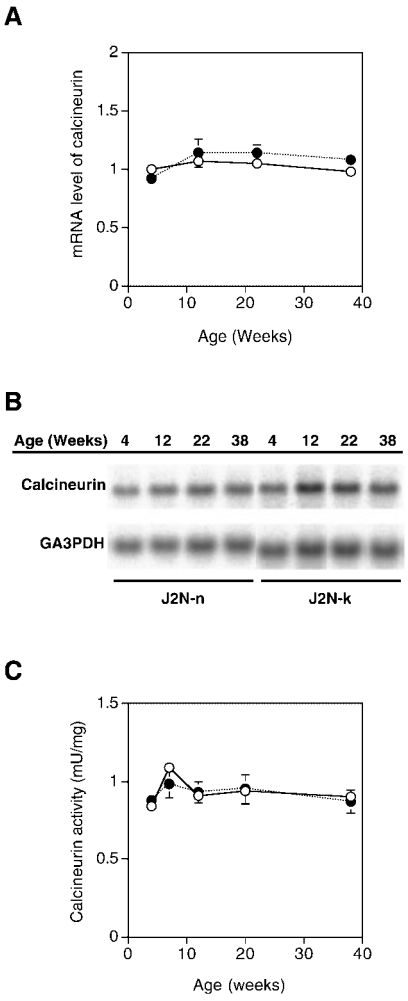

Fig. 6. **mRNA expression and phosphatase activities of CN in the hearts of J2N-n and J2N-k hamsters.** (A) CN regulatory subunit (CNBα1) and glyceraldehyde 3-phosphate dehydrogenase (GAP) mRNA levels were quantified by the Northern blot analyses of total heart RNA derived from J2N-n (open circles, $n = 4$) and J2N-k (closed circles, $n = 4$) hamsters. Values are means ± SD. (B) A representative result of panel A is shown. The transcription sizes of CNBα1 and GAP were approximately 4.2 and 1.3 kb, respectively. (C) CN activity was measured from hearts of J2N-n (open circles, $n = 7$) and J2N-k (closed circles, $n = 7$) hamsters. Values are means ± S.E.M. Northern blot analyses and CN activity assays were performed as described in "MATERIALS AND METHODS."

CN could change with abnormal Ca^{2+} handling. In cardiomyocytes, increasing Ca^{2+} influx was shown to augment transcription of Ca^{2+} response genes such as atrial natriuretic factor (ANF) and b-type natriuretic peptide (BNP) mRNA (*18*, *31*, *32*). Thus, detection of higher transcription levels of ANF and BNP would indicate a rise of Ca^{2+} concentration and activation of CN *in vivo*. To examine transcription levels of ANF and BNP mRNAs, we performed Northern blot analysis with animals aged 4–37 weeks (Fig. 7). Although considerable fibrotic changes appeared from 8 weeks (data not shown), transcription levels of ANF and BNP did not significantly differ in J2N-n and J2N-k hamsters at 4–11 weeks of age. Cardiac dilatation appeared at 20 weeks. At 22 and 38 weeks of age, ANF mRNA level in heart of J2N-k was 70 and 204% higher than that of J2N-n, respectively, and BNP mRNA level in heart of J2N-k was 70 and 109% higher than that of J2N-n, respectively.

DISCUSSION

We found that the δ-SG gene is deleted in J2N-k strain, and further analysis using F2 hamsters clearly demonstrated that homozygous deletion at the δ-SG gene locus correlated to the onset of DCM followed by death by congestive heart failure. In human patients, it was known that disruption of δ-SG induces a significant incidence of DCM (*7*). Recently, δ-SG-deficient mice were established, but they were shown to develop severe skeletal muscle dystrophy in addition to cardiac lesions, whereas hamsters lacking δ-SG showed slightly reduction of skeletal muscle force as human patients do (*33*). In this study, we confirmed that J2N-k hamsters did not develop severe skeletal muscle lesions. Since symptoms of J2N-k hamsters are more similar to those of human DCM-patients than are those of δ-SG-null mice, and J2N-k hamsters have J2N-n hamsters as an appropriate healthy control, we propose that J2N-k hamsters are one of the best animal models for X-linked DCM and many DCMs in humans. Usage of J2N-k and J2N-n hamsters will be very beneficial to understanding pathogenesis, development of therapy, and prevention of this disease.

Although Bio 53.58 hamsters have been extensively used as a DCM model, myocardial lesions such as focal necrosis and fibrosis are more severe than those of human DCM (*32*). In addition, it has been suggested that myocardial ischemia induced by microvascular spasm is also implicated in the pathogenesis of Bio 53.58 hamsters (*34*). Therefore, Bio 53.58 hamsters are not appropriate as a model of DCM in humans.

A technical problem in clarifying the role of CN in pathogenesis was difficulty in measurement of CN activity (*23*). To overcome this problem, we developed a new method for CN activity. Using this method, we demonstrated firstly a close correlation between the amount and activity of CN *in vitro* (*23*). In the present study, no alteration in CN activity in heart of J2N-k hamster was observed using our newly developed method, and the CN mRNA expression did not change during the course of the disease. Thus, regulation of the amount of CN does not play an important role in DCM caused by a defect of δ-SG in the hamster. CN activities in various other DCMs should be reexamined as potential full activity of CN using our method.

It has been reported that Ca^{2+} concentration is elevated in CM due to abnormal Ca^{2+} handling (*34–38*). To clarify this point further in J2N-k and J2N-n hamsters, we examined mRNA levels of ANF and BNP. These natriuretic peptides are thought to be the products of Ca^{2+} response genes, because activity of CN and Ca^{2+}-calmodulin dependent protein kinases (CaMKs) induce augmentation of these peptides (*18*, *31*, *32*, *39*). Our present results using J2N-k and J2N-n hamsters clearly demonstrated that mRNA levels of the calcium-response genes are associated with DCM based on δ-SG deletion and suggest that a defect in δ-SG leads to a rise in intracellular Ca^{2+}.

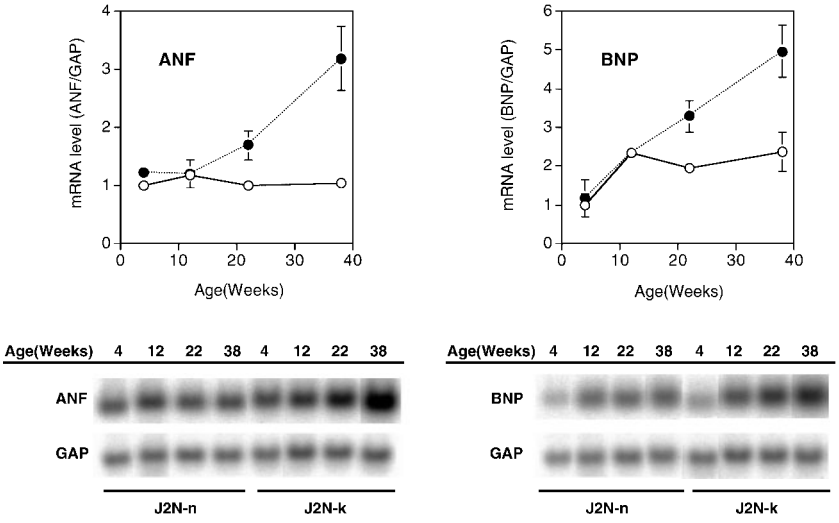

Fig. 7. **mRNA levels of ANP and BNP in the hearts of J2N-n and J2N-k hamsters.** Upper panels: ANP and BNP mRNA levels were quantified by the Northern blot analyses of total heart RNA derived from J2N-n (open circles, n = 4) and J2N-k (closed circles, n = 4) hamsters. Values are means ± SD. Lower panels: Representative results of Northern blot analyses are shown. The transcription sizes of ANP and BNP were 0.9 kb and 0.8 kb, respectively. The procedures used are described in "MATERIALS AND METHODS."

Recently, an attractive hypothesis for a compensation mechanism of cardiomyopathy has been proposed. Abnormal myofibrillar or cytoskeletal architecture would provoke dysfunction of intracellular Ca^{2+} regulation, leading to CN activation. An increase in CN activity sometimes masks cardiac dysfunction by promoting hypertrophy (*34*, *40*). The present study suggested that CN was activated by Ca^{2+} influx in cardiomyocytes of J2N-k hamsters. However, the heart did appear hypertrophied. Presumably, there are important unknown factor(s) other than activation of CN pathway for development of cardiac hypertrophy. We propose that CN expression and activities in various DCMs should be examined for profiles of altered regulation of CN in relation to heart failure in an attempt to design novel therapeutic strategies.

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