vol.3

豊岡照彦編集委員が語る

MMJは2015年創刊10周年を迎える。この10年間の医療の歩みについて、6人の編集委員が各回1テーマで概観する。

循環器病の診断と治療の10年

東京大学名誉教授　豊岡照彦

とよおか・てるひこ　1945年生まれ。72年東京大学医学部卒、同病院内科系研修医などを経て、76年東京都立臨床医学研究所非常勤研究員。78年米カリフォルニア大学留学。西独ハイデルベルク大学客員教授、自治医科大学講師などを経て、東京大学教授。MMJでは2002年編集委員。

遺伝子解析の驚異的進歩

過去10年間、循環器領域では遺伝子解析を中心に診断や治療の開発が驚異的な進歩を遂げた。2005～2014年のノーベル医学生理学賞は、RNA干渉の発見による遺伝子ノックアウト(06年)、胚性幹細胞を用いた遺伝子改変動物による発現蛋白の機能解析(07年)、telomereによる細胞老化(09年)から成人細胞にリプログラミングするiPS細胞作製(12年)――と、多くが遺伝子工学的手法を用いた研究に贈られた。基礎研究の臨床応用も急速に進んだ。血管内皮細胞増殖因子(VEGF)受容体の遺伝子クローニングによるアミノ酸配列決定によって作製されたヒト型モノクローナル抗体が、加齢黄斑変性や糖尿病性網膜症などの血管病治療に結実した。

循環器系は他領域と比較して、遺伝性疾患が少ないと誤解されていた。しかし、循環器疾患と直結する内臓肥満、高血圧、高脂血症、糖尿病の4疾患に代表される代謝(性)症候群(メタボ)は罹患者数が多く、家族的な負荷も強い。分析が複雑で環境要因の影響が強いと推定されたが、解析が急速に進んだ。研究対象も最近は複数遺伝子が絡む「稀(rare)な単一性遺伝子疾患(monogenic)から高頻度な(common)多遺伝子性(polygenic)疾患へ」移行しつつある。

膨大な遺伝子配列の決定を目指し、1990年に始まった日米欧共同のヒトゲノム計画は、high throughputのmicroarray、高速演算機の急速な進歩と国際協力に支えられ、2004年に終了宣言された。その後の10年は配列情報を基に単一塩基多型(single nucleotide polymorphism, SNP)による関連遺伝子候補を対照群と網羅的に比較するGenome-Wide Association Study(GWAS)に向けられた。その成果の中で、メタボを中心に以下、年代順に概説する。

なお、SNPのχ^2検定による遺伝統計では、解析試料数が増すと指数的に危険率が減少するため、今回は国際的な診断基準に基づき、各群10万人以上のマス報告に限って、紹介する。現在、循環器関連領域で、これを超える解析は報告されていない。この解析法はピンポイントで責任SNP(locus)を明らかにするとは限らず、近隣部位を示す場合も多い。また、臨床的な重篤さとは別に多数集団の中で罹患者が少なければ、国際的な判定閾値($P<5\times10^{-8}$)に達せず、医学的に重要な変異が除外される危険もはらんでいる。これらのlociは責任遺伝子に加え、表現型、すなわち病態を増悪させ、逆に軽減する種々の修飾遺伝子(modifier gene)も含む。この点からGWASが総合的解析法として評価される所以である。

脂質、肥満、血圧関連遺伝子の同定

1. 血中脂質レベルの関連遺伝子解析(Teslovich, et al., *Nature*, 2010)

欧米中心の解析で、95のSNP部位(loci)が同定された。この中にはHDL、LDL等のコレステロール類と中性脂肪(TG)代謝に関連する遺伝子で既知のLDL受容体(LDLR)、アポリポ蛋白の一部(apo B、apo E)、食品や

薬物の代謝酵素(CYP7A)、コレステロールの吸収/排出関連蛋白(ABCG 5/8)が含まれる。本論文では予想以上のlocus数が報告されたことは、それぞれの寄与率は小さくても、複数の関連遺伝子の相乗効果で脂質代謝障害を来すことが示された。また、複数の脂質に関連する非特異的なlociが同定され、複合脂質代謝障害の臨床病態にも合致する。

2. 体格(BMI)などの肥満関連遺伝子(Speliotes, et al., *Nat-Genet*, 2010)

BMIに関しても興味ある報告が発表された。かつてBMIは家族や地域の食生活や嗜好品が類似することから、環境要因が大きいとされたが、実際は脂肪·肥満関連遺伝子(fat mass & obesity associated gene)やgastric inhibitory polypeptide受容体遺伝子(GIPR)など、新たに18部位を示し、計32 SNPに有意なlociが同定された。

インクレチンの一部であるGIPは、食品を視認した際に食欲中枢が刺激され、さらに食後血糖上昇時に十二指腸や小腸上部のk細胞から門脈に内分泌されて、膵臓のβ細胞表面に存在するGIP受容体(GIPR)から細胞内信号伝達系を介して、インスリン分泌を増加させるユニークな機能を持つ。この視覚→食欲中枢→k細胞→β細胞→インスリン分泌に至る多臓器間の血糖調節機能は、肥満の成立機序を説明する上で興味深い(Lock, et al., *Nature* 2015)。

3. 血圧制御遺伝子のマス遺伝子データ(Ehret, et al., *Nature*, 2011)

単純に見えた血圧関連の解析結果が最も遅れて、29カ所のSNPが最後に公表された。この研究には日本の貢献が特に多い。この結果は耳目を引く内皮細胞由来昇圧物質のendothelin-3(EDN3)に加え、ANPやBNP等のNa利尿ペプチドの受容体(NPR3)など降圧物質にも変異を認め、低圧系の遺伝子変異による機能喪失(loss-of-function, LOF)も示唆された。Endothelinと利尿ペプチドの発見やstatinの開発は日本でなされ、世界的にも注目される。

こうした内因性物質に加え、電位依存性チャネルCa^{2+} β_2(CACNB2)、α刺激性グアニンヌクレオチド結合蛋白(GNAS)や細胞内Ca^{2+}輸送酵素β_1(ATP2B1)などの心筋や血管平滑筋収縮を制御するCa^{2+}調節や細胞内信号伝達系に、T-box転写因子(TBX3)など発現蛋白用のmRNA量を左右する変異や、まだ機能不明蛋白の変異も同定され、血圧調節の奥深さを見せる。

4. 2型糖尿病のSNPの解析

上記のメタボを構成する3疾患に比べ、成人発症の2型糖尿病のSNP解析データは症例数が不足し、上記の判断基準を満たさなかった。これにはインスリン耐性、糖代謝やグルカゴン、インクレチンなど糖代謝のほかに、未発見の因子が多数残り、機能同定が不十分なことと、血管障害、神経系、腎障害など多彩な病態が複雑に影響することを反映している。

これらのSNPをそれぞれのメタボ構成疾患の間で有している事実は、メタボ特有の重複症状の多さの傍証でもある。今後、複数のSNPによる病態修飾と機能解析が望まれる。なお、一般的に遺伝子解析をexonに限ったexon解析では、家族性疾患の4分の1程度の関連SNPが同定されたに過ぎない(Yang, et al., *NEJM*, 2013)。今後、intronや非翻訳領域のlocusの相互作用も俯瞰する新たな遺伝統計用のalgorismが必要となる。また遺伝子配列が同じでも、発現量が変わりうるコピー数変異(CNV)、EpigeneticsやmicroRNA(miR)の重要さも示唆された。

画像診断、薬物治療の進展

画像診断の進歩も著しい。冠動脈疾患の予後判定には動脈石灰化像が重要だが、近年開発された非侵襲性64列冠動脈CTは従来の冠動脈造影に匹敵する感度·特異度を持ち、検査入院が不要となりつつある。ステントによる血管内治療も内科、外科の枠を越えた新たな治療領域として急速に発展している。対象も脳血管、腎血管性高血圧など全身に拡大し、薬剤溶出性ステント(DES)の登場により、再狭窄率が激減した。不整脈対策として、心房細動やWPW症候群のカテーテルアブレーション発作性頻脈の治療について再発予防には厳密な適用検討が求められる。

薬物治療も心房細動に伴う脳塞栓予防に抗血小板薬と抗凝固薬(ワルファリンなど)の比較、Ca拮抗薬、β遮断薬、アンジオテンシン変換酵素(ACE)阻害薬、アンジオテンシンII受容体遮断薬(ARB)や、究極のレニン拮抗薬やstatin類の選別について議論が深化した。最近は1人の患者が多種の疾患を合併する結果、他の薬剤や食品との併用問題も表面化している。

Association of Hypocalcemia With Mortality in Hospitalized Patients With Heart Failure and Chronic Kidney Disease

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ABSTRACT

Background: Chronic kidney disease—mineral and bone disorders (CKD-MBD) are associated with vascular calcification and abnormal electrolytes that lead to cardiovascular disease and mortality. CKD-MBD is identified by imbalances in serum calcium (Ca), phosphate, and parathyroid hormone (PTH). Although the relation of phosphate and PTH with the prognosis of HF patients has been reported, the association of Ca with prognosis in patients with heart failure (HF) and CKD remains unclear.

Methods and Results: We examined 191 patients admitted for HF and CKD (estimated glomerular filtration rate < 60 mL min^{-1} 1.73 m^{-2}), and they were divided into 2 groups based on levels of corrected Ca: low Ca (Ca < 8.4 mg/dL; n = 32) and normal-high Ca (8.4 ≦Ca; n = 159). We compared laboratory and echocardiographic findings, as well as followed cardiac and all-cause mortality. The low-Ca group had 1) higher levels of alkaline phosphatase (308.9 vs 261.0 U/L; $P = .026$), 2) lower levels of 1,25-dihydroxy vitamin D (26.1 vs 45.0 pg/mL; $P = .011$) and hydrogen carbonate (22.4 vs 24.5 mmol/L; $P = .031$), and 3) a tendency to have a higher PTH level (87.5 vs 58.6 pg/mL; $P = .084$). In contrast, left and right ventricular systolic function, estimated glomerular filtration rate, urine protein, phosphate, sodium, potassium, magnesium, and zinc did not differ between the 2 groups. In the Kaplan-Meier analysis, cardiac and all-cause mortality were significantly higher in the low-Ca group than in the normal-high—Ca group ($P < .05$). In the multivariable Cox proportional hazard analyses, hypocalcemia was an independent predictor of all-cause mortality in HF and CKD patients ($P < .05$).

Conclusions: Hypocalcemia was an independent predictor of all-cause mortality in HF and CKD patients. (*J Cardiac Fail 2015;21:621–627*)

Key Words: Heart failure, chronic kidney disease, chronic kidney disease-mineral and bone disorders, hypocalcemia, prognosis.

Heart failure (HF) is the major cause of death among the elderly in many countries. Chronic kidney disease (CKD) frequently coexists with HF and increases mortality and morbidity.[1] Recently, chronic kidney disease—mineral and bone disorders (CKD-MBD) have gained attention and have been associated with vascular calcification and abnormal electrolytes that lead to cardiovascular disease and mortality in patients, not only with hemodialysis but also with predialysis.[2,3] Mineral disorders are associated with abnormal bone metabolism,[4] progressive CKD,[5] cardiovascular events,[6] and mortality.[2,6–9] CKD-MBD is identified by imbalances in serum calcium (Ca), phosphate, and parathyroid hormone (PTH).[2]

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See page 626 for disclosure information.

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Furthermore, Ca plays an important role in myocardial contraction and relaxation.[10] Recently, serum Ca levels have been reported to be associated with left ventricular diastolic function in CKD patients[11] and left ventricular ejection fraction (LVEF) in HF patients.[12] Although the relationships of increased phosphate and PTH with the prognosis of HF patients[13–15] have been reported, little is known about the association of Ca levels with prognosis in patients with HF and CKD.[12,16] On the other hand, hypocalcemia progresses as CKD develops[3] and is assumed to cause systolic and diastolic dysfunction, elongation of QT interval, and increasing PTH, which may be associated with cardiac mortality (pump failure death and arrhythmic death) in HF and/or CKD. On another note, the renin-angiotensin-aldosterone system and sympathetic nervous activity are related to progressive HF and/or CKD, and are reported to be predictors of HF patients. The presence of other mineral disorders, including hyponatremia[16,17] and hypomagnesemia,[16,18] is common in HF patients with advanced HF.

Therefore, the aims of the present study were to investigate the association of hypocalcemia with 1) cardiac function, 2) electrocardiogram findings (QT interval), 3) PTH, 1,25-dihydroxy vitamin D [1,25$(OH)_2$D], and other mineral factors (phosphate, sodium, potassium, magnesium, and zinc), 4) neurohumoral factors (plasma noradrenalin, plasma renin activity, renin concentration, aldosterone, B-type natriuretic peptide), and 5) prognosis (cardiac and all-cause mortality).

Methods

Subjects and Study Protocol

This was a prospective observational study which enrolled consecutive symptomatic HF and CKD patients (CKD grade 3–5, defined as estimated glomerular filtration rate (eGFR) < 60 mL min^{-1} 1.73 m^{-2} according to the Modification of Diet in Renal Disease formula[19]) who were hospitalized to treat decompensated HF at Fukushima Medical University from 2009 to 2012 (Fig. 1). The diagnosis of decompensated HF was defined according to the Framingham criteria.[20] Patients with acute coronary syndrome, dialysis, and documented cancer were excluded (Fig. 1). Blood sample was obtained within the 1st 24 hours of admission. Patients were divided into 2 groups based on corrected Ca levels of this single measure (defined as measured [Ca + 0.8] × [4.0 − serum albumin]): low Ca, <8.4 mg/dL; and normal-high Ca, ≥8.4 mg/dL).[21,22] We compared the clinical features and results from several examinations of each group, such as laboratory tests, echocardiography, and electrocardiography, performed during hospitalization. Hypertension was defined as the recent use of antihypertensive drugs, systolic blood pressure ≥140 mm Hg, and/or diastolic blood pressure ≥90 mm Hg. Diabetes was defined as the recent use of insulin or antidiabetic drugs, fasting blood glucose ≥126 mg/dL, and/or hemoglobin A_{1c} ≥6.5%. Dyslipidemia was defined as the recent use of cholesterol-lowering drugs, triglyceride ≥150 mg/dL, low-density lipoprotein cholesterol ≥140 mg/dL, and/or high-density lipoprotein cholesterol <40 mg/dL. The eGFR was determined according to the Modification of Diet in Renal Disease formula.[19] Anemia was defined as hemoglobin <12.0 g/dL in women and <13.0 g/dL in men.[23] Reduced LVEF was defined as <50%. The primary outcome of our study was all-cause mortality. Patients were followed for cardiac death and all-cause mortality. Survival time was calculated from the date of hospitalization until the date of death or last follow-up. Status and dates of deaths were obtained from the patients' medical records. If those data were unavailable, status was ascertained by a telephone call to the patient's referring hospital physician. These survey was performed blindly to the analyses of this study. Written informed consent was obtained from each study subject. The study protocol was approved by the Ethical Committee of Fukushima Medical University. The investigation conforms to the principles outlined in the Declaration of Helsinki. Reporting of the study conforms to STROBE along with references to STROBE and the broader EQUATOR guidelines.[24]

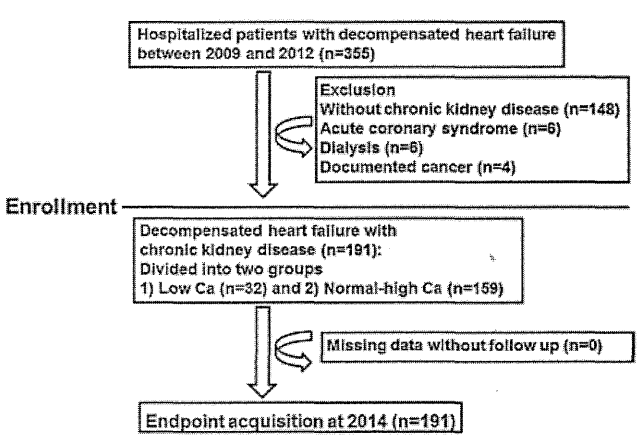

Fig. 1. Patient flow chart.

Echocardiography

Echocardiography was performed blindly by an experienced echocardiographer with the use of the standard techniques. Echocardiographic parameters investigated included interventricular septum thickness, left ventricular dimension, posterior wall thickness, LVEF, left atrial volume, the ratio of early transmitral flow velocity to mitral annular velocity (mitral valve E/e′), inferior vena cava diameter, right ventricular fractional area change (RV-FAC), and tissue Doppler–derived tricuspid lateral annular systolic velocity (tricuspid valve S′).[25] The LVEF was calculated with the use of a modification of the Simpson method. Mitral valve E/e′ was calculated with the use of transmitral Doppler flow and tissue Doppler imaging. Tissue Doppler imaging was obtained from the average of lateral and septal annulus velocities. The RV-FAC, defined as ([end-diastolic area − end-systolic area]/end-diastolic area) × 100, is a measure of right ventricular systolic function.[25] All recordings were performed on ultrasound systems (Acuson Sequoia; Siemens Medical Solutions USA, Mountain View, California).

Measurement of QT and QTc Intervals

The standard resting ECG was recorded in the supine position with the use of the Cardiostar FCP-7541 (Fukuda Denshi Co, Tokyo, Japan) and stored digitally. This system allows automatic measuring of QT and QTc intervals. The QT interval was measured from the beginning of the QRS complex until the T-wave returns to the isoelectric line. Then the median QT interval was calculated and corrected for the heart rate.

Measurement of Ca, Phosphate, Magnesium, Zinc, PTH, and 1,25(OH)$_2$D

A blood sample was obtained from each patient on admission. The level of serum Ca was measured by means of the Arsenazo III method (Aquaauto Kainos Ca reagent; Kainos, Tokyo, Japan). The levels of phosphate, zinc, and magnesium were also measured with the use of standard methods in our laboratory [phosphate: enzymatic method (Pi-L; Serotec, Hokkaido, Japan); zinc: colorimetric method (Accuras Auto Zn; Shino-Test Corp, Kanagawa, Japan); magnesium: atomic absorption spectrophotometer method (magnesium Flex Reagent Cartridge; Siemens Healthcare Diagnostics, Tokyo, Japan)]. Serum intact PTH was measured with the use of an electrochemiluminescence immunoassay kit (Access Intact PTH; Beckman Coulter, Tokyo, Japan). The level of 1,25(OH)$_2$D was measured by means of radioimmunoassay (1,25(OH)$_2$D RIA kit; Fujirebio, Tokyo, Japan). Assays were performed by a laboratory technician blinded to the patients' clinical data.

Statistical Analysis

Normally distributed data are presented as mean ± SD, and nonnormally distributed data are presented as median (interquartile range [IQR]). Categoric variables are expressed as number and percentage. The chi-square test was used for comparisons of categoric variables. Characteristics and data of the 2 groups were compared by means of the independent Student t test for normally distributed data and the Mann-Whitney U test for nonnormally distributed data. The Kaplan-Meier method was used for presenting the event-free rate, and the log-rank test was used for initial comparisons. Cox proportional hazards analyses were used to evaluate Ca levels as a predictor of all-cause mortality. We constructed 2 models: in the first, we analyzed Ca levels as a categoric variable model (low Ca vs normal-high Ca); and in the second, we analyzed Ca levels as a continuous variable model. To prepare for potential confounding, we considered the following clinical factors, which are known to affect the risk of all-cause mortality in HF patients: age, sex, New York Heart Association (NYHA) functional class III or IV, body mass index, systolic blood pressure, presence of ischemic etiology, diabetes, atrial fibrillation, anemia, reduced LVEF (<50%), B-type natriuretic peptide (BNP), and sodium. Among these, factors that independently predicted all-cause mortality with a value of $P < .05$ were selected in the final adjusted model: age, body mass index, presence of ischemic etiology, BNP, and sodium. The proportional hazards assumption for the model was checked by examining log–minus-log transformed Kaplan-Meier estimates of the survival curves for 2 groups plotted against time to follow-up period. These curves help in identifying nonproportionality patterns in hazard function, such as convergent (difference in risk between the 2 groups decreases with time), divergent, or crossing of the curves. In addition, Schoenfeld test for the violation of proportional hazards, which assesses the correlation between scaled residuals and time, was conducted. Because the proportional hazards assumptions were violated in the above-mentioned diagnostic test, the extended Cox hazards model was used for time-varying exposure of the adjusting variable. There was no significant multicollinearity between Ca levels and these confounding factors. A value of $P < .05$ was considered to be significant for all comparisons. These analyses were performed with the use of a statistical software package (SPSS v 21.0; IBM, Armonk, New York).

Results

Clinical features of the study subjects are summarized in Table 1. The Low Ca group had higher levels of blood pressure and heart rate, longer length of hospital stay, higher prevalence of anemia, and less use of β-blockers. Comparisons of laboratory data between the 2 groups are shown in Table 2. The Low Ca group had higher levels of alkaline

Table 1. Comparisons of Clinical Features (n = 191)

Group	Low Ca (Ca <8.4 mg/dL; n = 32)	Normal-high Ca (Ca ≥8.4 mg/dL; n = 159)	P Value
Age, y	70.8 ± 12.4	70.3 ± 11.4	.528
Male gender, n (%)	25 (78.1)	110 (69.2)	.311
Body mass index, kg/m^2	23.4 ± 4.8	23.0 ± 3.7	.660
NYHA III or IV	12 (37.5)	46 (28.9)	.539
Systolic blood pressure, mm Hg	146.3 ± 40.2	126.0 ± 28.6	<.001
Diastolic blood pressure, mm Hg	83.5 ± 25.8	70.9 ± 19.9	.004
Heart rate, beats/min	91.8 ± 28.0	81.1 ± 26.4	.006
Length of hospitalization, d	28.9 ± 22.7	19.4 ± 14.4	.029
Ischemic etiology, n (%)	11 (34.4)	46 (28.9)	.539
Reduced LVEF, n (%)	19 (59.4)	89 (56.0)	.723
Comorbidity, n (%)			
Hypertension	23 (71.9)	126 (79.2)	.358
Diabetes	15 (46.9)	60 (37.7)	.334
Dyslipidemia	23 (71.9)	120 (75.5)	.669
Atrial fibrillation	13 (40.6)	65 (40.9)	.979
Anemia	26 (81.3)	84 (52.8)	.003
Medications, n (%)			
RAAS inhibitors	24 (75.0)	113 (71.1)	.652
β-blockers	20 (62.5)	132 (83.0)	.009
Diuretics	17 (53.1)	91 (57.2)	.669
Inotropic agents	3 (9.4)	21 (13.2)	.551
Activated vitamin D_3 drugs	2 (6.3)	16 (10.1)	.501
Phosphate binders	2 (6.3)	16 (10.1)	.501
Erythropoiesis stimulating agent	0 (0)	6 (3.8)	.264
Iron drug	6 (18.8)	22 (13.8)	.473

NYHA, New York Heart Association (NYHA) functional class; LVEF, left ventricular ejection fraction; RAAS, renin-angiotensin-aldosterone system.

Table 2. Laboratory Data

Group	Low Ca (n = 32)	Normal-high Ca (n = 159)	*P* Value
Hemoglobin, g/dL	11.2 ± 2.4	12.5 ± 2.5	.008
B-Type natriuretic peptide, pg/mL*	469.3 (788)	415.0 (655)	.201
Blood urea nitrogen, mg/dL	30.9 ± 17.4	27.9 ± 12.1	.215
Creatinine, mg/dL	2.3 ± 2.5	2.0 ± 1.9	.176
eGFR, mL min^{-1} 1.73 m^{-2}	39.8 ± 23.8	41.7 ± 18.0	.346
Alkaline phosphatase, U/L	308.9 ± 152.0	261.0 ± 105.1	.026
C-reactive protein, mg/dL*	0.80 (1)	0.45 (1)	.132
Sodium, mmol/L	137.8 ± 6.0	138.2 ± 4.7	.213
Potassium, mmol/L	4.2 ± 1.1	4.3 ± 0.6	.849
Chloride, mmol/L	101.4 ± 6.1	101.8 ± 4.4	.668
Corrected calcium, mg/dL	7.9 ± 0.4	9.2 ± 0.5	<.001
Phosphate, mg/dL	3.8 ± 1.3	3.6 ± 0.8	.271
Magnesium, mEq/L	1.8 ± 0.5	1.8 ± 0.3	.713
Zinc, mg/dL	59.3 ± 14.5	68.0 ± 14.5	.124
Intact PTH, pg/mL	87.5 ± 46.0	58.6 ± 21.5	.084
1,25-dihydroxy vitamin D, pg/mL	26.1 ± 11.6	45.0 ± 19.8	.011
Iron, μg/dL	39.4 ± 19.5	78.2 ± 40.5	<.001
Ferritin, ng/mL	207.3 ± 74.3	172.1 ± 99.5	.232
UIBC, μg/dL	184.5 ± 80.6	218.8 ± 65.4	.109
Erythropoietin, mIU/mL	142.9 ± 79.0	122.9 ± 63.6	.225
Urine protein, −/±/1+/≥2+	20/2/2/8	104/25/15/15	.060
HCO_3^-, mmol/L	22.4 ± 5.7	24.5 ± 4.1	.031
Plasma noradrenalin, pg/mL	754.0 ± 305.5	978.3 ± 483.1	.437
Plasma renin activity, ng mL^{-1} h^{-1}*	0.6 (2.7)	1.1 (3.9)	.284
Renin concentration, pg/mL*	7.5 (68.3)	14.0 (44.2)	.330
Aldosterone, pg/mL*	61.1 (85.6)	88.0 (72.2)	.713

eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone; UIBC, unsaturated iron-binding capacity; HCO_3^-, hydrogen carbonate.
*Data presented as median (interquartile range).

phosphatase, and lower levels of hemoglobin, $1,25(OH)_2D$, iron, and hydrogen carbonate. Furthermore, the Low Ca group tended to have higher PTH levels. In contrast, eGFR, urine protein, phosphate, sodium, potassium, magnesium, zinc, plasma noradrenalin, renin activity, renin concentration, and aldosterone did not differ between the 2 groups. Echocardiographic parameters and QT intervals are summarized in Table 3. Left and right ventricular systolic function did not differ between the 2 groups. The Low Ca group had a higher heart rate and tended to have a longer QTc interval.

During the follow-up period (mean 643 days, median 627 days [IQR 311−929 days]), there were 29 cardiac deaths and 26 noncardiac deaths [respiratory failure and/or pneumonia (n = 6), renal failure (n = 5), cancer (n = 5), infection/sepsis (n = 3), aneurysm (n = 2), stroke (n = 2), digestive hemorrhage (n = 1), and other problems (n = 2)]. These events in each group are summarized in Table 4. As shown in Figure 2, the event-free rates from cardiac death and all-cause mortality were significantly lower in the Low Ca Group than in the Normal-high Ca Group ($P < .05$). Median survival time of the Low Ca

Table 3. Comparisons of Echocardiographic and Electrocardiographic Data

Group	Low Ca (n = 32)	Normal-high Ca (n = 159)	*P*-Value
Echocardiography			
Interventricular septum thickness, mm	11.3 ± 3.4	11.1 ± 2.8	.966
Left ventricular end-diastolic dimension, mm	50.4 ± 8.1	53.4 ± 10.9	.225
Left ventricular end-systolic dimension, mm	38.2 ± 11.1	40.6 ± 11.8	.472
Posterior wall thickness, mm	11.4 ± 2.6	11.4 ± 2.6	.872
LVEF, %	45.5 ± 16.1	46.9 ± 15.3	.683
Left atrial volume, mL	82.6 ± 39.6	88.3 ± 51.7	.416
Mitral valve E/e′	17.7 ± 7.4	17.7 ± 9.9	.960
Inferior vena cava diameter, mm	17.6 ± 3.8	15.2 ± 4.5	.066
RV-FAC, %	44.9 ± 23.4	45.6 ± 12.5	.653
Tricuspid valve S′, cm/s	8.9 ± 2.2	9.8 ± 4.3	.704
Electrocardiography			
Heart rate, beats/min	93.8 ± 29.8	78.6 ± 19.9	.008
QRS, ms	117.9 ± 32.7	120.2 ± 29.7	.658
QT, ms	383.4 ± 55.9	401.8 ± 50.6	.096
QTc, ms	464.1 ± 43.3	451.2 ± 40.5	.127

LVEF, left ventricular ejection fraction; mitral valve E/e′, ratio of the peak transmitral velocity during early diastole to the peak mitral valve annular velocity during early diastole; RV-FAC, right ventricular fractional area change; tricuspid valve S′, Doppler-derived tricuspid lateral annular systolic velocity.

Table 4. Numbers and Proportion of Events (n = 191)

Group	Low Ca (n = 32)	Normal-high Ca (n = 159)	Total (n = 191)
Cardiac death, n (%)	10 (31.3)	19 (11.9)	29 (15.2)
All-cause mortality, n (%)	16 (50.0)	39 (24.5)	55 (28.8)
In-hospital mortality, n (%)	7 (21.9)	4 (2.5)	11 (5.8)
Time frame of events, n (%)			
Rehospitalization within 30 d*	1 (4.0)	1 (0.6)	2 (1.1)
Rehospitalization within 90 d*	2 (8.0)	6 (3.9)	8 (4.4)
Rehospitalization within 1 y*	2 (8.0)	10 (6.5)	12 (6.7)
All-cause mortality within 30 days	6 (27.3)	3 (1.9)	9 (4.7)
All-cause mortality within 90 days	7 (31.8)	4 (2.5)	11 (5.8)
All-cause mortality within 1 year	9 (40.9)	9 (5.7)	18 (9.4)

*Patients without in-hospital mortality (n = 180).

group was 521 days and of the Normal-high Ca was 657 days.

The Cox proportional hazards model was used to examine the prognostic value of hypocalcemia (low Ca) and continuous Ca levels in patients with HF and CKD (Table 5). We checked that the Cox models support the assumption of proportional odds. In multivariable analysis, both low Ca and Ca levels were independent predictors of all-cause mortality ($P < .05$).

Discussion

To the best of our knowledge, the present study is the first to show the association between hypocalcemia and all-cause mortality in hospitalized patients with HF and CKD. Patients with hypocalcemia had higher levels of alkaline phosphatase (as a marker of bone turnover), lower levels of $1,25(OH)_2D$, hydrogen carbonate, and hemoglobin, and a tendency to having higher PTH and longer QTc interval. In contrast, and unexpectedly, left and right ventricular systolic function, BNP, renal function, urine protein, phosphate, plasma noradrenalin, and parameters of RAAS did not differ between the 2 groups.

In patients with CKD, altered Ca-phosphate metabolism is present, along with altered myocardial and vascular function. In CKD patients, depending on the CKD stage, there is decreased serum Ca, decreased hydrogen carbonate and $1,25(OH)_2D$, and elevated phosphate and PTH levels.[3] Decreased vitamin D (25-OH vitamin D) is associated with poor prognosis in HF.[14,26] Elevated PTH levels are reportedly associated with 1) lower cardiac index and higher pulmonary capillary wedge pressure and heart rate in HF,[27] 2) predictors of worsening HF in chronic HF,[15] and 3) predictors of all-cause mortality in HF.[14]

The direct association with hypocalcemia and the vascular system and the complicated metabolic circumstances occurring in CKD remain unclear. Calcium is required for myocardial contraction and relaxation. After it is diffused into the region of myofibrils, free Ca^{2+} induces a reversal of the inhibition of actin-myosin contraction by the troponin-tropomyosin complex,[28] an effect that allows the interaction of actin and myosin with the resultant sliding of filaments and production of myocardial contraction.

To date, few studies regarding association with Ca levels and HF prognosis exist.[12,16] Cubbon et al found that levels of Ca were inversely correlated with LVEF and associated with all-cause mortality, but not with sudden death, in HF patients.[12] However, their HF patients were outpatients included regardless of CKD, were within almost normal levels of serum Ca, and differed from our hospitalized patients with HF and CKD. On the other hand, Zitterman et al reported that Ca levels were significantly higher in event-free survivors than in nonsurvivors of end-stage HF, and that derangements in parameters of Ca, phosphate, and magnesium metabolism predict poor outcome in end-stage HF.[16] We also think that extremely low or extremely high Ca levels seem to be related to cardiovascular mortality,[29] concordant with CKD-MBD management.[21,22]

We could not explain the reason why left ventricular systolic function and QT interval were not different between our 2 groups of subjects, or why the Low Ca group had more

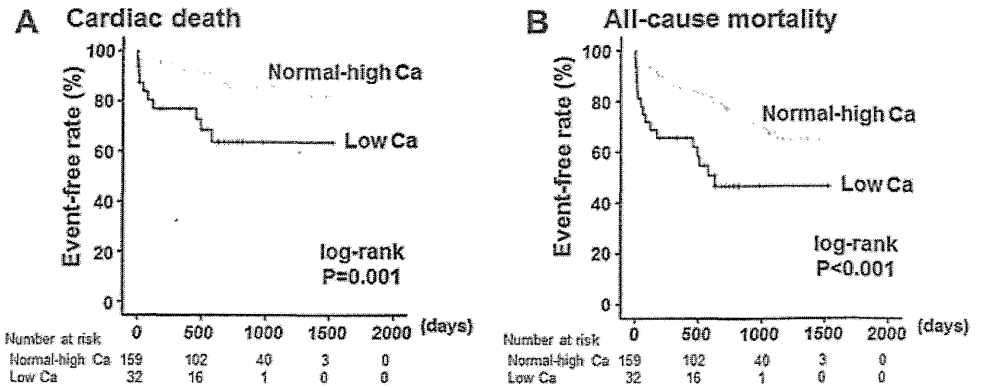

Fig. 2. Kaplan-Meier analysis for (A) cardiac death and (B) all-cause mortality between the 2 groups (Low Ca and Normal-high Ca).

Table 5. Cox Proportional Hazards Model of Cardiac Death and All-Cause Mortality in Heart Failure

	Categoric Variable Model (Low Ca vs Normal-high Ca)			Continuous Variable Model (Ca 1 mg/dL Decrease)		
Event	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Cardiac death (n = 29)						
Unadjusted	3.404	1.576–7.352	.002	1.165	1.032–1.505	.002
All-cause mortality (n = 55)						
Unadjusted	2.895	1.608–5.215	<.001	1.387	1.060–1.816	<.001
Adjusted model*	1.749	1.014–3.414	.045	1.352	1.029–1.775	.021

HR, hazard ratio; CI, confidence interval.
*Adjusted for age, body mass index, presence of ischemic etiology, B-type natriuretic peptide, and sodium.

cardiac deaths. Hypocalcemia may cause decreased pump function, which we could not evaluate with the use of LVEF or RV-FAC, and abnormal repolarization, which we could not evaluated with the use of QTc. Hypocalcemia progresses as the CKD develops,[5] with advanced acidemia, decreased $1,25(OH)_2D$,[14,26] and increased PTH,[14,15] fibroblast growth factor 23,[13] and bone turnover. Higher mortality in hypocalcemia may reflect a relationship severity not only of cardiac dysfunction but also of comorbidities, including CKD. These mechanisms may in part explain the poor prognosis of hypocalcemia in patients with HF and CKD.

Also, various CKD-MBD control drugs were recently reported to affect mortality in CKD patients by, for example, acting on the vitamin D receptor activator[30] and phosphate binders (Ca component or noncomponent).[31] It is recommended that these control drugs should be given priority over the Ca-phosphate control of PTH in CKD-MBD management.[21,22] Although using appropriate management to control Ca levels as well as phosphate (CKD-MBD balance) may be important in patients with HF and CKD, attention for the complex network including fibroblast growth factor 23, vitamin D, and PTH is necessary.

Study Strengths and Limitations

Several limitations remain in the present study. First, as a prospective observational study in a single institution, the number of subjects was relatively small and the study may have been underpowered to accurately estimate the association between hypocalcemia and outcomes. The numbers of cardiac deaths and all-cause deaths were also small. For these reasons, results from the multivariable Cox proportional hazards regression models, in particular, should be viewed as preliminary. Prospective studies with larger populations are needed. However, diagnosis of HF and causes of death including cardiac and all-cause mortality were accurately made by our experienced cardiologists. Second, we did not measure and consider changes in Ca levels, and only baseline Ca levels within the 1st 24 hours after admission were used for the analyses. Third, we used only index hospitalization variables, without consideration of change in medical parameters and post-discharge treatment. Fourth, there is no way of knowing the detailed causal nature of relationships between poor prognosis and hypocalcemia with elevated PTH and lowered $1,25(OH)_2D$. Fifth, 25-OH vitamin D might be a better presentation of true body deficiency. Sixth, activated vitamin D_3 drugs or phosphate binders were given in only a few patients among our study subjects. Seventh, levels of plasma renin activity, concentrations of renin and aldosterone might be affected by taking RAAS inhibitors.

Conclusion

Hypocalcemia was an independent predictor of all-cause mortality in patients with HF and CKD. These findings provide an important insight for the management of Ca levels and for prospectively assessing the comparative yield of strategies designed to optimize treatment in patients with HF and CKD. Further study is required to elucidate the mechanisms underlying the adverse association with hypocalcemia and to determine whether controlling Ca levels improves the prognosis in HF and CKD patients.

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Disclosures

None.

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Long-Term Prognostic Role of the Diagnostic Criteria for Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia

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ABSTRACT

OBJECTIVES The aim of this study was to evaluate the long-term prognostic role of the 2010 task force criteria (TFC)-based scoring in patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D).

BACKGROUND Categories of the 2010 TFC include the risk factors for cardiovascular mortality and sudden cardiac death in patients with ARVC/D.

METHODS Ninety patients with ARVC/D who met the definitive diagnosis of the 2010 TFC were retrospectively studied. ARVC/D risk score was calculated as the sum of major (2 points) and minor (1 point) criteria in all 6 subdivided categories of the TFC and was divided into tertile groups of scores; group A (4 to 6 points), group B (7 to 9 points), and group C (10 to 12 points). The primary endpoints were major adverse cardiovascular events: cardiovascular death, heart failure hospitalization, and sustained ventricular tachycardia or ventricular fibrillation.

RESULTS During the follow-up period of 10.2 ± 7.1 years, 19 patients died because of cardiovascular causes, 28 patients were admitted because of worsened heart failure, and 47 patients experienced sustained ventricular tachycardia or ventricular fibrillation. Patients in groups B and C were at increased risk for major adverse cardiovascular events compared with those in group A (hazard ratio [HR]: 4.80; 95% confidence interval [CI]: 1.87 to 12.33; p = 0.001; and HR: 6.15; 95% CI: 2.20 to 17.21; p = 0.001, respectively). Patients in groups B and C were at increased risk for sustained ventricular tachycardia or ventricular fibrillation compared with those in group A (HR: 6.64; 95% CI: 2.00 to 22.03; p = 0.002; and HR: 9.18; 95% CI: 2.60 to 32.40; p = 0.001, respectively).

CONCLUSIONS Our study suggests that risk scoring based on the 2010 TFC is useful to predict major adverse cardiovascular events in patients with ARVC/D. (J Am Coll Cardiol EP 2016;2:107–15) © 2016 by the American College of Cardiology Foundation.

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a cardiomyopathy characterized by ventricular arrhythmias and fibrofatty replacement of the right ventricular (RV) myocardium (1,2). ARVC/D slowly progresses to more diffuse RV and left ventricular (LV) dysfunction (2,3). In the early (concealed) phase, structural change is absent or minor, but patients may be at risk for sudden cardiac death (SCD) caused by sustained ventricular tachycardia (VT) or ventricular fibrillation (VF) in younger patients and athletes (1–5). Life-threatening ventricular arrhythmia and SCD can constitute the initial presentation of ARVC/D (2,3). In the overt (electric) phase, patients have symptomatic ventricular arrhythmia with manifested structural abnormalities of the right and/or left ventricle (2).

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ABBREVIATIONS AND ACRONYMS

ARVC/D = arrhythmogenic right ventricular cardiomyopathy/dysplasia

CI = confidence interval

ECG = electrocardiography

HF = heart failure

HR = hazard ratio

ICD = implantable cardioverter-defibrillator

LV = left ventricular

MACE = major adverse cardiovascular event(s)

RV = right ventricular

SAECG = signal-averaged electrocardiography

SCD = sudden cardiac death

TFC = task force criteria

VF = ventricular fibrillation

VT = ventricular tachycardia

In the later phase, patients experience progressed right or biventricular heart failure (HF) with or without the presence of ventricular arrhythmia (2).

Thus, there is no single diagnostic tool for ARVC/D, and the clinical diagnosis of ARVC/D is often complex and difficult. The 1994 task force criteria (TFC) first provided the clinical diagnosis of ARVC/D on the basis of several categories, such as structure, function, histology, electrocardiography (ECG), arrhythmia, and family history (6). These criteria were modified in 2010 to improve diagnostic sensitivity by advances in the diagnostic modalities and the genetic knowledge of ARVC/D (7).

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Several electrocardiographic and electrophysiological abnormalities and structural features of both ventricles have been reported as clinical markers of a worse prognosis in patients with ARVC/D, but the predictive value of each factor itself is not high (8). The categories of TFC also include risk factors of cardiovascular mortality and SCD in patients with ARVC/D (7,8). We hypothesized that risk scoring on the basis of the 2010 TFC for the diagnosis of ARVC/D has a role in predicting ARVC/D-specific outcomes: cardiovascular death, worsening HF, and sustained VT or VF. The aim of this study was to evaluate the long-term prognostic role of 2010 TFC-based scoring in patients with ARVC/D.

METHODS

SUBJECTS. We retrospectively studied 90 patients with ARVC/D who met the definitive diagnosis of the 2010 TFC. All patients admitted to the Department of Cardiology, Tokyo Women's Medical University Hospital, for evaluation of sustained VT or VF and/or cardiomyopathy between 1974 and 2012 and with available follow-up were included in this study. All patients underwent 12-lead ECG and echocardiography or magnetic resonance imaging or RV angiography. Eighty-one patients also underwent endomyocardial biopsy of the right ventricle. Eighty-five patients underwent signal-averaged ECG (SAECG) (Predictor BSM-32, Arrhythmia Research Technology, Fitchburg, Massachusetts), and 79 patients underwent 24-h Holter ECG.

Antiarrhythmic drugs were prescribed for ventricular and supraventricular arrhythmias. In Japan, amiodarone and sotalol were approved in 1992 and 1998, respectively, and the implantable cardioverter-defibrillator (ICD) was approved in 1996.

Available data were obtained retrospectively from the medical records of our hospital. The patients were followed until December 31, 2013. Information regarding deceased subjects was obtained from medical records, family members, their local hospitals or general practitioners, and the admitting hospital. The patients were followed until the end of the follow-up period (December 31, 2013). The protocol was approved by the Institutional Review Board of Tokyo Women's Medical University.

ARVC/D RISK SCORE. ARVC/D risk score was calculated as the sum of major and minor criteria in all 6 subdivided categories of the 2010 TFC, with major criteria given 2 points and minor criteria given 1 point. The definite diagnosis of ARVC/D according to the 2010 TFC was fulfilled by the presence of 2 major criteria, 1 major plus 2 minor criteria, or 4 minor criteria from different categories. Thus, the minimum score of the ARVC/D risk was 4, and the range of this score was between 4 and 12. The patients were divided into 3 subgroups on the basis of the ARVC/D risk score tertiles: group A (first tertile, 4 to 6 points), group B (second tertile, 7 to 9 points), and group C (third tertile, 10 to 12 points).

OUTCOMES. The primary endpoints were major adverse cardiovascular events (MACEs), a composite of cardiovascular death, hospitalization for worsened HF, and sustained VT or VF. Worsened HF was defined by signs and symptoms, such as dyspnea, rales, and ankle edema, as well as the need for treatment with diuretic agents, vasodilators, positive inotropic drugs, or an intra-aortic balloon pump. Sustained VT was defined as a rate of more than 100 beats/min or more than 30 s in duration (or less if treated by electrocardioversion within 30 s) of VT on ECG, VT that required external defibrillation, intravenous antiarrhythmic agents such as amiodarone, and ICD therapy for termination. The occurrence of these events was validated through a review of medical records by 3 investigators (N.K., D.Y., and A.S.). The details of cardiovascular death were based on the clinical history obtained from medical charts or information from other hospitals. Sudden death was defined as unexpected endogenous death within 24 h after last having been observed alive, unrelated to a specific cause of circulatory failure.

STATISTICAL ANALYSIS. Summary data are presented either as the median and range or the number of patients. Cumulative probabilities of cardiovascular death, first HF hospitalization, and first

sustained VT or VF after the diagnosis were estimated using the Kaplan-Meier method and by means of a comparison of cumulative events according to 3 groups on the basis of the ARVC/D risk score with a log-rank test. The risk for MACEs associated with each increase of 1 point in the ARVC/D risk score was assessed using a Cox proportional hazards model, with adjustment for age and sex. The proportionality assumption was checked by inspection of log-log plots. Univariate Cox regression analysis was used to estimate the relationship between each major or minor criterion of the 2010 TFC and the long-term outcomes: cardiovascular death, HF hospitalization, and sustained VT or VF. A p value <0.05 was considered to indicate statistical significance. Data analyses were performed using SPSS version 11.01 (SPSS, Inc., Chicago, Illinois).

RESULTS

PATIENT CHARACTERISTICS. The patients' baseline characteristics are shown in **Table 1**. The mean age of the cohort at diagnosis of ARVC/D was 44 ± 15 years, and 68 (76%) were men.

Sustained VT or VF was documented in 64 patients (71%), and 3 patients had cardiac arrest. Nine patients (10%) had family histories of definite ARVC/D, which were confirmed in first-degree relatives who met the 2010 TFC. Nonsustained or sustained VT with left bundle-branch block was the most frequent. Electrocardiographic abnormalities were observed, especially T-wave inversion in the right precordial leads (V_1 through V_3) in 47 patients (52%) and epsilon waves in 39 patients (43%). SAECG showed ventricular late potentials in 85 patients (94%). RV angiography revealed signs suggesting ARVC/D, such as RV dilation, reduction of the RV ejection fraction, and RV aneurysms, in 98% of the patients. Histological examinations were performed in 81 patients using myocardial tissue. Any abnormality of the myocardium, such as fibrofatty replacement, was observed (**Table 1**). At the end of the follow-up period (December 31, 2013), 16 patients (18%) were lost to follow-up because they did not visit our hospital and provided no explanation.

ARVC/D RISK SCORE AND LONG-TERM OUTCOMES. The mean ARVC/D risk score of our patients was 8 ± 2 points. The distribution of patients according to the score is shown in **Figure 1**. The number of patients in each group was as follows: 25 patients in group A, 49 patients in group B, and 16 patients in group C.

TABLE 1 Clinical Characteristics in Patients With Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia (n = 90)

Male	69 (76)
Age at diagnosis, yrs	44 ± 15
Family history of ARVC/D	9 (10)
Previous sustained VT	64 (71)
Previous VF/cardiac arrest	3 (3)
Nonsustained or sustained VT of LBBB	74 (82)
LVEF, %	52 ± 14
RVEF, %	30 ± 12
NYHA functional class at diagnosis	
I	74 (82)
II	12 (13)
III/IV	4 (4)
ECG	
Inverted T-wave in right precordial leads (V_1–V_3)	47 (52)
Epsilon wave	39 (43)
SAECG (n = 85)	
Ventricular late potential	79 (92)
Holter recording (n = 79)	
VPB (>500/24 h)	63 (93)
Right ventricular biopsy (n = 81)	
Regional myocytes <60% with fibrous replacement	40 (49)
Regional myocytes 60%–75% with fibrous replacement	41 (51)
ICD implantation	16 (18)
Catheter ablation	31 (34)
Medications	
Beta-blockers	22 (24)
ACE inhibitors/ARBs	28 (31)
Amiodarone	38 (42)
Sotalol	2 (2)
Other antiarrhythmic agents	17 (19)
Digitalis	4 (4)
Diuretic agents	13 (14)
Anticoagulation	14 (16)

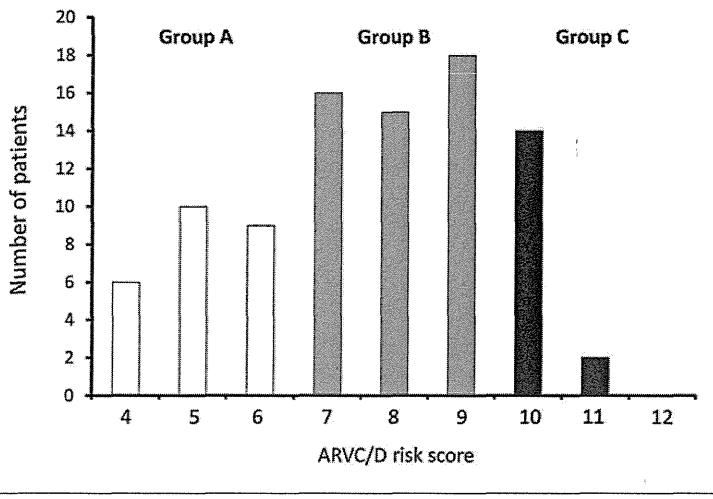
Values are n (%) or mean ± SD.

ACE = angiotensin-converting enzyme; ARB = angiotensin II receptor blocker; ARVC/D = arrhythmogenic right ventricular cardiomyopathy/dysplasia; ECG = electrocardiography; ICD = implantable cardioverter-defibrillator; LBBB = left bundle branch block; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association; RVEF = right ventricular ejection fraction; SCD = sudden cardiac death; VF = ventricular fibrillation; VPB = ventricular premature beat; VT = ventricular tachycardia.

During the follow-up period of 10.2 ± 7.1 years, 19 patients died because of cardiovascular causes, 28 patients were admitted because of worsened HF, and 47 patients experienced sustained VT or VF (**Figure 2**). The 19 cardiovascular deaths included HF death (n = 16) and SCD (n = 3).

The Kaplan-Meier curves for MACEs in the 3 groups are shown in **Figure 3**. Patients in groups B and C were at increased risk for MACEs compared with those in group A (**Table 2**). There was a significantly higher

FIGURE 1 Distribution of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia Risk Scores



ARVC/D = arrhythmogenic right ventricular cardiomyopathy/dysplasia.

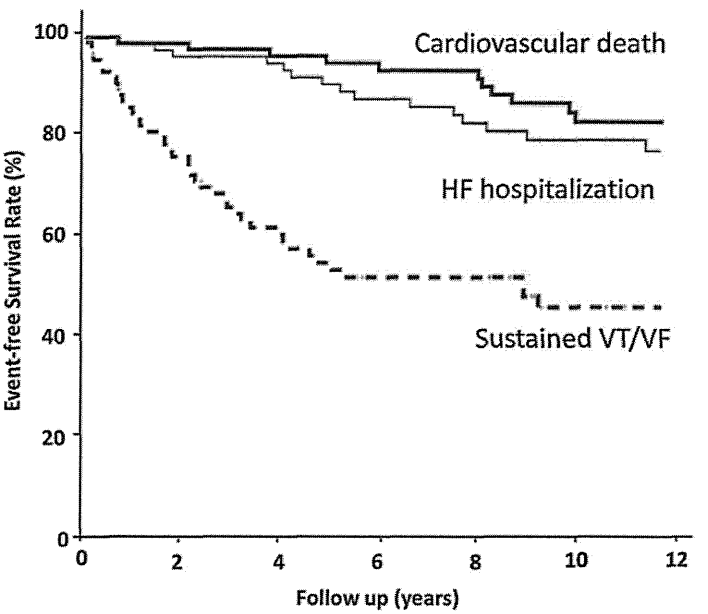
incidence of MACEs with a higher score (per 1 point; hazard ratio [HR]: 2.29; 95% confidence interval [CI]: 1.52 to 3.45; $p < 0.001$). After adjusting for age and sex, the score was associated with MACEs (per 1 point; HR: 2.29; 95% CI: 1.51 to 3.46; $p < 0.001$).

Patients in groups B and C were at increased risk for sustained VT or VF compared with those in group A. Patients in group C, but not those in group B, were also at increased risk for cardiovascular death and HF hospitalization compared with those in group A (**Table 3, Figure 4**).

CATEGORIES OF TFC AND LONG-TERM OUTCOMES. Univariate analysis for each major and minor criterion in the 6 categories of MACEs and each event showed that repolarization and depolarization abnormalities were significantly related to all events. The major criteria, but not the minor criteria, of RV dysfunction (global and/or regional dysfunction and structural alterations with dilation of the RV outflow tract or severely reduced RV systolic function) and the major criteria of depolarization abnormalities (epsilon waves) were significantly related to the occurrence of sustained VT or VF, as well as the major criteria of arrhythmias (nonsustained or sustained VT with left bundle branch block with superior axis) (**Table 3**).

DRUG THERAPY AND LONG-TERM OUTCOMES. Our study failed to show the benefit of beta-blockers in the prevention of sustained VT or VF (HR: 1.05; 95% CI: 0.53 to 2.07; $p = 0.891$) or cardiovascular death (HR: 0.43; 95% CI: 0.10 to 1.88; $p = 0.557$). In 64 patients who had previous sustained VT, there was no difference in the first recurrence rate of sustained VT or VF between amiodarone users ($n = 31$) and nonamiodarone users ($n = 33$) (8.4% per year vs. 9.6% per year, $p = 0.457$).

FIGURE 2 Kaplan-Meier Analysis of Cardiovascular Death, First Heart Failure Hospitalization, and First Sustained Ventricular Tachycardia or Ventricular Fibrillation After Diagnosis in Patients With Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia



HF = heart failure; VF = ventricular fibrillation; VT = ventricular tachycardia.

DISCUSSION

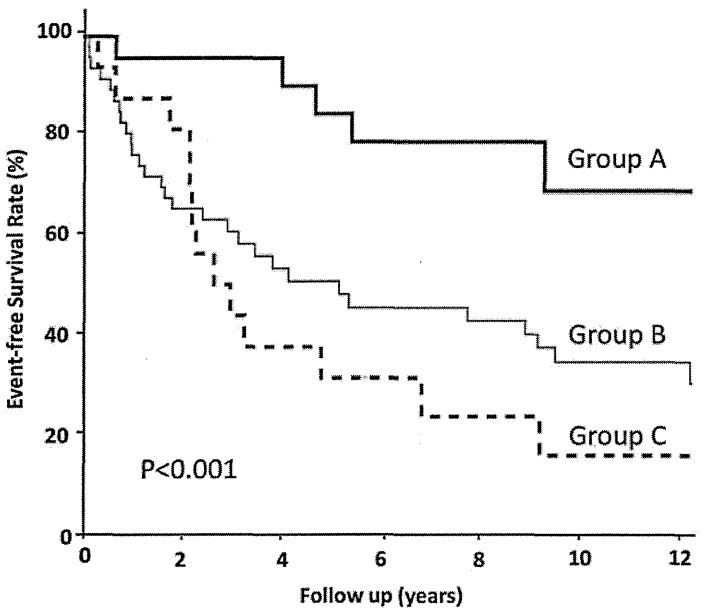
Our long-term observational study suggested that risk scoring on the basis of the 2010 TFC is useful to predict adverse events in patients with ARVC/D: 1) the categories of the TFC include the risk factors of cardiovascular mortality and SCD in patients with ARVC/D; 2) a higher incidence of MACEs was associated with a higher score; 3) higher incidences of cardiovascular death, HF hospitalization, and sustained VT or VF were associated with the highest score; and 4) electrocardiographic (repolarization and depolarization) abnormalities were related to all major events. Additionally, RV dysfunction was related to the occurrence of sustained VT or VF, and family history was related to cardiovascular death.

ARVC/D DIAGNOSTIC CRITERIA AND RISK FACTORS FOR SCD. SCD is the most common cause of death in

patients with ARVC/D (1-8). For risk stratification of SCD in patients with ARVC/D, previous reports suggested prior cardiac arrest caused by hemodynamically unstable VT and VF, a history of syncope, electrocardiographic depolarization abnormalities, such as QRS prolongation in the right precordial leads, QRS dispersion and ventricular late potential on SAECG, electrocardiographic repolarization abnormalities such as a significant extent of negative T and epsilon waves, RV dysfunction, and LV involvement (8). Most of these clinical findings overlapped the major or minor criteria in 4 of the 6 categories of the 2010 TFC for the diagnosis of ARVC/D (7). Thus, most patients who met the definitive diagnosis of ARVC/D also have the risk factors for SCD. The 2010 TFC does not include aborted SCD, hemodynamically unstable sustained VT, and syncope, which are strong predictors of SCD, because these criteria were developed only for the diagnosis of ARVC/D. In these specific patients, ICD therapy should be considered regardless of the diagnostic criteria.

ARVC/D SCORE AND RISK STRATIFICATION OF MACES. This approach can be challenging for the risk stratification of MACEs in patients with ARVC/D. There are no powerful risk factors for SCD and other cardiovascular events in patients with ARVC/D, and there is no standard diagnostic tool. However, a high score indicates multiple risk factors. In fact, our results suggested that the high-score group showed a higher incidence of MACEs. Recently, ICDs have been placed to avoid SCD in high-risk patients with ARVC/D with primary or secondary indications (9,10). Amiodarone is used as a first-line antiarrhythmic drug to suppress ventricular arrhythmia because it has superior efficacy in preventing sustained VT or VF compared with beta-blockers or sotalol (11). Therefore, the cause of death in patients who were recently diagnosed with ARVC/D may shift from SCD to death due to HF in the later phase. Our study showed that the most common cause of cardiovascular death was worsened HF. Risk factors of death in patients with ARVC/D will change in the future if the strategies for the prevention and treatment of SCD are recognized.

FIGURE 3 Kaplan-Meier Analysis of Major Adverse Cardiovascular Events According to the 3 Groups on the Basis of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia Risk Score



Although the RV abnormalities were not significantly related to HF hospitalization and cardiovascular death, other markers, including parameters that show RV and/or LV failure, are risk factors of cardiovascular death.

CATEGORIES AND LONG-TERM OUTCOMES. Our findings show that the ARVC/D score is an effective predictor of adverse events, including ventricular arrhythmia, HF, and cardiovascular mortality. Although more than one-half of the patients did not undergo magnetic resonance imaging, because our study included historical cases, most patients underwent RV angiography. In our study, the majority of patients met the major criteria of RV dysfunction. They had severely reduced RV systolic function, and this criterion was a significant factor for the occurrence of sustained VT or VF. Hulot et al. (12)

TABLE 2 Groups According to Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia Risk Score and Long-Term Outcomes

	MACEs		Cardiovascular Death		HF Hospitalization		Sustained VT/VF	
Group	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value
A (4-6 points) (n = 25)	1.00 (reference)		1.00 (reference)		1.00 (references)		1.00 (references)	
B (7-9 points) (n = 49)	4.80 (1.87-12.33)	0.001	3.45 (0.44-27.37)	0.240	7.24 (0.97-54.38)	0.054	6.64 (2.00-22.03)	0.002
C (10-12 points) (n = 16)	6.15 (2.20-17.21)	0.001	8.10 (1.01-65.02)	0.049	10.91 (1.38-86.61)	0.024	9.18 (2.60-32.40)	0.001

CI = confidence interval; HF = heart failure; HR = hazard ratio; MACE = major adverse cardiac event; other abbreviations as in Table 1.

TABLE 3 The 2010 Task Force Criteria and Long-Term Outcomes

	MACEs		Cardiovascular Death		HF Hospitalization		Sustained VT/VF	
Criteria	**HR (95% CI)**	**p Value**	**HR (95% CI)**	**p Value**	**HR (95% CI)**	**p Value**	**HR (95% CI)**	**p Value**
RV systolic function and structure								
Major (n = 71)	4.79 (1.73–13.28)	0.003	26.80 (0.09–8094)	0.259	5.10 (0.69–37.73)	0.111	5.12 (1.59–16.48)	0.006
Minor (n = 17)	0.26 (0.09–0.72)	0.010	0.04 (0.00–17.44)	0.275	0.24 (0.03–1.75)	0.157	0.24 (0.07–0.77)	0.017
Tissue characterization								
Major (n = 40)	0.75 (0.42–1.33)	0.325	0.44 (0.15–1.25)	0.122	0.59 (0.27–1.29)	0.187	0.91 (0.49–1.67)	0.760
Minor (n = 41)	1.33 (0.75–2.36)	0.325	2.29 (0.80–6.53)	0.122	1.69 (0.78–3.69)	0.187	1.10 (0.60–2.02)	0.760
Repolarization abnormalities								
Major (n = 47)	1.02 (0.50–2.08)	0.966	1.31 (0.37–4.58)	0.676	2.31 (0.93–5.77)	0.072	0.78 (0.33–1.84)	0.565
Minor (n = 14)	2.10 (1.35–4.29)	0.003	3.18 (1.05–9.65)	0.041	1.96 (0.87–4.41)	0.104	2.72 (1.42–5.20)	0.002
Depolarization abnormalities								
Major (n = 39)	1.71 (0.99–2.95)	0.054	1.67 (0.67–4.18)	0.270	2.56 (1.17–5.61)	0.019	1.86 (1.03–3.34)	0.039
Minor (n = 79)	1.93 (0.47–8.00)	0.364	22.00 (0.00–54104)	0.604	0.95 (0.12–7.24)	0.957	1.66 (0.40–6.93)	0.484
Arrhythmias								
Major (n = 74)	1.55 (0.66–3.65)	0.315	0.84 (0.19–3.70)	0.816	0.43 (0.14–1.30)	0.134	3.29 (1.02–10.61)	0.046
Minor (n = 63)	1.43 (0.66–3.09)	0.366	1.01 (0.28–3.67)	0.989	0.74 (0.28–1.91)	0.529	1.84 (0.71–4.76)	0.210
Family history								
Major (n = 8)	0.87 (0.12–6.33)	0.887	0.05 (0.00–11266)	0.734	3.35 (0.44–25.40)	0.242	0.05 (0.00–445.0)	0.514
Minor (n = 1)	1.59 (0.63–4.00)	0.330	1.53 (0.35–6.66)	0.572	2.17 (0.74–6.31)	0.157	1.02 (0.32–3.30)	0.972

RV = right ventricular; other abbreviations as in Tables 1 and 2.

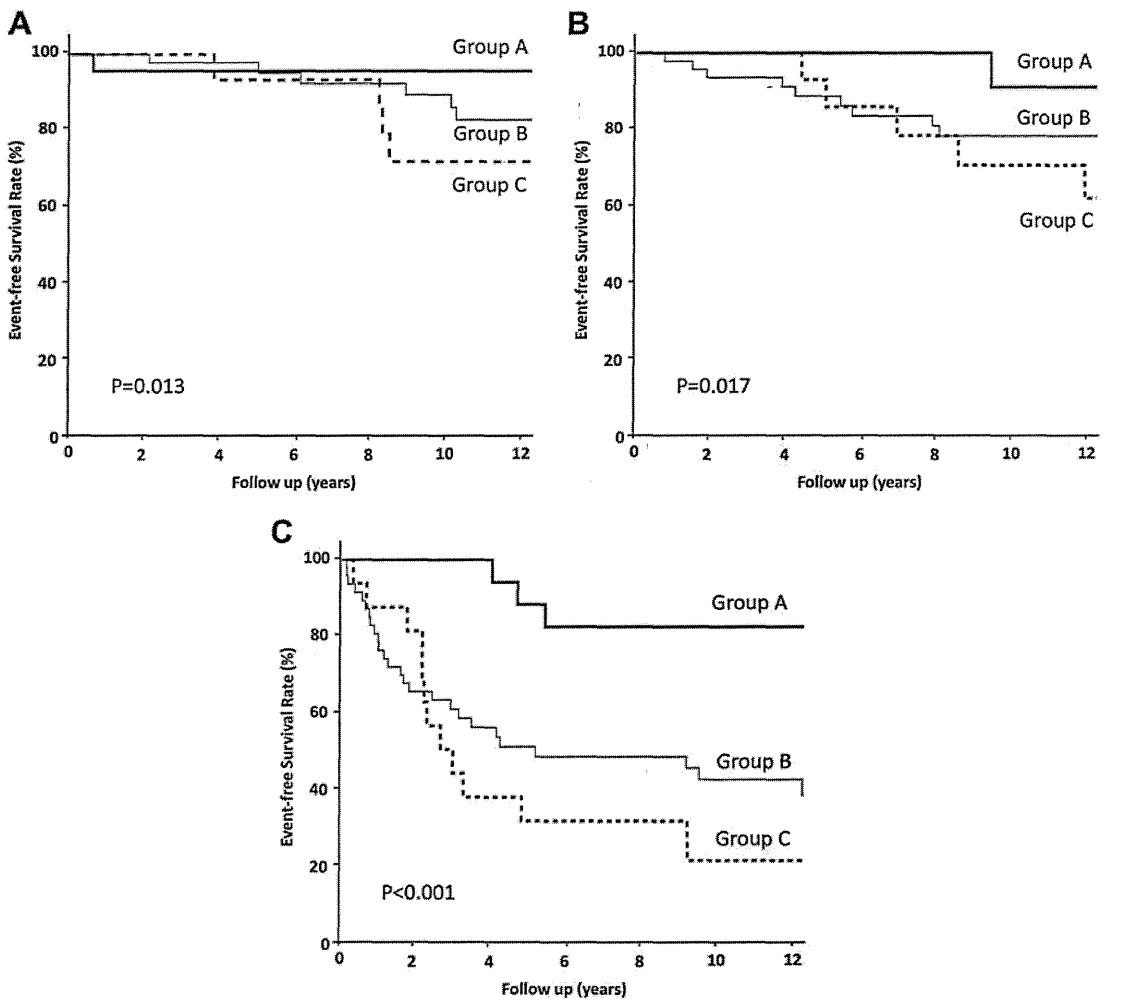
reported that clinical signs of RV and LV dysfunction were associated with cardiovascular death. Other studies reported that RV dysfunction is associated with VT or VF and SCD (13–15). Because RV dysfunction is related to the presence of late potentials (13), it may show the presence of the arrhythmogenic substrate of the right ventricle. However, RV dysfunction as a category of the 2010 TFC for the diagnosis of ARVC/D did not include RV dilation or hemodynamic state. Meanwhile, the minor criterion of RV dysfunction was a negative factor for the occurrence of sustained VT or VF. Minor structural abnormalities, such as mild segmental dilation of the right ventricle or regional RV hypokinesis, might contribute less to the development or recurrence of sustained VT, and the small number of patients who met this criterion (n = 17) might have influenced this result.

Electrocardiographic repolarization abnormalities have 2 meanings: the extent of the RV scar and the arrhythmogenic substrate of the right ventricle, which lead to the occurrence of arrhythmia (13,16–18). The former may be related to the hemodynamic state of the right ventricle as well as the presence of the arrhythmogenesis. T-wave inversion in leads V_1, V_2, and V_3 and beyond in patients >14 years of age as a major criterion is reasonable for the diagnosis of ARVC/D, especially in the early stage (19). Previous reports showed that the extent of T-wave inversion is related to RV enlargement and the progressed stage (1,20,21). However, it is not clear whether major or minor criteria for repolarization abnormalities as a diagnostic tool on the basis of the extent of T-wave inversion and the presence or absence of complete right bundle-branch block indicate the degree of arrhythmogenesis.

Late potentials on SAECG (minor criteria of depolarization abnormalities) have diagnostic value for ARVC/D, but there is no evidence of their predictive value for arrhythmic events (8,22–24). The epsilon wave is a potential electrocardiographic feature of ARVC/D that indicates delayed RV activation, but it is not common (3,17,20,25–28). It appears in patients with diffuse RV involvement, but not in the early phase (27,28). In our study, the presence of an epsilon wave, but not a late potential on SAECG, was significantly associated with the occurrence of VT or VF and HF hospitalization. However, its prognostic value remains unclear (12,28).

DRUG THERAPY AND LONG-TERM OUTCOMES. Beta-blocker therapy to prevent the occurrence of sustained VT or VF and delay disease progression in patients with ARVC/D is controversial. Our retrospective analysis failed to show the clear benefit of beta-blockers in the prevention of sustained VT or

FIGURE 4 Kaplan-Meier Analysis of Cardiovascular Death, Heart Failure Hospitalization, and Sustained Ventricular Tachycardia or Ventricular Fibrillation According to the 3 Groups on the Basis of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia Risk Score



(A) Cardiovascular death; **(B)** HF hospitalization; **(C)** sustained VT or VF. HF = heart failure; VF = ventricular fibrillation; VT = ventricular tachycardia.

VF or cardiovascular death. The role of antiarrhythmic drugs, such as sotalol or amiodarone, is considered to prevent the recurrence of VT in low- or intermediate-risk patients and reduce VT-required ICD therapy in high-risk patients (11,29). One-half of our patients received amiodarone therapy, and the others received class I antiarrhythmic drugs or sotalol. We could not prove the benefit of amiodarone to prevent the first recurrence of sustained VT in patients with sustained VT. However, these results could not counteract the effect of these drugs in the prevention of worsening of arrhythmia or disease progression, because this study was retrospective and small in size, and the backgrounds of these compared patients were not identical. Further evaluation will be required to confirm this issue.

STUDY LIMITATIONS. First, it was a retrospective, observational study at a single center. Some patients were lost to follow-up. Data concerning clinical condition at the time of MACEs were not available. In addition, there was a treatment bias.

Second, we could not assess the patients with ARVC/D whose first presentation of the disease was with SCD. In the early stage of ARVC/D, the diagnosis may be challenging (2).

Third, we used the parameters at the diagnosis of ARVC/D as the ARVC/D risk scoring. In this study, the time-dependent changes in markers, such as ECG and

the structures of the right and left ventricles, were not evaluated.

Fourth, the outcome assessment of each criterion in the 6 categories was limited by the small number of patients in our study.

Fifth, this study included patients from a single referral hospital who were enrolled over a long sampling period. The treatments that were administered were not controlled for during this long sampling period and may thus have influenced the outcome and prognoses of these patients. Moreover, the treatment strategies used for each patient were changed during the follow-up period. The potential confounding factors associated with time and era could not be completely excluded.

CONCLUSIONS

Our long-term observational study suggested that risk scoring on the basis of the 2010 TFC for diagnosis is useful to predict MACEs in patients with ARVC/D.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE 1: MACEs in patients with ARVC/D include cardiovascular death and worsened HF, as well as sustained VT or VF, which may lead to SCD.

COMPETENCY IN MEDICAL KNOWLEDGE 2: Risk scoring on the basis of the 2010 TFC for diagnosis is useful to predict MACEs in patients with ARVC/D.

TRANSLATIONAL OUTLOOK: Additional studies are needed to develop the risk stratification and management of cardiovascular death and worsening HF for patients with ARVC/D in the ICD era.

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KEY WORDS **arrhythmia, arrhythmogenic right ventricular cardiomyopathy/dysplasia, diagnosis, heart failure, prognosis, risk stratification**

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SHORT COMMUNICATION

Screening of sarcomere gene mutations in young athletes with abnormal findings in electrocardiography: identification of a *MYH7* mutation and *MYBPC3* mutations

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There is an overlap between the physiological cardiac remodeling associated with training in athletes, the so-called athlete's heart, and mild forms of hypertrophic cardiomyopathy (HCM), the most common hereditary cardiac disease. HCM is often accompanied by unfavorable outcomes including a sudden cardiac death in the adolescents. Because one of the initial signs of HCM is abnormality in electrocardiogram (ECG), athletes may need to monitor for ECG findings to prevent any unfavorable outcomes. HCM is caused by mutations in genes for sarcomere proteins, but there is no report on the systematic screening of gene mutations in athletes. One hundred and two genetically unrelated young Japanese athletes with abnormal ECG findings were the subjects for the analysis of four sarcomere genes, *MYH7*, *MYBPC3*, *TNNT2* and *TNNI3*. We found that 5 out of 102 (4.9%) athletes carried mutations: a heterozygous *MYH7* Glu935Lys mutation, a heterozygous *MYBPC3* Arg160Trp mutation and another heterozygous *MYBPC3* Thr1046Met mutation, all of which had been reported as HCM-associated mutations, in 1, 2 and 2 subjects, respectively. This is the first study of systematic screening of sarcomere gene mutations in a cohort of athletes with abnormal ECG, demonstrating the presence of sarcomere gene mutations in the athlete's heart.
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INTRODUCTION

Cardiac hypertrophy is an adaptive response of the heart, in which myocardial mass is increased beyond the normal range. Various causes could lead to cardiac hypertrophy, but in general it occurs in response to hemodynamic (volume or pressure) overload.[1] Although the most common etiologies of pathological cardiac hypertrophy are high blood pressure (hypertension) and valvular heart diseases, intensive endurance or strength training may result in physiological cardiac hypertrophy, called as 'athlete's heart'. Intensive prolonged exercise increases the aerobic capacity and oxygen consumption of skeletal muscles, followed by increases of diastolic ventricular filling and stroke volume, leading to a rise in maximum cardiac output and peak oxygen consumption.[2] Induced volume overload increases not only end-diastolic and end-systolic ventricular volumes but also the corresponding diameters. Consequently, compensatory myocardial hypertrophy develops without external stimuli.[2] The physiological left ventricular (LV) remodeling occasionally mimics certain pathological conditions associated with sudden cardiac death (SCD), such as hypertrophic cardiomyopathy (HCM).[2,3] Indeed, it is often difficult to distinguish the 'athlete's heart' from HCM, because there are overlaps in phenotypes between 'athlete's heart' and mild form of HCM.[2,4] It was reported that HCM had consistently been the most common cause of SCD in young athletes.[3] Therefore, early differential diagnosis of HCM from the physiological cardiac hypertrophy is important to prevent the unfavorable outcome in the athletes.

Participation in intense competitive sports can represent a potential risk factor of SCD in athletes with HCM, even when conventional markers are absent.[5] It was strongly suggested that the diagnostic ambiguity should be solved to reduce the risk for SCD, by using the paradigm of noninvasive parameters including findings in electrocardiogram (ECG), echocardiogram and genetic analysis of HCM-causing sarcomere gene mutations.[4,6,7] It has been reported that ECG

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is useful to detect early signs of HCM, but there is no report on the systematic screening of sarcomere gene mutations in a panel of trained athletes.[8] In this study, we analyzed four major HCM-causing sarcomere genes, *MYH7*, *MYBPC3*, *TNNT2* and *TNNI3*,[9] in 102 unrelated young Japanese athletes to clarify the genetic predisposing factors.

MATERIALS AND METHODS

Subjects

A total of 102 genetically unrelated Japanese trained athletes between the ages of 18 and 28 were the subjects in this study. None of the athletes in this study had apparent family history of HCM or SCD in first-degree relatives, but they had shown abnormal ECG changes or mild cardiomegaly found in a chest X-ray examination. ECG abnormalities include LV hypertrophy, ST-T changes and/or conduction block determined by the criteria as reported previously,[8] in which signs of LV hypertrophy in ECG were observed in 62 subjects. Echocardiographic-based LV hypertrophy was noted in only 7 out of the 102 athletes. In addition, genotyping data from our HCM cohort composing of 162 familial cases and 100 sporadic cases[10] were retrieved for specific mutations and previously obtained samples from patients with specific mutations and their family relatives were analyzed.

Written informed consent was obtained from each subject and the research protocol conformed to the ethical guidelines of the Declaration of Helsinki as reflected in *a priori* approval by the Ethics Committees of Juntendo University School of Health and Sports Science and Medical Research Institute of Tokyo Medical and Dental University.

Genetic analyses

DNA samples extracted from peripheral blood of subjects were used as templates to amplify each coding exon of *MYBPC3* (NM_000256), *TNNT2* (NM_001001430) and *TNNI3* (NM_000363), and exons 3–25 of *MYH7* (MN_000257) by PCR and the PCR products were analyzed for sequence variations by direct DNA sequencing on both strands by using Big Dye Terminator (version 3.1) Cycle Sequencing kit and ABI3130xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA), as described previously.[9]

Haplotype analyses of sarcomere gene mutations

Microsatellite analyses were done for MYOI with primers of 5′-NED-CAGG TCTAAACATGGGCG-3′ and 5′-ACATACCCTTTCTCACATTCAG-3′ and MYOII with primers of 5′VIC-GTGAGTAGATTGAGAGTTGTGGG-3′ and 5′-TCCTCTAACCCTACCCCCC-3′ in *MYH7* locus[11] and MYBPC3-CA with primers of 5′-FAM-GGTCAGGTCACCTAGCATAGCAT-3′ and 5′-GGCAGAT TCCTGATTTATTTGGC-3′ and D11S4109 with primers of 5′-PET-AGTTAGG AGACCTGGCATTTCTC-3′ and 5′-GAAGATCCCTCACAGACTCTCTCT-3′ in *MYBPC3* locus.[12] PCR fragments amplified with the primers were electrophoresed in the ABI3130xl DNA sequencer (Applied Biosystems) and the microsatellite alleles were defined as length of PCR fragments. The microsatellite polymorphisms were confirmed by sequencing PCR fragments amplified with nonlabeled primers.

In silico prediction of functional impacts

Pathogenicity of the identified mutation was predicted by using online programs, PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), Mutation Taster (http://www.mutationtaster.org/) and PROVEN (http://provean.jcvi.org/index.php). We searched for data concerning pathogenicity of mutations in the ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar/).

RESULTS

Identification of sarcomere gene mutations in trained athletes

To explore the possible presence of HCM-causing mutations in Japanese athletes, 102 trained athletes were tested for mutations in four sarcomere genes—*MYH7*, *MYBPC3*, *TNNT2* and *TNNI3*—because these four genes are the major HCM-causing genes in Japan.[9,10] In addition to 10 synonymous polymorphisms (rs2069540, rs2069542, rs735712, rs2231126 and rs7157716 in *MYH7*, rs11570058, rs379953 and rs1052373 in *MYBPC3*, rs3729547 in *TNNT2* and rs3729841 in TNNI3), four missense variations were identified (Table 1). Among the missense variations, Lys253Arg (c.758A>G, rs3730238) in *TNNT2* was found in seven subjects and it was a common polymorphism registered in the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/), the 1000 genomes database (http://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/) and the human genetic variation database (http://www.genome.med.kyoto-u.ac.jp/SnpDB/). The other three missense variants, Glu935Lys (c.2803G>A, rs121913639) in *MYH7* found in one case (Jun-49), and Arg160Trp (c.478C>T, rs193068692) and Thr1046Met (c.3137C>T, rs371061770) in *MYBPC3* found in two (Jun-3 and Jun-16) and two (Jun-75 and Jun-78) subjects, respectively, were previously reported as HCM-associated mutations.[13,14] The *MYBPC3* Thr1046Met mutation was found at rare frequencies in healthy subjects in the 1000 genomes database and human genetic variation database, although the *MYH7* Glu935Lys and *MYBPC3* Arg160Trp mutations were not found in Japanese populations registered in the 1000 genomes database and human genetic variation database (Table 1).

Table 1 Sarcomere gene mutations found in Japanese young athletes with abnormal ECG findings

Gene	*Exon number*	*Nucleotide change*	*Amino-acid change*	*PolyPhen-2*	*Mutation taster*	*PROVEN*	*dbSNP ID*	*ClinVar database description*	*1000 genomes database*[a]	*HGV database*[b]
MYH7	23	c.2803G>A	Glu935Lys	Possibly damaging (score: 0.766)	Disease causing	Deleterious (score: −2.691)	rs121913639	Pathogenic OMIM: 160760.0019	Not found	Not found
MYBPC3	4	c.478C>T	Arg160Trp	Probably damaging (score: 0.999)	Disease causing	Deleterious (score: −4.970)	rs193068692	Conflicting interpretation of pathogenicity	GMAF: 0.0006 JPT: 0.0000	Not found
MYBPC3	27	c.3137C>T	Thr1046Met	Benign (score: 0.007)	Polymorphism	Neutral (score: −1.416)	rs371061770	Conflicting interpretation of pathogenicity	GMAF: 0.0004 JPT: 0.0048	0.0110
TNNT2	14	c.758A>G	Lys253Arg	Benign (score: 0.146)	Polymorphism	Neutral (score: −1.523)	rs3730238	Benign	GMAF: 0.0974 JPT: 0.0721	0.0622

Abbreviations: ECG, electrocardiogram; GMAF, global minor allele frequency; HGV, Human genome variation; JPT, minor allele frequency in Japanese panels.
[a]Frequency of minor allele (variant) in 1000 genomes.
[b]Frequency of variant in Japanese subjects retrieved from human genetic variation database.