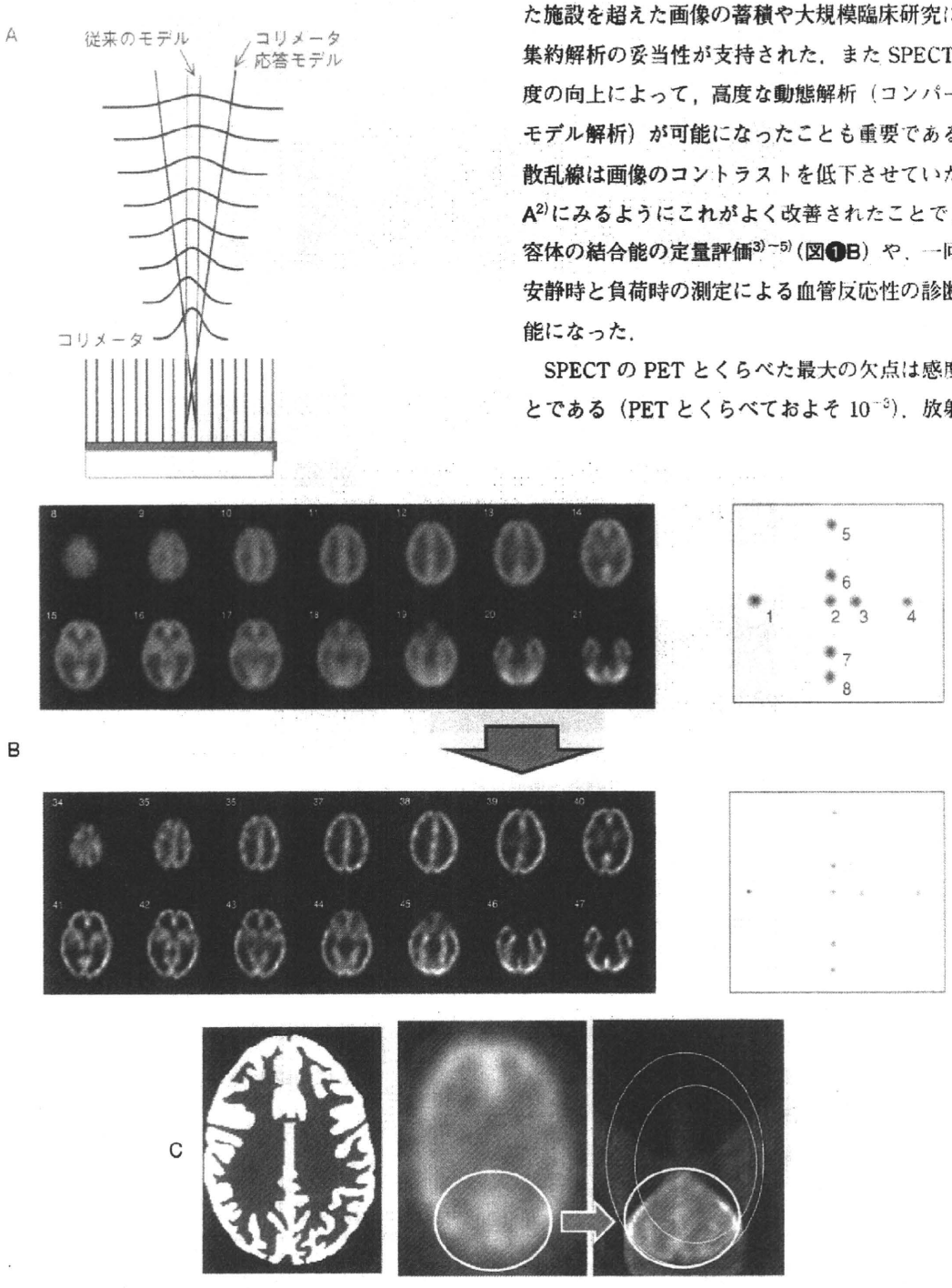
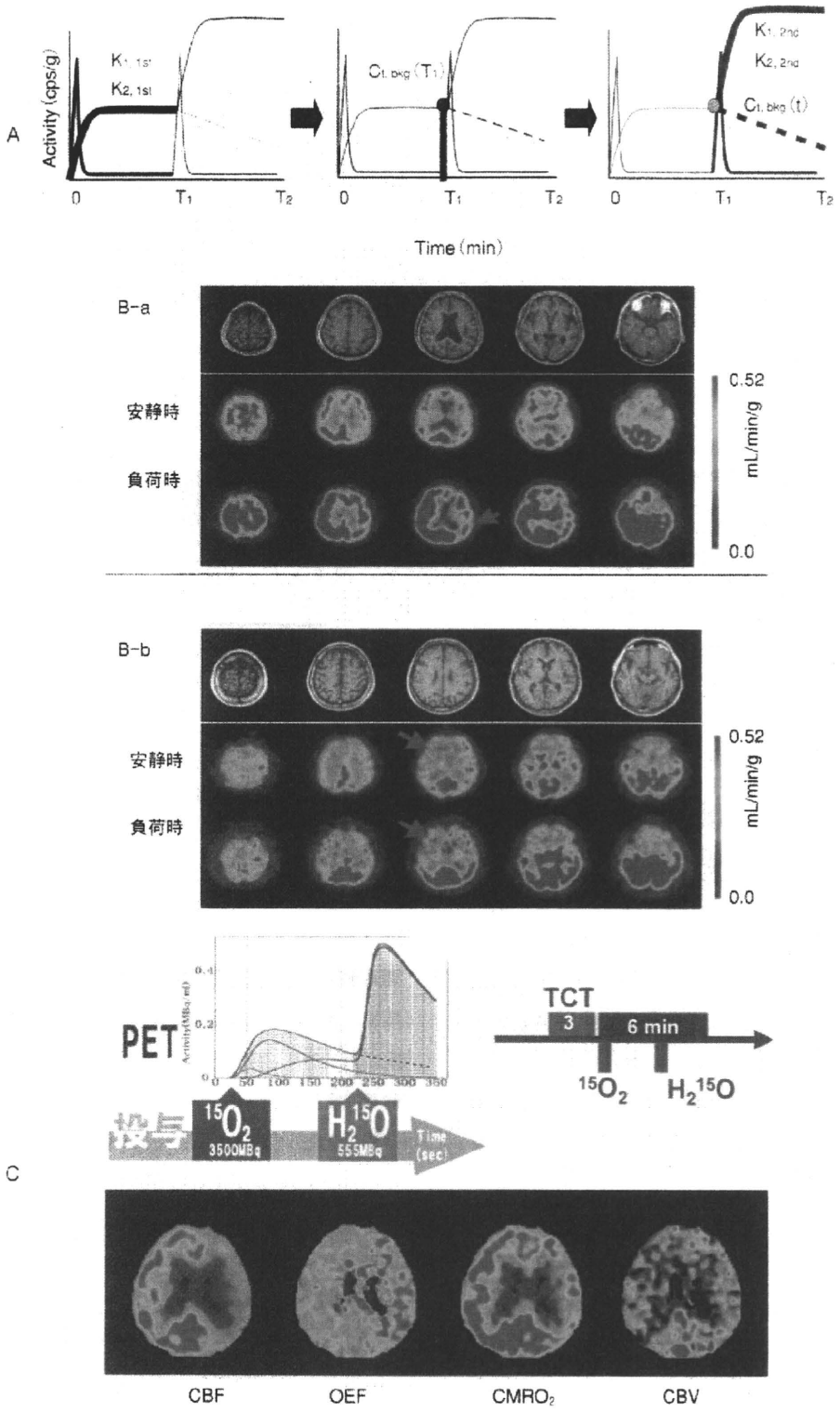
超えてデータが共有できる点においても，また既存の装置がそのまま利用できる点においても，PET に勝る重要な特長である．SPECT を使った多施設臨床研究は困難とされてきたが[1]，当該研究者らが中心となっておこなった施設を超えた画像の蓄積や大規模臨床研究において，集約解析の妥当性が支持された．また SPECT の定量精度の向上によって，高度な動態解析（コンパートメントモデル解析）が可能になったことも重要である．とくに散乱線は画像のコントラストを低下させていたが，図❶A[2]にみるようにこれがよく改善されたことで，脳神経受容体の結合能の定量評価[3)~5)]（図❶B）や，一回の撮像で安静時と負荷時の測定による血管反応性の診断などが可能になった．

SPECT の PET とくらべた最大の欠点は感度が低いことである（PET とくらべておよそ 10^{-3}）．放射線計測の

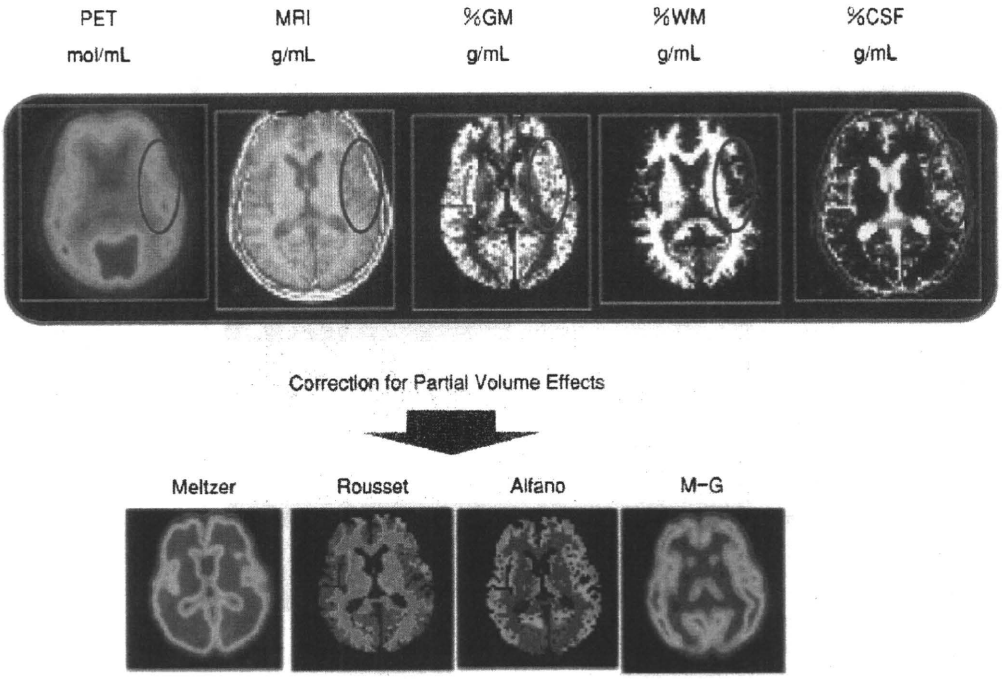


図❷ SPECT 画像再構成における空間解像度の補正
A：コリメータ開口にもとづく空間解像度劣化のモデル．B：コリメータ開口の補正を組み込むことで，空間解像度は 9.0 mm から 5.0 mm 以下に改善し，また統計ノイズも軽減された．今後臨床診断での実用化が望まれる．C：局所領域にピンホールコリメータの焦点を設定して得た 3D Hoffman ファントムの Tc-99m 画像．従来の画像撮像よりも局所において空間解像度の上昇が確認される．



図❸ 複数薬剤投与法の効果

A：複数トレーサ投与時の入力関数と脳内放射能濃度曲線を示す．バックグランド画像を推定し，さらに2回目投与後の脳内放射能濃度から機能画像を推定することが可能である．B：I-123 IMP の2回連続投与法にもとづく典型的な安静時とダイアモックス負荷後の局所脳血流量画像．症例 a では軽度血管狭窄を予測し，症例 b では高リスク血管狭窄を予測した．C：迅速^{15}O-ガス PET 検査への利用．従来1時間以上を要していた一連の検査が，10分間以内のスキャンで可能になった．



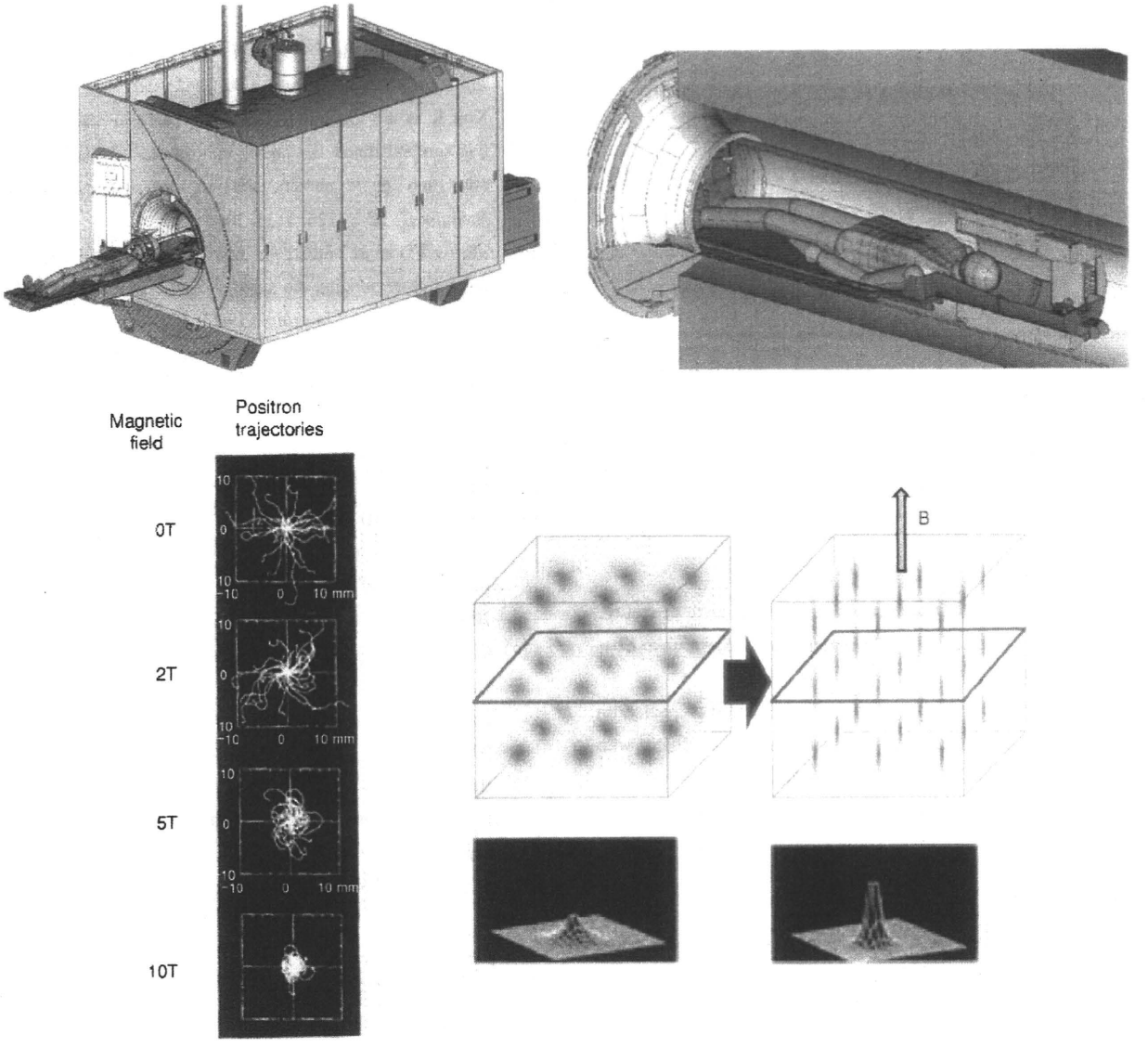
図❹ MRI 形態画像の部分容積効果の補正への利用

一般におこなって感度の高いトレーサ計測技術ではあるが，精細な画像を得るにはやはり限界とされてきた．コリメータを工夫することで，原理的には限りなく高い空間解像度を得ることができるが，実際には空間解像度を犠牲にして現実的な感度を確保してきたのがこれまでの現況である．しかし近年になって，コリメータ開口による解像度劣化を補償するような立体的な画像再構成が試みられるようになり（**図❷A, B**），これは空間解像度の向上だけでなく統計ノイズの抑制の効果を有することが明らかになり注目されている．視野全体の計測データを局所の画像再構成に組み入れられることの効果であると理解できる．空間解像度と画質の両方が同時に改善できることの意義は，実際の臨床診断ではきわめて重要である．多くの応用領域に貢献することが期待される．

実験小動物の SPECT イメージングではピンホールコリメータを使って高い空間解像度が実現でき，また従来から問題とされた空間解像度の不均一さは撮像軌道の工夫[6]や，複数ホールコリメータの利用によってほぼ解決した．さらに，ヒトなど大きな対象においてもトランケーションによるアーチファクトを回避する理論が開発され，局所を高解像度撮像できることが示された（**図❷C**）．更なる技術整備によって実用化が待たれる．

3 動態解析の進歩

核医学の動態解析における課題の一つは，動的な機能変化の検出であろう．従来から，核医学イメージングでは 1 回の放射性薬投与に対してひとつの機能をみるに限られていたが，**図❸A** に示すように複数投与した動態解析において残存薬剤の影響を動態に組み込む理論が提案された[7][8]．また，PET や SPECT で撮像した画像は，検査中における平均ではなく過渡的な重みを有すること[9][10]を応用して，検査中の組織血流量やシナプス間隙の内因性神経伝達物質の濃度の時間変化の画像化が試みられている．また，脳賦活によるドーパミンリリース変化のタイミングをとらえる試みもなされ，この遅れがある種の疾患の本質であるとしている[11][12]．**図❸B** には SPECT 検査中に I-123 標識 iodoamphetamine（IMP）を 2 回投与し，安静時と血管拡張薬（ダイアモックス）投与による血管反応性の検査結果の例を示す．明らかに脳虚血の程度や脳梗塞発症のリスクを診断できるとして期待されている．また**図❸C** には，短時間の間に ^{15}O-標識酸素ガスと ^{15}O-標識水を連続投与し，従来 1 時間以上要していた検査が全体で 6〜9 分間の PET 撮像のみから局所脳血流量（CBF），局所脳酸素代謝量（$CMRO_2$），酸



図❺ MRI と PET の一体化
上段：ドイツで開発中の 9.4 テスラ全身 MRI 装置と高性能 PET 装置の一体化のシェーマ．
下段：高磁場中で PET 撮像をおこなうとポジトロン飛程が減少する．これは空間解像度というよりも画像コントラストの向上に貢献するとされる．

素摂取率（OEF），局所脳血液量（CBV）の画像を計算した例を示す．これを脳虚血の重症度診断に貢献する種々の脳神経イメージングにおいてこのような撮像法の応用が可能であり，これは生体機能の調節機能の解明に貢献すると考えられる．

4 マルチモダリティイメージング

核医学画像と MRI などの形態画像との融合処理は，多くの自動化プログラムが開発され，脳萎縮の評価や部分容積効果の補正などに応用されている（図❹）．また，CT/PET や CT/SPECT 一体型装置につづいて，MRI/PET 一体化装置の実用化が進んでいる．図❺A には，ドイツ国ユーリッヒ研究所で開発中の 9.4 テスラ全身 MRI への高解像度・高感度 PET 組み込みのシェーマを示す．MRI 装置の外に磁気シールドを設置し，さらに外側の RF シールドとの間に血液分析システム一式を設置するなど，きわめて大がかりなシステムである．最新の PET 動態解析手法を駆使することで，fMRI や神経連絡イメージングと同時に PET 受容体賦活検査や，MRS，

PET 代謝イメージングなど多くの撮像が試みられる．このような検査においては，とくに上で述べたような複数核医学イメージングが望まれる．また，高磁場中ではポジトロン飛程が短くなることが予測され（図❺B）[13]，これは空間解像度というよりも画像コントラストの向上に貢献することが予測されている．

おわりに

PET，SPECT 機器，動態解析技術の現状と将来への期待について述べた．撮像や画像解析にかかる地道な技術整備が必要であり，この分野における物理工学研究者の活躍を期待したい．

●文　献●

1) Hapdey S *et al*：Quantification in SPECT：myth or reality? A multicentric study. *IEEE Nuclear Science Symposium Conference Record* **5**：3170-3173, 2004
2) Iida H *et al*：Effects of scatter and attenuation correction on quantitative assessment of regional cerebral blood flow with SPECT. *J Nucl Med* **39**：181-189, 1998
3) Fujita M *et al*：Widespread decrease of nicotinic acetylcholine receptors in Parkinson's disease. *Ann Neurol* **59**：174-177, 2006
4) Fujita M *et al*：Effect of scatter correction on the compartmental measurement of striatal and extrastriatal dopamine D_2 receptors using [^{123}I] epidepride SPET. *Eur J Nucl Med Mol Imaging* **31**：644-654, 2004
5) Kim KM *et al*：Contribution of scatter and attenuation compensation to SPECT images of nonuniformly distributed brain activities. *J Nucl Med* **44**：512-519, 2003
6) Zeniya T *et al*：A new reconstruction strategy for image improvement in pinhole SPECT. *Eur J Nucl Med Mol Imaging* **31**：1166-1172, 2004
7) Nishizawa S *et al*：Validation of the dual-table autoradiographic method to quantify two sequential rCBFs in a single SPET session with *N*-isopropyl- [^{123}I] p-iodoamphetamine. *Eur J Nucl Med Mol Imaging* **30**：943-950, 2003
8) Kim K *et al*：Quantitative mapping of basal and vasareactive cerebral blood flow using split-dose ^{123}I-iodoamphetamine and single photon emission computed tomography. *Neuroimage* **33**：1126-1135, 2006
9) Morris ED *et al*：ntPET：a new application of PET imaging for characterizing the kinetics of endogenous neurotransmitter release. *Mol Imaging* **4**：473-489, 2005
10) Iida H *et al*：Rapid measurement of cerebral blood flow with positron emission tomography. In：*Exploring the brain functional anatomy with positron tomography*, ed. by Chadwick DJ *et al*, John Wiley & Sons, Chichester, 1991, pp.23-42
11) Normandin MD *et al*：Temporal resolution of ntPET using either arterial or reference region-derived plasma input functions. *Conf Proc IEEE Eng Med Biol Soc* **1**：2005-2008, 2006
12) Constantinescu CC *et al*：Nonparametric extraction of transient changes in neurotransmitter concentration from dynamic PET data. *IEEE Trans Med Imaging* **26**：359-373, 2007
13) Iida H *et al*：A simulation study of a method to reduce positron annihilation spread distributions using a strong magnetic field in positron emission tomography. *IEEE Trans Nucl Sci* **33**：597-600, 1986

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Antiproteinuric Effect of Cilnidipine in Hypertensive Japanese Treated with Renin-Angiotensin-System Inhibitors - A Multicenter, Open, Randomized Trial Using 24-Hour Urine Collection

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Abstract

Sustained proteinuria is an important risk factor for not only renal but also cardiovascular morbidity and mortality. Although inhibitors of the renin-angiotensin system (RAS) have been shown to reduce proteinuria. Monotherapy with those drugs is often insufficient for optimal blood pressure (BP)-lowering and therefore, combined therapy is needed. Recent reports suggested that cilnidipine, a dual L-/N-type calcium channel blocker, has renoprotective effect by dilating both efferent and afferent arterioles. In this study, a multicenter, open, randomized trial was designed to compare the antiproteinuric effect between cilnidipine and amlodipine when coupled with RAS inhibitors in hypertensive patients with significant proteinuria. Proteinuria was evaluated by 24-h home urine collection for all patients. A total of 35 proteinuric (>0.1 g/day) patients with uncontrolled BP (>135/85 mmHg) were randomized to receive either cilnidipine (n = 18) or amlodipine (n = 17) after a 6-month treatment with RAS inhibitors and were followed for 48 weeks. At baseline, the cilnidipine group was older and had lower body mass index (BMI) compared to the amlodipine group. After 32 weeks of treatment, diastolic blood pressure (DBP) was slightly, but significantly reduced, in the cilnidipine group, although systolic blood pressure (SBP) and mean BP did not differ. The urinary protein did not differ at baseline (cilnidipine group 0.48 g/day, amlodipine group 0.52 g/day); however, it significantly decreased in the cilnidipine group (0.22 g/day) compared to the amlodipine group (0.50 g/day) after 48 weeks of treatment. Our findings suggest that cilnidipine is superior to amlodipine in preventing the progression of proteinuria in hypertensive patients even undergoing treatment with RAS inhibitors.

Keywords: proteinuria, cilnidipine, hypertension, renin-angiotensin system (RAS) inhibitor, randomized trial

INTRODUCTION

The presence of proteinuria is a well-established marker for renal dysfunction and furthermore, recent studies have demonstrated that proteinuria is an independent risk factor for cardiovascular disease (CVD) in patients with hypertension or diabetes (1–4). Therefore, the strict control of urinary protein is important to suppress not only the progression of renal dysfunction but also that of CVD.

Accumulating evidence has revealed that the renin-angiotensin system (RAS) plays an important role in the progression of the renal dysfunction (5). A number of clinical trials showed the renoprotective effect of RAS inhibitors such as angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARB) in patients with renal insufficiency (6–8), and therefore, RAS inhibitors are assumed to be a first-line agent to the hypertensive patients with renal dysfunction. However, to control both hypertension and proteinuria, monotherapy using RAS inhibitors is generally insufficient. Most cases need the combined therapy and indeed, the combined therapy is recommended in the guidelines (9–11).

Calcium channel blockers (CCBs), the most commonly used anti-hypertensive agents in Japan, have a stable and powerful blood pressure (BP)-lowering

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effect and are often used in combination with RAS inhibitors for the treatment of hypertension. Although the effect of CCBs on proteinuria is controversial, recent studies have reported the renoprotective effects of some kinds of CCBs. Cilnidipine, a dihydropyridine CCB, represents slow-onset, long-lasting BP-lowering effects by inhibiting L-type calcium channels as well as other conventional CCBs, and furthermore, has been reported to inhibit N-type calcium channels, which are densely distributed in the sympathetic nervous system (12). In the kidney, sympathetic nerves are distributed to both afferent and efferent arterioles in the glomeruli and contribute to the regulation of renal blood flow and the glomerular firtration rate (13, 14). The sympathetic nerve activity is increased in chronic renal insufficiency and thus, several researchers have tested the effect of cilnidipine on renal injury in animal models and have reported its benefit (15, 16).

Based on these clinical observations, several studies have reported the beneficial effect of cilnidipine on proteinuria (17–19). We also previously reported that switching conventional CCBs to cilnidipine decreased urinary protein excretion in the hypertensive patients with proteinuria (20). However, the additional renoprotective effect of cilnidipine to RAS inhibitors has not been fully investigated. In the present study, we compared the antiproteinuric effects between cilnidipine and amlodipine, a most commonly used CCB, when co-treated with RAS inhibitors in hypertensive patients with significant proteinuria.

METHODS

Participants

Participants were hypertensive patients who visited the outpatient clinic of the National Kyushu Medical Center, Kyushu Central Hospital, and National Cardiovascular Center. Each patient underwent 24-h home urine collection using a partition cup (proportional sampling method) (21) that collects a 1/50 portion of 24-h urine. Inclusion criteria of this study were: taking RAS inhibitors for more than 6 months; in-office BP levels more than 135 mmHg systolic and 85 mmHg diastolic during the observation period; and presence of proteinuria greater than 0.1 g/day. Exclusion criteria were malignant hypertension, severe heart failure (≥NYHA grade III), within 6 months following cardiovascular events, severe diabetes (HbA1c ≥ 8.0%), possibility of pregnancy, and history of adverse effects when using CCBs.

Study Protocol

Between April 2003 and December 2005, 41 patients met the criteria and enrolled in this study (Figure 1). The patients were randomly assigned to receive cilnidipine (21 patients) or amlodipine (20 patients) using a computer-generated schedule. The randomization was stratified according to sex, causal disease (essential or renal), and serum creatinine level. Renal hypertension was defined as having granular casts in the urine. Since other causes of secondary hypertension were clinically ruled out, other patients were diagnosed as essential hypertension. If the patients were already treated with other CCBs, they were switched to cilnidipine or amlodipine. The patients who were taking other types of anti-hypertensive drugs continued to take them without changing the doses. Blood pressure was measured in the sitting position every month in each hospital throughout the study period. During the study, three patients in the cilnidipine group and three patients in the amlodipine group dropped out. The main reason of the withdrawal was unsuccessful urine collection and no adverse effect was observed in both groups. Thus, 17 patients in the cilnidipine group and 18 patients in the amlodipine group completed the protocol and were used for the analysis. The doses of cilnidipine or amlodipine ranged from 5 to 15 mg/day (mean 8.6 mg) and 2.5 to 5 mg/day (mean 4.6 mg), respectively.

This study protocol was approved by the ethical review committee of each hospital or institute. The

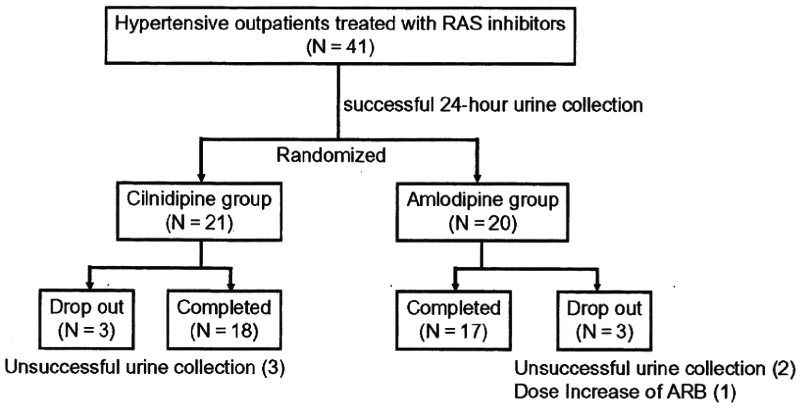

Figure 1. Profile of the trial.

detail procedure of the study was well explained and the written informed consent was obtained from all participants.

Statistical Analysis

Values are expressed as the mean ± SE. The student's paired *t*-test was used to analyze the changes of variables from the baseline. Differences in variables between the groups were analyzed by ANOVA. The Mann-Whitney U test was also used to compare the changes in the variables during the study period between the cilnidipine and amlodipine groups. A value of $P < 0.05$ was considered statistically significant. All analyses were performed using JMP6.0J software (SAS Institute Inc. Cary, NC, USA)

RESULTS

Baseline characteristics of each group are shown in Table 1. Three of 18 patients in the cilnidipine group and 3 of 17 patients in the amlodipine group were diagnosed as renal hypertensive. Patients treated with cilnidipine were significantly older and had lower BMI than those treated with amlodipine. There were no significant differences in other parameters including BP, heart rate, serum creatinine, serum lipid levels, and urinary protein excretion. Baseline RAS inhibitors and preceding CCBs are shown in Table 2. The cilnidipine group showed a relatively higher usage of ACE inhibitors such as imidapril, enalapril, and alacepril; however, they did not show large differences. Preceding CCBs also did not show significant differences between groups.

Figure 2 shows the changes in BP during the 48 months of the follow-up periods. Systolic and mean BP did not change in either the cilnidipine or amlodipine group, while DBP showed a small, but significant decrease, at 40 weeks and 48 weeks after treatment compared to baseline (0 week) in the cilnidipine group.

Table 1. Characteristics of the subjects

	Cilnidipine group	Amlodipine group	*P*
Number	18	17	
Age (years old)	66.8 ± 2.1	57.9 ± 2.4	0.008
Sex (men/women)	9/9	9/8	NS
Hypertensive	15	14	NS
Chronic nephritis	3	3	NS
Systolic BP (mmHg)	140.9 ± 3.0	135.6 ± 2.4	NS
Diastolic BP (mmHg)	75.9 ± 2.0	75.9 ± 2.7	NS
Heart rate (beat/min)	71.1 ± 1.4	72.7 ± 1.7	NS
Body mass index (kg/m^2)	24.7 ± 0.7	27.0 ± 0.7	0.037
Serum creatinine (mg/dL)	0.82 ± 0.10	0.91 ± 0.08	NS
BUN (mg/dL)	14.7 ± 1.1	16.8 ± 1.3	NS
Total cholesterol (mg/dL)	188.1 ± 7.7	205.0 ± 5.9	NS
HDL cholesterol (mg/dL)	51.5 ± 3.2	52.7 ± 2.5	NS
Urinary protein (g/day)	0.46 ± 0.14	0.52 ± 0.11	NS

Data are means ± SE.
Abbreviations: BP - blood pressure; BUN - blood urea nitrogen; HDL - high-density lipoprotein.

Table 2. Baseline RAS inhibitors and preceding CCBs

Drugs	Cilnidipine group	Amlodipine group
Baseline RAS inhibitors		
Losartan (25–100 mg/day)	10	7
Candesartan (4–12 mg/day)	2	5
Valsartan (40–120 mg/day)	1	4
Imidapril (5 mg/day)	2	0
Enarapril (5 mg/day)	1	0
Alacepril (25 mg/day)	1	1
Losartan (75 mg/day) & Imidapril (2.5 mg/day)	1	0
Preceding CCBs		
Amlodipine (2.5–5 mg/day)	6	6
Nilvadipine (8 mg/day)	3	2
Nifedipine CR (20–40 mg)	3	2
Nifedipine L (20 mg)	1	0
Benidipine (4–8 mg)	1	1
Nitrendipine (10 mg)	1	0
Diltiazem (100 mg/day)	1	0
Efonidipine (40 mg/day)	0	1
Azelnidipine (16 mg/day)	1	1
Cilnidipine (5–10 mg)	1	3

Abbreviations: RAS - renin-angiotensin system; CCBs - calcium channel blockers.

At 32, 40, 44, and 48 weeks after treatment, DBP was significantly lower in the cilnidipine group than in the amlodipine group (at 48 weeks: cilnidipine group 137.9/70.6 mmHg, amlodipine group 131.9/77.6 mmHg). As for the heart rate, a significant change was not observed after treatment and there were no significant differences in each period between groups (data not shown).

Changes in serum creatinine levels are shown in Figure 3. Serum creatinine levels did not show significant differences during the study period in both the cilnidipine group and the amlodipine group. No significant differences were observed between groups in each period. Although urinary protein excretion did not show differences between groups at baseline, it tended to decrease in the cilnidipine group at 24 weeks after treatment (Figure 4). Furthermore, 48 weeks after treatment, urinary protein excretion was lower in the cilnidipine group (0.22 ± 0.05 g/day) compared to the amlodipine group (0.50 ± 0.13 g/day).

DISCUSSION

In the present study, we showed a superior antiproteiuric effect of cilnidipine to amlodipine in hypertensive patients treated with RAS inhibitors by multicenter, open, randomized trial. Although several previous studies have reported similar results (18, 19), we firstly confirm the suppressive effect of cilnidipine on proteinuria using 24-h urine collection. Our results support the recent suggestion that cilnidipine, rather than conventional L-type CCBs, should be recommended as a second agent in hypertensive patients with significant proteinuria who are already treated with RAS inhibitors (19).

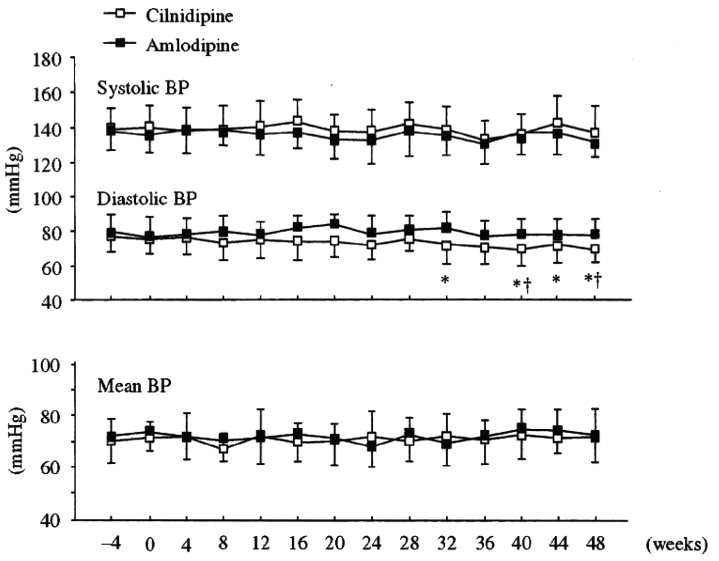

Figure 2. Changes in BP. Systolic BP and mean BP were almost the same in cilnidipine (open square) and the amlodipine (closed square) group. Diastolic BP was slightly decreased at 40 and 48 weeks compared to baseline (0 week) in the cilnidipine group and was siginificantly decreased at 32, 40, 44, and 48 weeks compared to the amlodipine group. Results are mean ± SE. $*P < 0.05$ vs. amlodipine group. $^{\dagger}P < 0.05$ vs. 0 week.

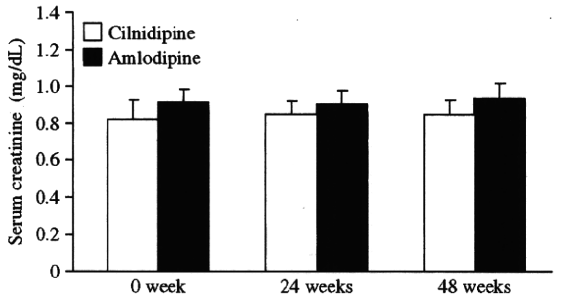

Figure 3. Changes in serum creatinine level. The serum creatinine level did not show significant differences during the study period in both the cilnidipine group (open bar) and the amlodipine group (closed bar). No significant difference was observed between groups.

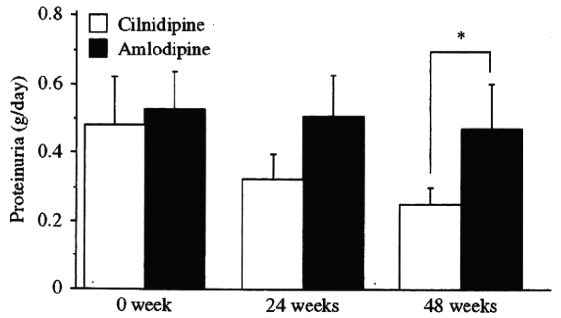

Figure 4. Changes in urinary protein. Urinary protein (g/day) was suppressed in the cilnidipine group (open bar) compared to the amlodipine group (closed bar) at 48 weeks after treatment. $*P < 0.05$.

Since the strict BP control significantly reduced proteinuria (22), it is clear that systemic BP influenced urinary protein excretion. In this study, most patients in both groups were pretreated with other CCBs and switched to cilnidipine or amlodipine, which has almost the same anti-hypertensive effects, resulting in the maintenance of similar BP levels during the study. However, cilnidipine elicited a small but significant decrease in DBP compared to amlodipine though systolic and mean BP showed no significant difference. In general, DBP is hard to reduce in young hypertensive patients compared to older patients. Age differences between groups may influence the difference in DBP. In addition, we are unable to exclude the possibility that the differences in BP level contributes to our result. However, proteinuria tended to decrease at 24 weeks after treatment when the BP level did not show a significant difference. Thus, the effect of cilnidipine seems to be attributable to its pharmacologic properties independent of BP-lowering effects.

A similar superior antiproteiuric effect of cilnidipine to amlodipine in Japanese has recently been reported by Fujita et al. (19). Although they evaluated proteinuria as urinary protein/Cr ratio, the study design was quite similar to ours. However, in their study, the participants had relatively severe renal damage and included many patients with diabetic nephropathy (39%) and primary renal disease (36%). Indeed, the values of urinary protein/Cr ratio were >1500 mg/g in more than half of the participants. Furthermore, in patients with hypertensive nephrosclerosis, antiproteiuric effect was relatively small and no difference was observed between cilnidipine and amlodipine. Conversely, in our

study, cilnidipine showed superiority against amlodipine though the etiology of most participants was essential hypertension and they had relatively small proteinuria. Our results indicate that cilnidipine is able to suppress proteinuria in patients with essential hypertension.

The characteristic renoprotective effect of cilnidipine seems to be related to its pharmacologic property to inhibit neuronal N-type calcium channels (23–25). It has been shown that most of conventional L-type CCBs preferentially dilate afferent glomerular arterioles by blocking the L-type, voltage-gated calcium channels that predominantly distribute in afferent arterioles (26). On the other hand, cilnidipine inhibits not only L-type but also N-type calcium channels, which are distributed to both afferent and efferent arterioles in the glomeruli (13, 14) and thus, cilnidipine is considered to dilate afferent and efferent arterioles. Actually, these effects of cilnidipine have confirmed in animal models, such as spontaneously hypertensive rats with hydronephrosis (27) and spontaneously hypertensive rat model of nephrosclerosis (15). Thus, the improvement of renal hemodynamics may assuredly account for the reduction of proteinuria in our study.

Previous studies suggest the other possible renoprotective effects of cilnidipine. In cultured rat mesangial cells, cilnidipine suppresses mesangial cell proliferation and the progression of extracellular matrix production (28). Cilnidipine has been shown to inhibit glomerular apoptosis and cellular proliferation in the nephrosclerosis model of spontaneously hypertensive rats (15). Although these nonhemodynamic actions may not be specific to cinlidipine, these actions may contribute to the renoprotective effects of cilnidipine. To elucidate the mechanism of antiproteinuric action of cilnidipine, further studies are required.

The renoprotective effect of RAS inhibitors has been supported by a number of clinical trials. A recent meta-analysis (29) indicated that the combination therapy of ACE inhibitor and ARB reduces proteinuria to a greater extent than either drug alone. However; this combined therapy might also lead excessive RAS inhibition resulting in the more frequent adverse events including acute renal failure and hyperkalemia. A recent study (30) reported that the combination therapy of ACE inhibitor ramipril and ARB telmisartan significantly reduces proteinuria compared to monotherapy, but it worsens major renal outcome such as dialysis, doubling of serum creatinine, and death. Which combination therapy is better in protecting kidney—ACE inhibitor and ARB, or RAS inhibitor and renoprotective CCB such as cilnidipine—should be examined in the future.

Small sample size and the varying characteristics at baseline between groups were important limitations in this study. However, patients in the cilnidipine group were older and had relatively higher SBP levels that might have led the higher possibility of progression of renal dysfunction. These factors may diminish the beneficial effect of cilnidipine. In addition, for BP evaluation, we used in-office BP but did not utilize home BP measurement or 24-h BP monitoring. Although we previously reported that the effects of cilnidipine on the 24-h BP profile are similar to nifedipine slow release using ambulatory BP monitoring (31), we were unable to exclude the possibility that cilnidipine decreased nocturnal or morning BP rather than daytime BP, which resulted in a greater reduction in proteinuria.

Proteinuria is not only a marker of the gromerular and vascular injuries but also facilitates progression of renal dysfunction. Indeed, the reduction of proteinuria has been reported to suppress the progression of renal dysfunction independent of BP lowering (32–34). Thus, to suppress the progression of renal dysfunction and CVD, strict control of proteinuria as well as BP is very important. This open randomized study using 24-h urine collection indicates that cilnidipine reduces proteinuria in hypertensive patients even while being treated with RAS inhibitors. When a single use of RAS inhibitors is insufficient for BP lowering or proteinuria reduction, additional treatment with cilnidipine should be considered as one of the useful therapies.

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Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

[1] Yudkin JS, Forrest RD, Jackson CA. Microalbuminuria as predictor of vascular disease in non-diabetic subjects. Islington Diabetes Survey. *Lancet* 1988;2:530–533.

[2] Bigazzi R, Bianchi S, Baldari D, Compese VM. Microalbuminuria predicts cardiovascular events and renal insufficiency in patients with essential hypertension. *J Hypertens* 1998;16: 1325–1333.

[3] Schrader J, Luders S, Kulschewski A, et al. Microalbuminuria and tubular proteinuria as risk predictors of cardiovascular morbidity and mortality in essential hypertension: final results of a prospective long-term study (MARPLE Study). *J Hypertens* 2006;24:541–548.

[4] Irie F, Iso H, Sairenchi T, et al. The relationships of proteinuria, serum creatinine, glomerular filtration rate with cardiovascular disease mortality in Japanese general population. *Kidney Int* 2006;69:1264–1271.

[5] Remuzzi G, Ruggenenti P, Benigni A. Understanding the nature of renal disease progression. *Kidney Int* 1997;51:2–15 (Review).

[6] Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. *N Engl J Med* 1993;329: 1456–1462.

[7] Brenner BM, Cooper ME, de Zeeuw D, et al. RENAAL Study Investigators. Effects of losartan on renal and cardiovascular

outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 2001;345:861–869.

[8] Parving HH, Lehnert H, Bröchner-Mortensen J, Gomis R, Andersen S, Arner P. Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria Study Group. The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med.*2001; 345:870–878.

[9] Mancia G, De Backer G, Dominiczak A, et al. Management of Arterial Hypertension of the European Society of Hypertension; European Society of Cardiology. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2007;25:1105–1187.

[10] Chobanian AV, Bakris GL, Black HR, et al. National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National High Blood Pressure Education Program Coordinating Committee.The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA* 2003;289:2560–2572.

[11] Guideline Subcommittee. Japanese society of hypertension guidelines for the management of hypertension (JSH 2004) *Hypertens Res* 2006;29(suppl):S1–S105.

[12] Hirning LD, Fox AP, McCleskey EW, et al. Dominant role of N-type Ca2+ channels in evoked release of norepinephrine from sympathetic neurons. *Science* 1988;239:57–61.

[13] Fogo AB. Glomerular hypertension, abnormal glomerular growth, and progression of renal diseases. *Kidney Int Suppl* 2000;75:S15–21 (Review).

[14] DiBona GF, Kopp UC. Neural control of renal function. *Physiol Rev* 1997;77:75–197 (Review).

[15] Zhou X, Ono H, Ono Y, Frohlich ED. N- and L-type calcium channel antagonist improves glomerular dynamics, reverses severe nephrosclerosis, and inhibits apoptosis and proliferation in an l-NAME/SHR model. *J Hypertens* 2002;20:993–1000.

[16] Konda T, Enomoto A, Matsushita J, Takahara A, Moriyama T. The N- and L-type calcium channel blocker cilnidipine suppresses renal injury in dahl rats fed a high-sucrose diet, an experimental model of metabolic syndrome. *Nephron Physiol* 2005;101:1–13.

[17] Rose GW, Kanno Y, Ikebukuro H, et al. Cilnidipine is as effective as benazepril for control of blood pressure and proteinuria in hypertensive patients with benign nephrosclerosis. *Hypertens Res* 2001;24:377–383.

[18] Kojima S, Shida M, Yokoyama H. Comparison between cilnidipine and amlodipine besilate with respect to proteinuria in hypertensive patients with renal diseases. *Hypertens Res* 2004; 27:379–385.

[19] Fujita T, Ando K, Nishimura H, et al. Cilnidipine versus Amlodipine Randomised Trial for Evaluation in Renal Desease(CARTER) Study Investigators. Antiproteinuric effect of the calcium channel blocker cilnidipine added to renin-angiotensin inhibition in hypertensive patients with chronic renal disease. *Kidney Int* 2007;72:1543–1549.

[20] Tsuchihashi T, Ueno M, Tominaga M, et al. Anti-proteinuric effect of an N-type calcium channel blocker, cilnidipine. *Clin Exp Hypertens* 2005;27:583–591.

[21] Tochikubo O, Uneda S, Kaneko Y. Simple portable device for sampling a whole day's urine and its application to hypertensive outpatients. *Hypertension* 1983;5:270–274.

[22] Peterson JC, Adler S, Burkart JM, et al. Blood pressure control, proteinuria, and the progression of renal disease. The Modification of Diet in Renal Disease Study. *Ann Intern Med* 1995;123: 754–762.

[23] Oike M, Inoue Y, Kitamura K, Kuriyama H. Dual action of FRC8653, a novel dihydropyridine derivative, on the Ba^{2+} current recorded from the rabbit basilar artery. *Circ Res* 1990;67: 993–1006.

[24] Fujii S, Kameyama K, Hosono M, Hayashi Y, Kitamura K. Effect of cilnidipine, a novel dihydropyridine Ca^{2+}-channel antagonist, on N-type Ca^{2+} channel in rat dorsal root ganglion neurons. *J Pharmacol Exp Ther* 1997;280:1184–1191.

[25] Uneyama H, Takahara A, Dohmoto H, Yoshimoto R, Inoue K, Akaike N. Blockade of N-type Ca^{2+} current by cilnidipine (FRC-8653) in acutely dissociated rat sympathetic neurones. *Br J Pharmacol* 1997;122:37–42.

[26] Loutzenhiser R, Hayashi K, Epstein M. Divergent effects of KCl-induced depolarization on afferent and efferent arterioles. *Am J Physiol* 1989;257:F561–564.

[27] Fujii S, Hosono M, Hayashi Y, Kimura K. Cilnidipine, a novel dihydropyridine derivative, dilates glomerular efferent arterioles by N-type Ca^{2+}-channel blockade. *J Hypertens* 1998;16 (suppl 2):S182 (abstract).

[28] Sugiura T, Imai E, Moriyama T, Horio M, Hori M. Calcium channel blockers inhibit proliferation and matrix production in rat mesangial cells: possible mechanism of suppression of AP-1 and CREB activities. *Nephron* 2000;85:71–80.

[29] Kunz R, Friedrich C, Wolbers M, Mann JF. Meta-analysis: effect of monotherapy and combination therapy with inhibitors of the renin angiotensin system on proteinuria in renal disease. *Ann Intern Med* 2008;148:30–48.

[30] Mann JF, Schmieder RE, McQueen M, et al. ONTARGET investigators. Renal outcomes with telmisartan, ramipril, or both, in people at high vascular risk (the ONTARGET study): a multicentre, randomised, double-blind, controlled trial. *Lancet* 2008;372:547–553.

[31] Minami J, Ishimitsu T, Kawano Y, Numabe A, Matsuoka H. Comparison of 24-hour blood pressure, heart rate, and autonomic nerve activity in hypertensive patients treated with cilnidipine or nifedipine retard. *J Cardiovasc Pharmacol* 1998;32:331–336.

[32] The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia) Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. *Lancet* 1997;349:1857–1863.

[33] Sarafidis PA, Khosla N, Bakris GL. Antihypertensive therapy in the presence of proteinuria. *Am J Kidney Dis.* 2007;49:12–26 (Review).

[34] Ruggenenti P, Perna A, Remuzzi G; GISEN Group Investigators. Retarding progression of chronic renal disease: the neglected issue of residual proteinuria. *Kidney Int* 2003;63: 2254–2261.

Ca^{2+}/Calmodulin-Dependent Kinase IIδ Causes Heart Failure by Accumulation of p53 in Dilated Cardiomyopathy

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Background—Dilated cardiomyopathy (DCM), characterized by dilatation and dysfunction of the left ventricle, is an important cause of heart failure. Many mutations in various genes, including cytoskeletal protein genes and contractile protein genes, have been identified in DCM patients, but the mechanisms of how such mutations lead to DCM remain unknown.

Methods and Results—We established the mouse model of DCM by expressing a mutated cardiac α-actin gene, which has been reported in patients with DCM, in the heart (mActin-Tg). mActin-Tg mice showed gradual dilatation and dysfunction of the left ventricle, resulting in death by heart failure. The number of apoptotic cardiomyocytes and protein levels of p53 were increased in the hearts of mActin-Tg mice. Overexpression of Bcl-2 or downregulation of p53 decreased the number of apoptotic cardiomyocytes and improved cardiac function. This mouse model showed a decrease in myofilament calcium sensitivity and activation of calcium/calmodulin-dependent kinase IIδ (CaMKIIδ). The inhibition of CaMKIIδ prevented the increase in p53 and apoptotic cardiomyocytes and ameliorated cardiac function.

Conclusion—CaMKIIδ plays a critical role in the development of heart failure in part by accumulation of p53 and induction of cardiomyocyte apoptosis in the DCM mouse model. **(*Circulation*. 2010;122:891-899.)**

Key Words: apoptosis ■ CaMKII ■ cardiomyopathy ■ heart failure ■ genes, p53

Heart failure is an important cause of morbidity and mortality in many industrial countries, and dilated cardiomyopathy (DCM) is one of its major causes.[1] Although treatments for heart failure have been progressed well in both pharmacological and nonpharmacological aspects, mortality of DCM patients remains high, and the only treatment for DCM patients with severe symptoms is heart transplantation. Because the number of hearts for transplantation is limited, the development of novel therapies for DCM has been awaited.

Clinical Perspective on p 899

DCM, characterized by dilatation and impaired contraction of the left ventricle, is a multifactorial disease that includes both hereditary and acquired forms. The acquired forms of DCM are caused by various factors.[2] Twenty percent to 35% of patients have hereditary forms,[1] and advances in molecular genetic studies during the last decade have revealed many mutations of various genes in DCM patients.[3–5]

Several hypotheses have been reported on the mechanisms of how gene mutations lead to DCM phenotypes. Mutations in genes encoding cytoskeletal proteins such as desmin and muscle LIM protein might disturb the interaction between the sarcomere and Z disk, resulting in impaired force transmission from the sarcomere to the surrounding syncytium.[4,6] On the other hand, mutations in genes encoding contractile proteins such as α-tropomyosin and cardiac troponin T have been reported to induce the decrease in myofilament calcium (Ca^{2+}) sensitivity.[7] An increase in apoptotic cardiomyocytes and/or destruction of membrane structure by calpain activa-

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tion have been reported to play a critical role in mutant gene–induced cardiac dysfunction.[8–10] However, the precise mechanisms remain largely unknown as a result, at least in part, of a lack of good animal models of DCM.

Several animal models of DCM have been reported.[11–13] The *mdx* mouse is a model of Duchenne muscular dystrophy, which has mutations in the dystrophin gene.[11] Unlike humans, *mdx* mice rarely show cardiac abnormality, which has limited the utility of *mdx* mice as a model to examine the pathogenesis of DCM. Although Golden Retriever–based muscular dystrophy dogs show DCM phenotypes,[12] the muscular dystrophy dogs are very difficult to maintain and handle. Although BIO 14.6 hamsters lacking δ-sarcoglycan are a good model of DCM,[13] it is difficult to apply genetic approaches to the hamster. To elucidate the molecular mechanisms of how gene mutations cause DCM, appropriate animal models, particularly mouse models, are necessary. We established here a mouse model of DCM by expressing a mutated cardiac α-actin gene (mActin-Tg), which has been reported in patients with DCM, in the heart.[5] mActin-Tg mice showed gradual dilatation and dysfunction of the left ventricle, resulting in death by heart failure. These phenotypes of mActin-Tg mice were quite similar to those of human DCM. In this study, we examined the underlying mechanisms of how this gene mutation leads to DCM using the new mouse model of DCM.

Methods

Detailed experimental methods are described in the online-only Data Supplement.

Mice

We generated transgenic mice (mActin-Tg) that expressed a mutated cardiac α-actin (R312H) with an HA tag in the heart. This mutation has been reported in patients with DCM.[5] Generation of transgenic mice with cardiac-restricted overexpression of human Bcl-2, AC3-I, or nuclear factor of activated T cell (NFAT)–luciferase has been described previously.[14–16] Heterozygous p53-deficient mice were purchased from The Jackson Laboratory (Bar Harbor, Me).[17] Wild-type littermates served as controls for all studies. KN-93 (10 $\mu mol \cdot kg^{-1} \cdot /d^{-1}$) was used to inhibit activation of Ca^{2+}/calmodulin-dependent kinase II (CaMKII). Echocardiography was performed on conscious mice.

Histology

For detection of apoptotic cardiomyocytes, we performed terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) staining, along with immunostaining for dystrophin.

Western Blot Analysis

Whole-cell lysates were resolved by SDS-PAGE. Western blot analyses were performed with some antibodies. The intensities of Western blot bands were measured with NIH ImageJ software (National Institutes of Health, Bethesda, Md).

Luciferase Assay

Left ventricles were homogenized in luciferase assay buffer as described previously.[15]

Force Measurements

A small fiber was dissected from the skinned left ventricular papillary muscle, and isometric force was measured as described previously.[7]

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction Analysis

Quantitative real-time polymerase chain reaction was performed with the LightCycler with the Taqman Universal Probe Library and Light Cycler Master. Relative levels of gene expressions were normalized to the mouse GAPDH expression with the ΔΔCt method.[18]

Statistical Analysis

Data are shown as mean±SEM. Multiple-group comparison was performed by 1-way ANOVA followed by the Bonferroni procedure for comparison of means. The F test was used to assess equal variances before comparison between 2 groups. Then, comparisons between 2 groups were performed with the Student t test (when $P>0.05$ in the F test) and the Welch t test (when $P<0.05$ in the F test). Survival rates were analyzed with the log-rank test. Values of $P<0.05$ were considered statistically significant.

Results

DCM Model Mouse

Because there are few useful DCM mouse models, we first generated transgenic mice that expressed a cardiac α-actin R312H mutant with an HA tag under the control of α-myosin heavy chain promoter (mActin-Tg). We obtained 3 independent founders of the transgenic mice (lines 301, 307, and 311). The protein levels of the cardiac α-actin R312H mutant were 1.6-fold in line 301, 3.3-fold in line 307, and 2.2-fold in line 311 compared with those of endogenous cardiac α-actin (Figure IA in the online-only Data Supplement). To confirm the expression of the transgene in cardiomyocytes, we performed immunohistological analyses with antibodies against HA and actinin. The mutated cardiac α-actin protein was colocalized with actinin, suggesting that the cardiac α-actin R312H mutant is incorporated into myofilaments (Figure IB in the online-only Data Supplement). Cardiac systolic function was decreased in mActin-Tg mice at 10 months of age, and the reduction was well correlated with protein levels of the cardiac α-actin R312H mutant (Figure IC in the online-only Data Supplement). To further investigate whether cardiac expression of the cardiac α-actin R312H mutant led to heart failure, we examined another transgenic mouse that expressed cardiac α-actin A331P mutant with an HA tag in the heart. This mutant has been reported to cause hypertrophic cardiomyopathy in human.[19] We obtained 2 independent founders of the transgenic mice that expressed almost the same levels of the cardiac α-actin A331P mutant protein. Although the protein levels of the mutant in the A331P mutant transgenic mice were almost same as those of the R312H mutant in line 307, which had the highest expression (Figure II in the online-only Data Supplement), echocardiography revealed that there were no significant differences in cardiac systolic function, wall thickness, and left ventricular dimension between cardiac α-actin A331P mutant transgenic mice and their wild-type littermates (Table I in the online-only Data Supplement). Although it is not known at present why the expression of cardiac α-actin A331P mutant did not induce hypertrophic cardiomyopathy, these results suggest that cardiac dysfunction of mActin-Tg mice is due to cardiac expression of the cardiac α-actin R312H mutant in the heart, not to high-level expression of the cardiac α-actin protein with the tag (lines 307 and 311).

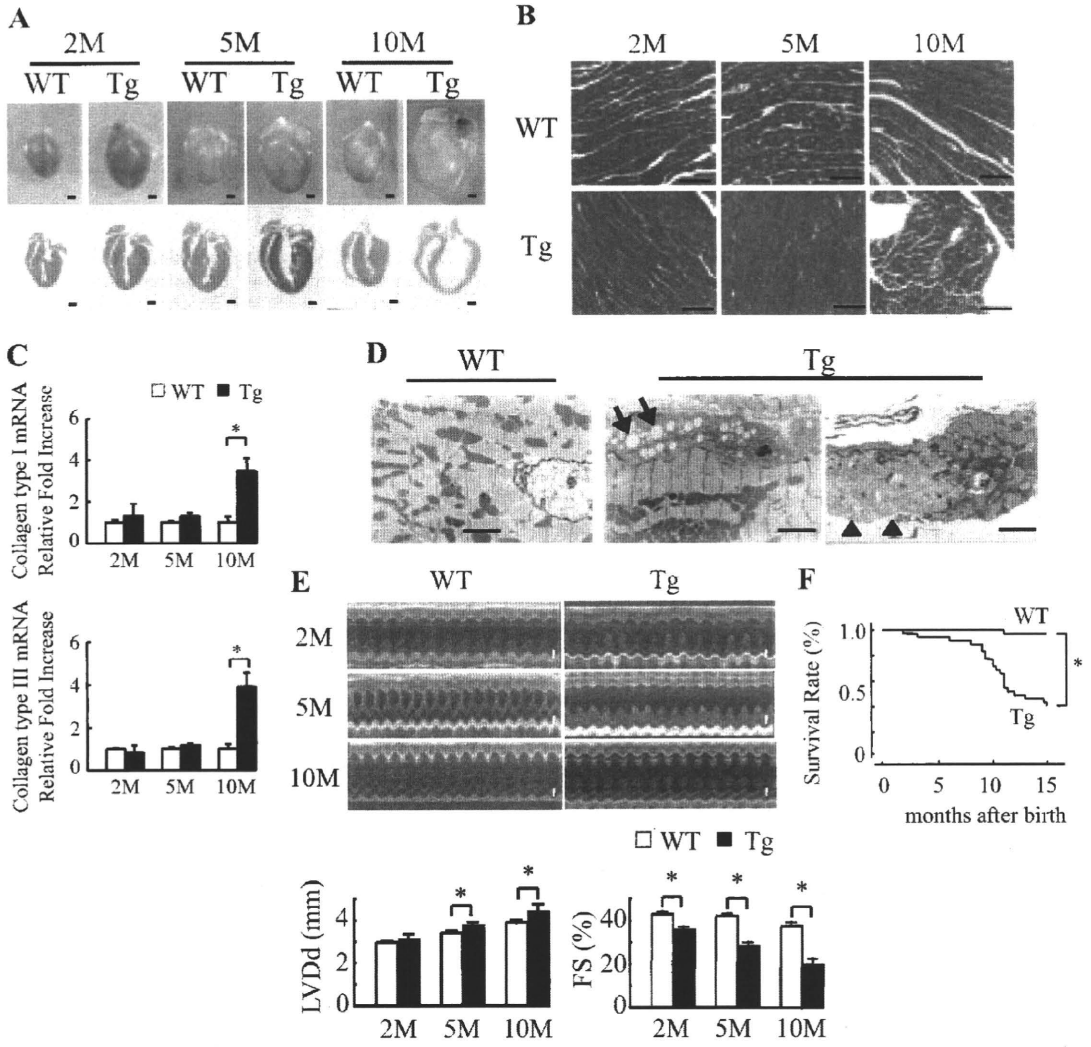

Figure 1. Mutated cardiac α-actin R312H transgenic mice. A, Gross morphology (top) and sections (bottom) of wild-type littermates (WT) or mActin-Tg (Tg) hearts at 2, 5, and 10 months (M) of age. Scale bar=1 mm. B, Masson trichrome staining. Scale bar=100 μm. C, Relative levels of collagen types I and III in hearts were normalized to GAPDH expression. *$P<0.05$ vs WT mice. n=4 in each group. D, Electron microscopic analyses. Cytoplasmic vacuolization (arrow) and lysis of myofibrils (arrowhead) were detected in the hearts of Tg mice. Scale bar=10 μm. E, Echocardiographic analysis. Scale bar=1 mm. LVDd indicates left ventricular end-diastolic dimension; FS, fractional shortening. *$P<0.05$. F, Kaplan-Meier survival curve. *$P<0.05$ vs WT mice. WT, n=32; Tg, n=37.

We used line 307, which expressed the cardiac α-actin R312H mutant at the highest levels, for further studies. The hearts in mActin-Tg mice were larger than those of wild-type littermates (Figure 1A), and heart weight and the ratio of heart weight to body weight were much increased in mActin-Tg mice (Table II in the online-only Data Supplement). Marked cardiac fibrosis was observed in mActin-Tg mice at 10 months of age, with increased expression of collagen types I and III (Figure 1B and 1C). Electron microscopic analyses showed that there were degenerated cardiomyocytes with an increase in vacuolar formation and lysis of myofibrils in mActin-Tg mice (Figure 1D). Echocardiography revealed that left ventricular dimension was gradually increased and that fractional shortening was reduced in mActin-Tg mice compared with wild-type littermates (Table II in the online-only Data Supplement and Figure 1E). The expression levels of ANP and SERCA2a were gradually increased and decreased in mActin-Tg mice, respectively (Figure III in the online-only Data Supplement). There was no significant difference in blood pressure, but heart rate was increased in mActin-Tg mice (Table II in the online-only Data Supplement), suggesting that the sympathetic nervous system is activated. Surface ECG monitoring showed low amplitude of the R wave in mActin-Tg mice (Table II in the online-only Data Supplement), which is often observed in human DCM patients. Many mActin-Tg mice died by 35 weeks of age (Figure 1F). Although telemetric ECG recording did not show life-threatening arrhythmia in mActin-Tg mice (data not shown), spontaneous Ca^{2+} sparks and Ca^{2+} waves were significantly increased in the cardiomyocytes of mActin-Tg mice (Table III in the online-only Data Supplement), suggesting that not only cardiac pump failure but also arrhythmia could be the cause of death. These phenotypes of mActin-Tg mice were quite similar to those of human DCM.

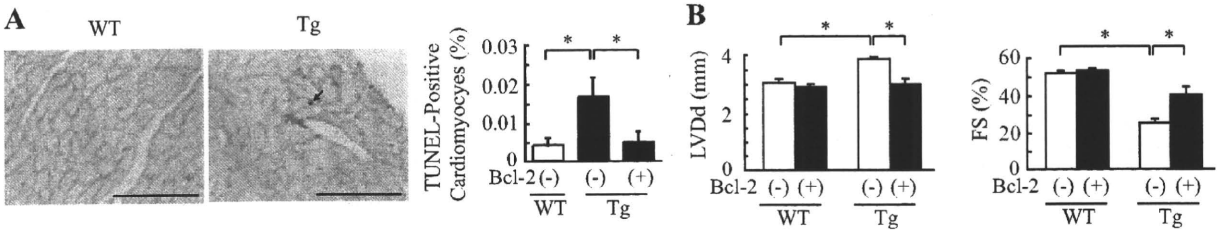

Figure 2. Increase in Bcl-2 preserves cardiac function in mActin-Tg mice. A, Double immunostaining for TUNEL (black) and dystrophin (red) of the heart (left). The graph indicates quantitative analyses of TUNEL-positive cardiomyocytes. Scale bar=100 μm. n=4 in each group. *$P<0.05$. B, Echocardiographic analyses at 5 months of age. *$P<0.05$. WT/Bcl-2(−), n=5; WT/Bcl-2(+), n=10; Tg/Bcl-2(−), n=10; Tg/Bcl-2(+), n=5. WT indicates wild-type littermates; Tg, mActin-Tg mice; LVDd, left ventricular end-diastolic dimension; and FS, fractional shortening.

Apoptotic Cardiomyocytes Are Increased in mActin-Tg Hearts

It has been reported that apoptosis of cardiomyocytes is observed in hearts of human DCM[10] and that cardiomyocyte death might cause cardiac dysfunction.[20] We thus examined apoptosis of cardiomyocytes by TUNEL labeling in left ventricular sections of wild-type littermates and mActin-Tg mice at 5 months of age. The number of TUNEL/dystrophin double-positive cardiomyocytes was significantly larger in mActin-Tg mice compared with wild-type littermates (Figure 2A). To examine whether the increase in apoptotic cardiomyocytes causes cardiac dysfunction in mActin-Tg mice, we generated double-transgenic mice by crossing mActin-Tg mice and the transgenic mice, which overexpress the antiapoptotic protein Bcl-2 in cardiomyocytes [mActin(+)/Bcl-2(+)-DTg].[14] The number of apoptotic cardiomyocytes in mActin(+)/Bcl-2(+)-DTg mice was significantly less compared with mActin-Tg mice (Figure 2A). Echocardiography revealed that the left ventricular dimension was smaller and fractional shortening was better in mActin(+)/Bcl-2(+)-DTg mice than in mActin-Tg mice at 5 months of age (Figure 2B), suggesting that the increase in apoptotic cardiomyocytes causes cardiac dysfunction in the DCM mouse model.

p53 Is Involved in Cardiomyocyte Apoptosis in mActin-Tg Mice

To clarify the mechanisms of how the cardiac α-actin R312H mutant induces apoptosis of cardiomyocytes, we examined expression levels of apoptosis-related proteins by Western blot analyses. The protein levels of p53 and Bax were higher in mActin-Tg mice compared with wild-type littermates (Figure 3A). Several key proapoptotic genes have been reported to be positively regulated by p53,[21] and increased expression of p53 induces left ventricular dilatation and dysfunction in several types of mice.[22,23] To determine the role of p53 in gene mutation–induced DCM, we crossed mActin-Tg mice and heterozygous p53-deficient mice [p53(+/−)]. Because many of homozygous p53-deficient mice [p53(−/−)] died of tumors before 5 months of age,[17] we used heterozygous p53-deficient mice [p53(+/−)] for this study. Echocardiography revealed that left ventricular dimension was smaller and fractional shortening was better in mActin-Tg/p53(+/−) mice than in mActin-Tg/p53(+/+) mice at 5 months of age (Figure 3B). Loss of a single p53 allele attenuated the increase of Bax (Figure 3C) and reduced the number of apoptotic cardiomyocytes in mActin-Tg mice (Figure 3D). These results suggest that p53-induced cardiomyocyte apoptosis induces dilatation and dysfunction of the left ventricle in the DCM mouse model.

Myofilament Calcium Sensitivity Is Decreased and Calcium-Dependent Enzymes Are Activated in mActin-Tg Mice

Many gene mutations associated with DCM have been reported to induce the decrease of myofilament Ca^{2+} sensi-

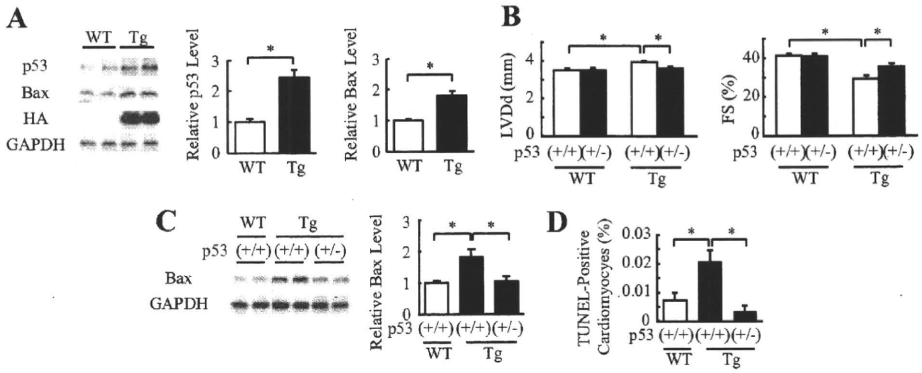

Figure 3. Inhibition of p53 preserves cardiac function in mActin-Tg mice. A, Western blot analyses in the hearts of wild-type littermates (WT) or mActin-Tg (Tg) mice at 5 months of age. The graph indicates relative protein levels of p53 (n=8 in each group) or Bax (n=10 in each group). *$P<0.05$. B, Echocardiographic analyses at 5 months of age. WT/p53(+/+), n=12; WT/p53(+/−), n=10; Tg/p53(+/+), n=19; Tg/p53(+/−), n=14. *$P<0.05$. C, Western blot analyses in the hearts. The graph indicates relative protein levels of Bax. n=6 in each group. *$P<0.05$. D, Quantitative analyses of TUNEL-positive cardiomyocytes. n=5 in each group. *$P<0.05$.

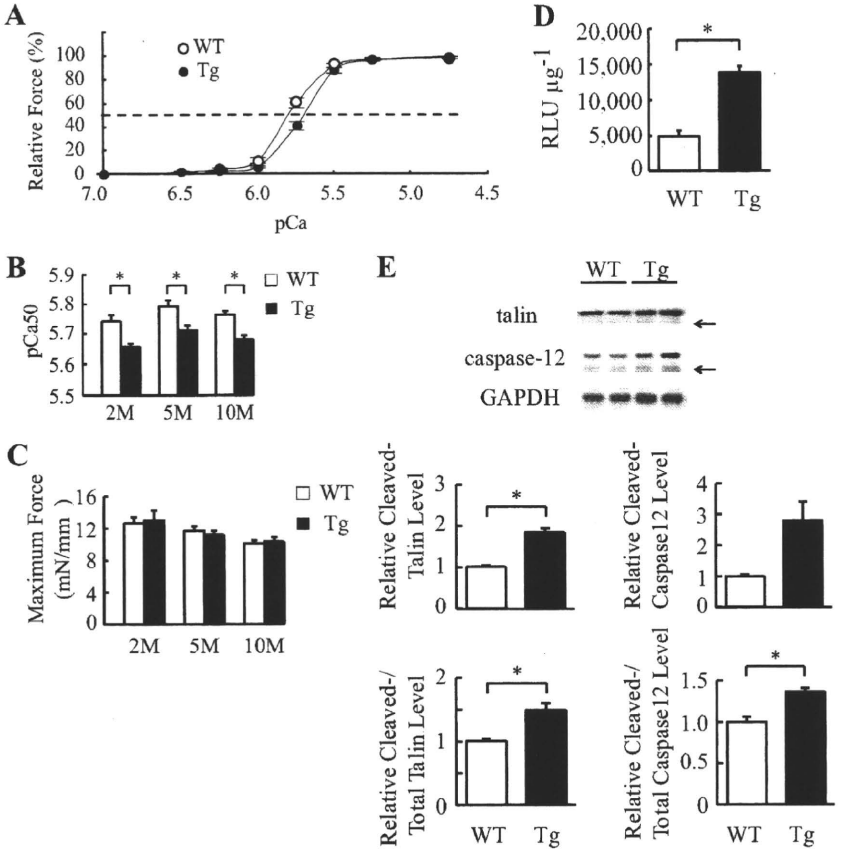

Figure 4. Myofilament Ca^{2+} sensitivity is decreased and Ca^{2+}-dependent enzymes are activated in mActin-Tg mice (Tg). A, Force-pCa relationship in skinned cardiac muscle fiber at 5 months of age. The broken line indicates pCa50. Wild-type (WT; n=11) and Tg (n=10) fibers were prepared from 3 isolated hearts. B, Ca^{2+} sensitivity (pCa50) of force-pCa relationships in skinned cardiac muscle fibers at 2, 5, and 10 months (M) of age. *P<0.05. C, Maximum force-generating capabilities. Fibers (n=9 to 11) were prepared from 3 isolated hearts of each group. D, The NFAT–luciferase reporter activity (RLU μg^{-1}) in the hearts at 5 months of age. n=4 in each group. *P<0.05. E, Western blot analyses in the hearts. Arrows indicate the calpain cleaved forms of talin and caspase-12. The graph indicates relative protein levels of cleaved talin or caspase-12 and ratio of cleaved forms to total proteins. n=4 in each group. *P<0.05.

tivity.[7] We examined myofilament Ca^{2+} sensitivity in mActin-Tg mice. The force-pCa relationship was shifted rightward in mActin-Tg mice compared with wild-type littermates (Figure 4A). The pCa value at half-maximal force generation (pCa50, an index of Ca^{2+} sensitivity) was significantly lower in mActin-Tg mice (Figure 4B), suggesting that skinned cardiac muscle fibers prepared from mActin-Tg mice show a decrease in Ca^{2+} sensitivity of force generation. The degree was the same between 2 and 10 months of age (Figure 4B), suggesting that the reduction in Ca^{2+} sensitivity is not a result of cardiac dysfunction. Despite the reduced Ca^{2+} sensitivity, there was no significant difference in maximum force-generating capabilities between wild-type littermates and mActin-Tg mice (Figure 4C). The decrease in myofilament Ca^{2+} sensitivity is expected to influence intracellular Ca^{2+} handling in cardiomyocytes of mActin-Tg mice. To clarify whether intracellular Ca^{2+} levels in cardiomyocytes are changed in mActin-Tg mice, we examined the activity of Ca^{2+}-dependent enzymes such as calcineurin and calpain. We generated double-transgenic mice by crossing mActin-Tg mice and the transgenic mice carrying a luciferase reporter driven by a cluster of NFAT binding sites, which is activated by calcineurin-dependent NFAT proteins.[15] The NFAT–luciferase reporter activity was higher in mActin-Tg mice than in wild-type littermates at 5 months of age (Table IV in the online-only Data Supplement and Figure 4D). Furthermore, the ratio of the calpain-induced cleaved forms of talin and caspase-12 to total proteins was significantly increased in mActin-Tg mice compared with wild-type littermates (Figure 4E). We next examined Ca^{2+} transients in cardiomyocytes using fluo-3AM (Figure IVA in the online-only Data Supplement). Although the time to peak amplitude of Ca^{2+} was significantly slower in mActin-Tg mice than in wild-type littermates (Figure IVB in the online-only Data Supplement), there was no significant difference in peak amplitude between wild-type littermates and mActin-Tg mice at 2 and 10 months of age (Figure IVC in the online-only Data Supplement). The expression levels of SERCA2a, but not Na^+/Ca^{2+} exchanger, were decreased in mActin-Tg mice (Figure III in the online-only Data Supplement).

CaMKIIδ Is Activated in mActin-Tg Mice

It has been reported that among Ca^{2+}-dependent proteins, expression of CaMKIIδ is increased in human DCM hearts[24] and that overexpression of CaMKIIδ induces heart failure in mice.[25,26] We thus examined the expression and phosphorylation of CaMKIIδ and phosphorylation of its target protein, phospholamban (Thr17). The protein levels of total (both CaMKIIδB and CaMKIIδC) and phosphorylated CaMKIIδ and of phosphorylated phospholamban (Thr17) were increased in mActin-Tg mice compared with wild-type littermates (Figure 5A and Figure VA in the online-only Data Supplement), suggesting that CaMKIIδ is activated in mActin-Tg mice. The protein levels of phosphorylated phospholamban (Ser16), which is activated by protein kinase A, were also increased in mActin-Tg mice (Figure 5A).

Because it has been reported that the sympathetic nervous system is activated in failing hearts and that β-adrenergic

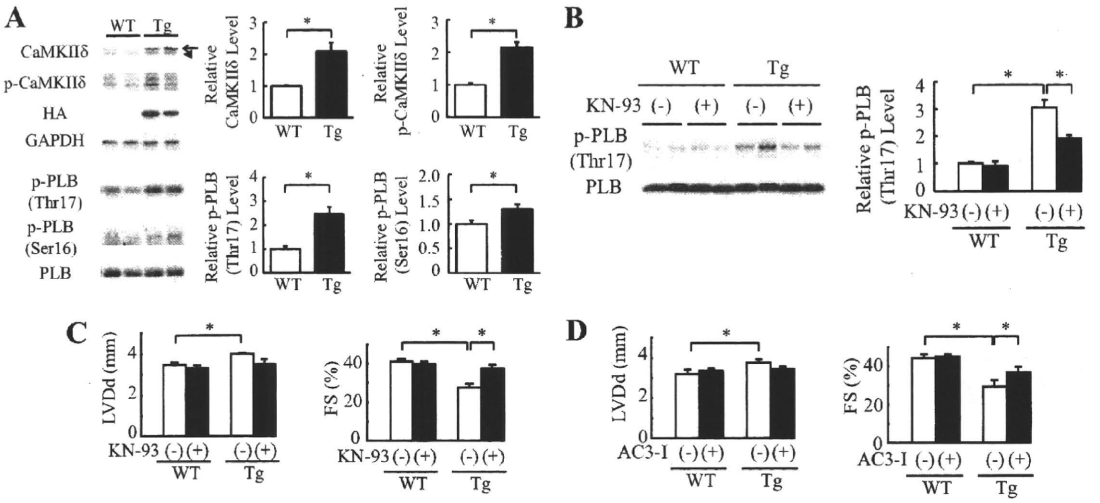

Figure 5. CaMKIIδ is activated in mActin-Tg mice. A, Western blot analyses in the hearts of wild-type littermates (WT) or mActin-Tg (Tg) mice at 5 months of age. The graph indicates relative protein levels of total and phosphorylated CaMKIIδ (p- CaMKIIδ) or phosphorylated phospholamban (p-PLB). Arrow and arrowhead indicate CaMKIIδB and CaMKIIδC, respectively. n=6 in each group. *P<0.05. B, Western blot analyses in the hearts at 5 months of age. The graph indicates relative protein levels of p-PLB (Thr17). n=4 in each group. *P<0.05. C and D, Echocardiographic analyses at 5 months of age. WT/KN-93(−), n=11; WT/KN-93(+), n=7; Tg/KN-93(−), n=8; Tg/KN-93(+), n=6; WT/AC3-I(−), n=8; WT/AC3-I(+), n=18; Tg/AC3-I(−), n=10; Tg/AC3-I(+), n=14. KN indicates KN-93; LVDd, left ventricular end-diastolic dimension; and FS, fractional shortening. *P<0.05.

receptor signal activates CaMKIIδ,[27] we treated mActin-Tg mice with the β-blocker bisoprolol to clarify the relationship between β-adrenergic receptor signal and activation of CaMKIIδ. The treatment with bisoprolol ameliorated cardiac dysfunction of mActin-Tg mice, and there was no significant difference in cardiac function between wild-type littermates and mActin Tg mice with bisoprolol treatment (Figure VB in the online-only Data Supplement). Furthermore, the increase in CaMKIIδ levels in mActin-Tg mice was prevented by bisoprolol treatment (Figure VC in the online-only Data Supplement), suggesting that the activation of CaMKIIδ in mActin-Tg mice might be due to activation of β-adrenergic receptor signaling.

To test whether activation of CaMKIIδ induces cardiac dysfunction, we first treated mActin-Tg mice with KN-93, a CaMKII inhibitor. Levels of both phosphorylated phospholamban (Thr17) and phospholamban (Ser16) were decreased by KN-93 treatment in mActin-Tg mice (Figure 5B and Figure VD in the online-only Data Supplement). Echocardiography revealed that KN-93 treatment prevented left ventricular dilatation and preserved cardiac function in mActin-Tg mice (Figure 5C). On the other hand, KN-92, an inactive derivative of KN-93, did not show any effects (Figure VE in the online-only Data Supplement). To confirm the role of CaMKIIδ in mActin-Tg mice, we crossed mActin-Tg mice and AC3-I mice, which expressed the CaMKII-inhibitory peptide AC3-I in the heart [mActin(+)/AC3-I(+)-DTg].[16] Echocardiography revealed that fractional shortening was better in mActin(+)/AC3-I(+)-DTg mice than in mActin(+)/AC3-I(−)-Tg mice (Figure 5D), suggesting that the activation of CaMKIIδ in the DCM mouse model induces left ventricular dilatation and contractile dysfunction.

We next examined the relation between CaMKIIδ activation and p53. The increase in p53 was attenuated by treatment with KN-93 or overexpression of AC3-I (Figure 6A and Figure VIA in the online-only Data Supplement). Furthermore, KN-93 treatment inhibited the increase in Bax expression and TUNEL-positive cardiomyocytes (Figure 6A and 6B). It has been reported that CaMKIIδC, but not CaMKIIδB, induces cardiomyocyte death.[27–29] To clarify the mechanism of how CaMKIIδ increases protein levels of p53 and which

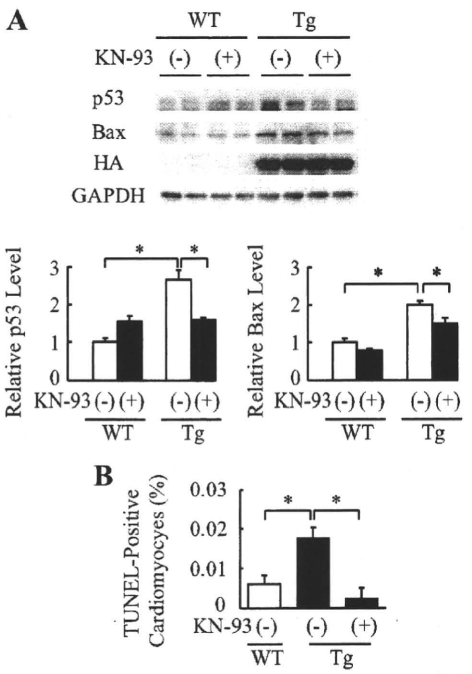

Figure 6. CaMKIIδ regulates expression of p53 in cardiomyocytes. A, Western blot analyses in the hearts of wild-type littermates (WT) or mActin-Tg (Tg) mice. The graph indicates relative protein levels of p53 or Bax. n=4 in each group. *P<0.05. B, Quantitative analyses of TUNEL-positive cardiomyocytes. n=5 in each group. *P<0.05.

CaMKIIδ, δB or δC, plays an important role in apoptosis of cardiomyocytes, we transfected constitutively active forms of CaMKIIδ (caCaMKIIδ) into cardiomyocytes. Only caCaMKIIδC, not caCaMKIIδB, increased protein levels of p53 (Figure VIB in the online-only Data Supplement). Furthermore, p53 protein levels in caCaMKIIδC-transfected cardiomyocytes did not increase with MG132 treatment compared with MOCK-treated cardiomyocytes (Figure VIC in the online-only Data Supplement). These results suggest that activation of CaMKIIδC increases apoptotic cardiomyocytes at least in part via stabilization of p53 in the DCM mouse model.

Discussion

In the present study, we established a novel mouse model of DCM to clarify the mechanisms of how mutant genes lead to DCM (Table II in the online-only Data Supplement and Figure 1). The mice expressing cardiac α-actin R312H mutant in the heart, which has been reported to cause DCM in humans,[5] showed dilatation and dysfunction of left ventricle with an increase in ANP messenger RNA levels, which is consistent with human heart failure (Figure 1A and 1E and Table II and Figure III in the online-only Data Supplement). Higher heart rate and hyperphosphorylated phospholamban (Ser16) (Table II in the online-only Data Supplement and Figure 5A) suggest the activation of the sympathetic nervous system to compensate for reduced cardiac systolic function, resulting in an increase in spontaneous Ca^{2+} sparks and Ca^{2+} waves (Table III in the online-only Data Supplement). Myofilament Ca^{2+} sensitivity was decreased in mActin-Tg mice even at 2 months of age (Figure 4B), when cardiac phenotypes such as left ventricular dilatation and cardiac fibrosis were not recognized (Table II in the online-only Data Supplement and Figure 1). These results suggest that the decrease in myofilament Ca^{2+} sensitivity is a primary cause of, not a secondary result from, cardiac dysfunction. Because these phenotypes were quite similar to those of human DCM, mActin-Tg mice are useful for examining the underlying mechanisms of how gene mutations lead to DCM.

There was no significant difference in the peak amplitude of Ca^{2+} transients between wild-type littermates and mActin-Tg mice (Figure IVC in the online-only Data Supplement), suggesting that global Ca^{2+} levels underlying each contractile cycle do not differ between the 2 groups. It has been reported that the peak amplitude of Ca^{2+} transients, which is associated with decreased Ca^{2+} sensitivity and systolic dysfunction, is higher in another mouse model of DCM, [7] suggesting that Ca^{2+} transients are augmented to compensate for decreased myofilament Ca^{2+} sensitivity in this model. In mActin-Tg mice, despite the preserved Ca^{2+} transients (Figure IVC in the online-only Data Supplement), global cardiac function was gradually impaired (Table II in the online-only Data Supplement). Local Ca^{2+} concentration has been reported to be important for the activation of Ca^{2+}-dependent enzymes such as calcineurin, calpain, and CaMKII in cardiomyocytes.[30] The activation of these molecules in mActin-Tg mice (Figures 4D, 4E, and 5A) might be attributed to an increase in local Ca^{2+} levels. It still remains to be determined whether local Ca^{2+} levels are really increased and, if so, how the decrease in Ca^{2+} sensitivity increases local Ca^{2+} levels.

Recent reports have shown that CaMKIIδ plays a crucial role in cardiovascular diseases.[16,31] The transgenic mice that overexpressed CaMKIIδ showed heart failure with systolic dysfunction and left ventricular dilatation.[25,26] In this study, CaMKIIδ was activated in the hearts of mActin-Tg mice (Figure 5A), and inhibition of CaMKIIδ by KN-93 or AC3-I ameliorated cardiac dysfunction in mActin-Tg mice (Figure 5C and 5D), suggesting that CaMKIIδ also plays an important role in gene mutation–induced cardiac dysfunction.

It has been reported that apoptosis of cardiomyocytes is observed in hearts of human DCM[10] and that cardiomyocyte death could cause cardiac dysfunction.[20] However, it remains unclear whether apoptosis of cardiomyocytes causes cardiac dysfunction and how cardiomyocyte apoptosis is induced in hearts of DCM. In this study, there were more apoptotic cardiomyocytes in mActin-Tg mice (Figure 2A), and cardiac function was improved by protecting cardiomyocytes from apoptosis through overexpression of Bcl-2 (Figure 2B). These results suggest that cardiomyocyte apoptosis plays a crucial role in the development of DCM. Several key proapoptotic and antiapoptotic genes have been reported to be positively or negatively regulated by p53, and increased expression of p53 induces left ventricular dilatation and dysfunction in mice deficient in MDM4, an E3 ligase for p53.[23] Furthermore, we have recently demonstrated that p53 is critically involved in pressure overload–induced cardiac dysfunction.[22] The protein levels of p53 were increased in mActin-Tg mice (Figure 3A), and loss of a single p53 allele reduced the number of apoptotic cardiomyocytes (Figure 3D) and improved cardiac function (Figure 3B). These results suggest that p53 is critically involved in induction of cardiomyocyte apoptosis, resulting in left ventricular dysfunction in the mouse model of DCM.

The present study indicates that p53 might be a therapeutic target for DCM. In this study, CaMKIIδ was activated in the hearts of mActin-Tg mice (Figure 5A), and the inhibition of CaMKIIδ attenuated the increase in p53 protein levels (Figure 6A and Figure VIA in the online-only Data Supplement), suggesting that CaMKIIδ regulates protein levels of p53 in the DCM model mice. Although it remains to be determined how CaMKIIδ regulates protein levels of p53, inhibition of CaMKIIδ may become a new therapeutic strategy for DCM patients by reducing p53 protein levels in the heart.

Limitations

This study has a couple limitations. First, we cannot completely rule out the nonspecific effects of overexpression of cardiac α-actin gene with tag because of a lack of transgenic mice that overexpress wild-type cardiac α-actin gene. However, we think the cardiac dysfunction observed in mActin-Tg was due to cardiac expressions of the cardiac α-actin R312H mutant in the heart, not to high-level expressions of the cardiac α-actin protein with tag because of the following reasons: We obtained 3 independent founders of the transgenic mice, and the reduction in cardiac function was well correlated with protein levels of the cardiac α-actin R312H mutant (Figure I in the online-only Data Supplement). An-

other transgenic mouse that expressed cardiac α-actin A331P mutant with an HA tag in the heart did not show cardiac dysfunction (Table I in the online-only Data Supplement), although the protein levels of the mutant in the A331P mutant transgenic mice were almost same as those of the R312H mutant in line 307, which had the highest expression (Figure II in the online-only Data Supplement). Second, we found that CaMKIIδC increases p53 protein levels mainly by its stabilization, but the underlying mechanisms remain to be determined.

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Disclosures

Dr Anderson is named on patents claiming to treat heart failure by CaMKII inhibition and is a cofounder of Allosteros. The other authors report no conflicts.

References

1. Michels VV, Moll PP, Miller FA, Tajik AJ, Chu JS, Driscoll DJ, Burnett JC, Rodeheffer RJ, Chesebro JH, Tazelaar HD. The frequency of familial dilated cardiomyopathy in a series of patients with idiopathic dilated cardiomyopathy. *N Engl J Med.* 1992;326:77–82.
2. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, Moss AJ, Seidman CE, Young JB. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association scientific statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. *Circulation.* 2006;113:1807–1816.
3. Ahmad F, Seidman JG, Seidman CE. The genetic basis for cardiac remodeling. *Annu Rev Genomics Hum Genet.* 2005;6:185–216.
4. Knoll R, Hoshijima M, Hoffman HM, Person V, Lorenzen-Schmidt I, Bang ML, Hayashi T, Shiga N, Yasukawa H, Schaper W, McKenna W, Yokoyama M, Schork NJ, Omens JH, McCulloch AD, Kimura A, Gregorio CC, Poller W, Schaper J, Schultheiss HP, Chien KR. The cardiac mechanical stretch sensor machinery involves a Z disc complex that is defective in a subset of human dilated cardiomyopathy. *Cell.* 2002;111:943–955.
5. Olson TM, Michels VV, Thibodeau SN, Tai YS, Keating MT. Actin mutations in dilated cardiomyopathy, a heritable form of heart failure. *Science.* 1998;280:750–752.
6. Towbin JA, Bowles NE. Genetic abnormalities responsible for dilated cardiomyopathy. *Curr Cardiol Rep.* 2000;2:475–480.
7. Du CK, Morimoto S, Nishii K, Minakami R, Ohta M, Tadano N, Lu QW, Wang YY, Zhan DY, Mochizuki M, Kita S, Miwa Y, Takahashi-Yanaga F, Iwamoto T, Ohtsuki I, Sasaguri T. Knock-in mouse model of dilated cardiomyopathy caused by troponin mutation. *Circ Res.* 2007;101:185–194.
8. Kawada T, Masui F, Tezuka A, Ebisawa T, Kumagai H, Nakazawa M, Toyo-Oka T. A novel scheme of dystrophin disruption for the progression of advanced heart failure. *Biochim Biophys Acta.* 2005;1751:73–81.
9. Kyoi S, Otani H, Matsuhisa S, Akita Y, Tatsumi K, Enoki C, Fujiwara H, Imamura H, Kamihata H, Iwasaka T. Opposing effect of p38 MAP kinase and JNK inhibitors on the development of heart failure in the cardiomyopathic hamster. *Cardiovasc Res.* 2006;69:888–898.
10. Olivetti G, Abbi R, Quaini F, Kajstura J, Cheng W, Nitahara JA, Quaini E, Di Loreto C, Beltrami CA, Krajewski S, Reed JC, Anversa P. Apoptosis in the failing human heart. *N Engl J Med.* 1997;336:1131–1141.
11. Bulfield G, Siller WG, Wight PA, Moore KJ. X chromosome-linked muscular dystrophy (mdx) in the mouse. *Proc Natl Acad Sci USA.* 1984;81:1189–1192.
12. Cooper BJ, Winand NJ, Stedman H, Valentine BA, Hoffman EP, Kunkel LM, Scott MO, Fischbeck KH, Kornegay JN, Avery RJ, Williams JR, Schmickel RD, Sylvester JE. The homologue of the Duchenne locus is defective in X-linked muscular dystrophy of dogs. *Nature.* 1988;334:154–156.
13. Nigro V, Okazaki Y, Belsito A, Piluso G, Matsuda Y, Politano L, Nigro G, Ventura C, Abbondanza C, Molinari AM, Acampora D, Nishimura M, Hayashizaki Y, Puca GA. Identification of the Syrian hamster cardiomyopathy gene. *Hum Mol Genet.* 1997;6:601–607.
14. Tanaka M, Nakae S, Terry RD, Mokhtari GK, Gunawan F, Balsam LB, Kaneda H, Kofidis T, Tsao PS, Robbins RC. Cardiomyocyte-specific Bcl-2 overexpression attenuates ischemia-reperfusion injury, immune response during acute rejection, and graft coronary artery disease. *Blood.* 2004;104:3789–3796.
15. Wilkins BJ, Dai YS, Bueno OF, Parsons SA, Xu J, Plank DM, Jones F, Kimball TR, Molkentin JD. Calcineurin/NFAT coupling participates in pathological, but not physiological, cardiac hypertrophy. *Circ Res.* 2004;94:110–118.
16. Zhang R, Khoo MS, Wu Y, Yang Y, Grueter CE, Ni G, Price EE Jr, Thiel W, Guatimosim S, Song LS, Madu EC, Shah AN, Vishnivetskaya TA, Atkinson JB, Gurevich VV, Salama G, Lederer WJ, Colbran RJ, Anderson ME. Calmodulin kinase II inhibition protects against structural heart disease. *Nat Med.* 2005;11:409–417.
17. Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA Jr, Butel JS, Bradley A. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature.* 1992;356:215–221.
18. VanGuilder HD, Vrana KE, Freeman WM. Twenty-five years of quantitative PCR for gene expression analysis. *Biotechniques.* 2008;44:619–626.
19. Olson TM, Doan TP, Kishimoto NY, Whitby FG, Ackerman MJ, Fananapazir L. Inherited and de novo mutations in the cardiac actin gene cause hypertrophic cardiomyopathy. *J Mol Cell Cardiol.* 2000;32:1687–1694.
20. Wencker D, Chandra M, Nguyen K, Miao W, Garantziotis S, Factor SM, Shirani J, Armstrong RC, Kitsis RN. A mechanistic role for cardiac myocyte apoptosis in heart failure. *J Clin Invest.* 2003;111:1497–1504.
21. Ryan KM, Phillips AC, Vousden KH. Regulation and function of the p53 tumor suppressor protein. *Curr Opin Cell Biol.* 2001;13:332–337.
22. Sano M, Minamino T, Toko H, Miyauchi H, Orimo M, Qin Y, Akazawa H, Tateno K, Kayama Y, Harada M, Shimizu I, Asahara T, Hamada H, Tomita S, Molkentin JD, Zou Y, Komuro I. p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload. *Nature.* 2007;446:444–448.
23. Xiong S, Van Pelt CS, Elizondo-Fraire AC, Fernandez-Garcia B, Lozano G. Loss of Mdm4 results in p53-dependent dilated cardiomyopathy. *Circulation.* 2007;115:2925–2930.
24. Hoch B, Meyer R, Hetzer R, Krause EG, Karczewski P. Identification and expression of delta-isoforms of the multifunctional Ca2+/calmodulin-dependent protein kinase in failing and nonfailing human myocardium. *Circ Res.* 1999;84:713–721.
25. Zhang T, Johnson EN, Gu Y, Morissette MR, Sah VP, Gigena MS, Belke DD, Dillmann WH, Rogers TB, Schulman H, Ross J Jr, Brown JH. The cardiac-specific nuclear delta(B) isoform of Ca2+/calmodulin-dependent protein kinase II induces hypertrophy and dilated cardiomyopathy associated with increased protein phosphatase 2A activity. *J Biol Chem.* 2002;277:1261–1267.

26. Zhang T, Maier LS, Dalton ND, Miyamoto S, Ross J Jr, Bers DM, Brown JH. The deltaC isoform of CaMKII is activated in cardiac hypertrophy and induces dilated cardiomyopathy and heart failure. *Circ Res.* 2003;92:912–919.
27. Zhu WZ, Wang SQ, Chakir K, Yang D, Zhang T, Brown JH, Devic E, Kobilka BK, Cheng H, Xiao RP. Linkage of beta1-adrenergic stimulation to apoptotic heart cell death through protein kinase A-independent activation of Ca2+/calmodulin kinase II. *J Clin Invest.* 2003;111:617–625.
28. Peng W, Zhang Y, Zheng M, Cheng H, Zhu W, Cao CM, Xiao RP. Cardioprotection by CaMKII-deltaB is mediated by phosphorylation of heat shock factor 1 and subsequent expression of inducible heat shock protein 70. *Circ Res.* 2010;106:102–110.
29. Zhu W, Woo AY, Yang D, Cheng H, Crow MT, Xiao RP. Activation of CaMKIIdeltaC is a common intermediate of diverse death stimuli-induced heart muscle cell apoptosis. *J Biol Chem.* 2007;282:10833–10839.
30. Wu X, Zhang T, Bossuyt J, Li X, McKinsey TA, Dedman JR, Olson EN, Chen J, Brown JH, Bers DM. Local InsP3-dependent perinuclear Ca2+ signaling in cardiac myocyte excitation-transcription coupling. *J Clin Invest.* 2006;116:675–682.
31. Ling H, Zhang T, Pereira L, Means CK, Cheng H, Gu Y, Dalton ND, Peterson KL, Chen J, Bers D, Heller Brown J. Requirement for Ca2+/calmodulin-dependent kinase II in the transition from pressure overload-induced cardiac hypertrophy to heart failure in mice. *J Clin Invest.* 2009;119:1230–1240.

CLINICAL PERSPECTIVE

Heart failure is an important cause of morbidity and mortality in many industrial countries, and dilated cardiomyopathy (DCM) is one of its major causes. Molecular genetic studies over the last 2 decades have revealed many mutations of various genes in DCM patients, but the precise mechanisms of how such mutations lead to DCM remain largely unknown partly because of a lack of good animal models of DCM. Here, we established the mouse model of DCM by expressing a mutated cardiac α-actin gene, which has been reported in patients with DCM, in the heart. The transgenic mice showed gradual dilatation and dysfunction of the left ventricle, resulting in death by heart failure. These phenotypes of the transgenic mice were quite similar to those of human DCM. The number of apoptotic cardiomyocytes and protein levels of p53 were increased in the hearts of the DCM mice. Overexpression of Bcl-2, an antiapoptotic factor, or downregulation of p53 decreased the number of apoptotic cardiomyocytes and improved cardiac function. The DCM mice showed activation of CaMKIIδ. The inhibition of CaMKIIδ prevented the increase in p53 and apoptotic cardiomyocytes and ameliorated cardiac function. These results suggest that CaMKIIδ plays a critical role in the development of heart failure in part by accumulation of p53 and induction of cardiomyocyte apoptosis in the DCM mouse model. The inhibition of CaMKIIδ may become a new therapeutic strategy for DCM patients.