治薬の開発を目指して，QT延長症候群モデルの作製と薬剤スクリーニングが活発になされている．

最初の報告はドイツのグループからのものであった[5]．カリウムチャネル遺伝子変異(KCNQ1 R190Q)を認める8歳の少年からLQT type1特異的iPS細胞を樹立している．同iPS細胞から心筋細胞を分化誘導し，電気的な活動を記録するとカリウム電流の著明な低下が観察され，イソプロテレノールを用いると活動電位持続時間が延長した．また，臨床的にLQT type1の治療目的で用いられる薬剤であるβブロッカーの投与で不整脈の出現が抑制されている．本報告における遺伝子変異の機能解析等は過去に報告されているものであり，臨床像を含めて患者由来iPS細胞で再現されたという報告である．新規性は低いようにも思えるかもしれないが，世界で初めてのQT延長症候群由来iPS細胞の機能解析である．

続いては，イスラエルのグループからのものであった[6]．前述とは別のカリウムチャネルの遺伝子変異(KCNH2 A614V)を認める28歳の女性からLQT type2特異的iPS細胞を樹立している．本変異に関しても，過去の報告でカリウムチャネルの異常によりカリウム電流の低下を来すことがわかっている．iPS細胞由来心筋細胞から電気的な活動を記録するとカリウム電流の著明な低下が観察され，心電図上のQT延長に相当する電気的な変化も認められた．続いて活動電位持続時間の短縮や不整脈の出現を抑制できる薬剤スクリーニングを行った．カルシウムの細胞内流入が活動電位持続時間や不整脈の出現に関与していることからカルシウムチャネル阻害薬であるニフェジピンを投与したところ，iPS細胞由来心筋細胞において治療効果が確認された．またカリウムチャネル開口薬であるピナシジルを投与したところ，同様にiPS細胞由来心筋細胞において治療効果が確認された．本報告はLQT type2患者からiPS細胞を樹立し，疾患モデルを作製し薬剤スクリーニングが可能であることを示唆した報告である．

次の報告は，アメリカのグループからのものである[7]．本研究においては，カルシウムチャネル遺伝子変異（CACNA1C G406R）を認める2症例からTimothy症候群（LQT type8）特異的iPS細胞を樹立している．本変異は電位依存性のカルシウムチャネルの不活性化障害を来すことが知られているが，どのようにQT延長症候群や不整脈を来すかなどは不明であった．iPS細胞由来心筋細胞から電気的な活動記録をすると，不規則な心筋細胞収縮，過剰なカルシウムの細胞内流入，活動電位持続時間の延長などが確認された．またロスコビチンという薬剤がカルシウムチャネルの電位依存性不活性化を増強させることが知られており，ロスコビチンをiPS細胞由来心筋細胞に添加したところ，不規則な心筋細胞収縮，過剰なカルシウムの細胞内流入，活動電位持続時間の延長などが改善されることが確認された．同薬剤を，そのまま患者に用いることは難しいと考えられるが，似た作用を有する薬剤の開発などが有益であることが示唆された．

われわれは新規変異（KCNQ1 1893delC）を有するLQT type1患者よりiPS細胞樹立を行い，機能解析を行った[8]．過去の疾患iPS細胞の機能解析の多くは既知の変異を有する患者からの樹立と機能解析で，過去の報告と整合性が得られているというものである．われわれは臨床でしばしば認める新規変異が疾患iPS細胞で機能解析できるかを検討した．iPS細胞を樹立し，心筋細胞に分化誘導を行い，電気生理学的性質を比較検討した．多電極記録（MEA）システムを用いて分化誘導した心筋細胞の細胞外電位を記録したところ，QT延長症候群患者由来の心筋細胞では，体表面心電図のQT時間に相当するとされる細胞外電位持続時間（FPD）の有意な延長が確認された．次に遅延整流性Kチャネル遮断薬の薬物反応試験を行ったところ，即時型遅延整流性Kチャネル（IKr）遮断薬であるE4031投与により，QT延長症候群患者由来の心筋細胞ではFPDの延長とともに，有意に早期後脱分極（EAD）様の不整脈が誘発された．さらに高濃度のE4031投与により，健常

者には見られない多形性心室頻拍（TdP）様の不整脈が誘発された．この結果は，本患者におけるK電流は，健常者に比しIKrに強く依存していることを示唆している．また緩徐型遅延整流性Kチャネル（IKs）遮断薬であるChromanol 293Bの投与を行ったところ，健常人由来の心筋細胞のFPDが有意に延長したのに対し，患者由来の心筋細胞ではFPDの延長が見られなかった．これらの結果より，IKsチャネルの機能喪失が疑われた．またβ受容体刺激薬の投与により，QT延長症候群患者由来の心筋細胞において心室頻拍（VT）様の不整脈が誘発され，β受容体遮断薬の投与により不整脈が停止する現象が確認され，同様にIKsチャネルの機能不全を示唆する所見と考えられた．直接IKs電流を測定するためにパッチクランプ法をiPS細胞由来心筋細胞に行いIKs電流を記録したところ，1893delCのヘテロ変異を有する患者特異的iPS細胞由来心筋細胞において有意なIKs電流の低下が確認された．また患者特異的iPS細胞由来心筋細胞のIKsチャネルの免疫染色により，1893delCのヘテロ変異において細胞膜表面のIKsチャネルの発現が著明に低下していることが確認され，チャネルのtrafficking異常が確認された．以上のことより，患者特異的iPS細胞を用いて新規変異の機能解析が可能であることが示唆された．

## 心筋症特異的iPS細胞の作製と解析

LEOPARD症候群は常染色体優性遺伝とされているが，孤発例が多い稀な疾患である．本症候群は多発性黒子，心伝導障害，眼間開離，肺動脈狭窄，外陰部異常，精神遅滞，感音性難聴などを特徴とし，心病変が最も生命予後に影響を与える．LEOPARD症候群の90％程度に，SHP2という脱リン酸化酵素をコードしているPTPN11遺伝子異常が認められる．PTPN11遺伝子の異常というのはSHP2という脱リン酸化酵素の機能異常を引き起こし，増殖因子やサイトカインなどの間違った情報が細胞内に伝わり，細胞の増殖，生存や分化などのさまざまな生命現象に異常を来す．本研究においてもPTPN11遺伝子異常を認めている典型的LEOPARD症候群である[9]．LEOPARD症候群の80％は肥大型心筋症を呈すことが知られ，なぜ肥大型心筋症を呈するかを解明すること，そして肥大型心筋症の発症を止める治療方法を研究・開発することが重要である．まずLEOPARD症候群iPS細胞由来心筋細胞が，試験管内で肥大するかどうかを検証している．LEOPARD症候群iPS細胞由来心筋細胞は，健常人と比し肥大していくことが判明した．LEOPARD症候群においてはRas-MAPキナーゼ経路という細胞内タンパクのリン酸化を調節して細胞の増殖，生存や分化などに強く影響を及ぼしているシグナル経路が特に異常を来していることが知られている．すなわちLEOPARD症候群においては，増殖因子が細胞に作用したときにこれらの細胞内シグナルの活性化が低下していると言われている．このことを検証するために，iPS細胞に線維芽細胞増殖因子を作用させ，Ras-MAPキナーゼ系の活性化が健常人とLEOPARD症候群に差があるかを検討した．健常人由来iPS細胞においては，線維芽細胞増殖因子を作用後には速やかにRas-MAPキナーゼ系の活性化が認められている．一方でLEOPARD症候群患者由来iPS細胞に線維芽細胞増殖因子を作用させると，Ras-MAPキナーゼ系の活性化反応性が著明に低下していた．LEOPARD症候群の原因というのはRas-MAPキナーゼ系の活性化の低下であると言われているが，このことがiPS細胞を用いた研究により試験管の中で再現可能であることが確認された．

拡張型心筋症は最も頻度が多く予後が悪い遺伝性心筋疾患の一つである．サルコメア構成タンパクの一つであるトロポニンTに変異のある（TNNT R173W）拡張型心筋症患者よりiPS細胞を作成した後に機能解析を行い報告されている．同報告においては，患者特異的iPS細胞由来心筋細胞において細胞内$Ca^{2+}$濃度の恒常性に異常を認め，また収縮能の

低下を認めている．同細胞においてβブロッカーの投与や，Serca2a遺伝子の過剰発現により機能回復を認めている．同報告においては疾患特異的iPS細胞を用いることにより，in vitroにおいて疾患表現形の再現ができ疾患発症機序の解析やドラッグスクリーニングの基盤的技術となりえる可能性が示唆されている．

## 今後の循環器疾患特異的iPS細胞の疾患解析における展望

これらの研究は一見すると似た研究のように見えるが，これまでの研究とは根本的に違う側面がある．これまでの研究は，患者の遺伝子変異を探索し，発見したとしてもヒトの生きた心筋細胞を用いた解析というのは不可能であった．そのために，これらの変異遺伝子を人工的に培養細胞（ヒト心筋細胞は用いることができないので，他の細胞種）に導入して変異遺伝子の電気生理学的解析や，実験動物（マウスなど）に遺伝子導入をすることによる遺伝子改変動物などを用いて解析することしかなかった．これらの実験系は極めて人工的なものであり，本当にヒトの病気の再現ができているかどうかはわからなかった．そのために疾患解析や治療方法の開発は思うように進んでこなかった．今後は，さまざまな難治性疾患を対照に患者特異的iPS細胞が作製され，疾患モデル作製の確認と薬剤スクリーニングが行われていくと思われる．1日も早く臨床応用され患者にフィードバックされていくことが期待される．

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Original Article

# The generation of induced pluripotent stem cells from a patient with *KCNH2* G603D, without LQT2 disease associated symptom

Shinichiro Okata[1,2], Shinsuke Yuasa[1], Teiichi Yamane[3], Tetsushi Furukawa[2] and Keiichi Fukuda[1]

*1) Department of Cardiology, Keio University School of Medicine, Japan.*
*2) Department of Bio-informational Pharmacology, Medical Research Institute, Tokyo Medical and Dental University, Japan.*
*3) Department of Cardiology, The Jikei University School of Medicine, Tokyo, Japan*

**The long QT syndrome type 2 (LQT2) is inheritable life threatening arrhythmic disorder and one of the most common genetic variants in long QT syndrome. There are some indications for treatment of the patients with LQT2 but it is impossible to completely prevent fatal arrhythmia. To develop novel therapy for the patients with LQT2, it has been desired to generate disease-specific and patient-specific disease model. Human induced pluripotent stem (iPS) cells are somatic cell-derived pluripotent stem cells with infinite proliferation ability and multipotency. Patient-specific iPS cells can be derived from patient somatic cells, have all genomic information encoded in patient's genome including mutation and all SNPs, and can be ideal disease models of the patients. To generate disease model for LQT2 by iPS cells, we should firstly generate iPS cells from the patient with LQT2 and confirm the genomic mutation in iPS cells. In this study, we showed the successful generation of iPS cells from a patient with *KCNH2* G603D mutation. The patient specific iPS cells properly expressed stem cell markers, such as NANOG and OCT3/4. We also confirmed that the *KCNH2* G603D (G1808A) mutation was taken over in patient specific iPS cells. These patient-specific iPS cells may contribute to the future analysis for disease pathogenesis and drug innovation.**

**Key words:** iPS cell, Long QT syndrome, Disease modeling

Corresponding Author: Shinsuke Yuasa, M.D, Ph.D.
Assistant Professor, Department of Cardiology, Keio University School of Medicine, 35 Shinanomachi Shinjuku-ku, Tokyo, 160-8582, Japan.
Tel: +81-3-5363-3373 Fax: +81-3-5363-3875
E-mail: yuasa@a8.keio.jp
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## Introduction

The generation of induced pluripotent stem (iPS) cell is firstly reported in 2006 with great surprise[1]. Human iPS cells are similar to human embryonic stem (ES) cells in terms of proliferation and differentiation ability, and can be generated from adult somatic cells[2,3]. Now we can easily generate iPS cells from patient's somatic cells and those iPS cells have all genomic information of the patient genome[4]. Many human diseases are caused by genomic mutation. Disease modeling using human iPS cells is newly emerged research field to analyze genetic human diseases[7]. Actually, there are many fatal genetic diseases without effective therapy. To develop newly effective therapies for those diseases, first of all we have to generate disease models. In the past, there had been solely animal models of human genetic disease, such as specific gene knockout mice, transgenic mice and autochthonous diseased animals. Although those models gave us many valuable information regarding to the mechanisms of human genetic diseases, most crucial problem is that those models are not human. So it is often difficult to model human diseases using experimental animals. In another important point of view, among humans, each individual shows highly rich in genomic diversity in terms of racial differences and single nucleotide polymorphisms (SNPs). So it has been highly expected to generate not only disease-specific models, but also disease-specific and patient-specific disease models. To

generate patient-specific disease models, now we can use iPS cells.

Long QT syndrome is one of the most common fatal cardiac arrhythmic disorders[8]. Recent molecular and electrophysiological examination established the fundamental disease concepts. But there is no definitive therapy invented so far. Here again, one of the most important points in drug discovery is to generate excellent disease models. Current experimental models for arrhythmic disorders mostly depend on animal model and heterologous expression system in human non-cardiomyocytes or non-human cardiomyocytes. The long QT syndrome type 2 (LQT2) is one of the most common genetic variants in long QT syndrome, and accounts for approximately 40% of genotyped patients[9]. LQT2 is caused by mutation of a potassium channel gene, *hERG* (human ether-a-go-go related gene), now referred to *KCNH2*. To generate the physiological cardiac action potential in human cardiomyocytes, in addition to inward sodium and calcium currents, several potassium currents are notably involved. The inward-rectifier background current (*I*K1), the rapidly activating and inactivating transient outward current (*I*to), and the ultrarapid (*I*Kur), rapid (*I*Kr), and slow (*I*Ks) components of delayed rectifier currents. Those potassium currents have pivotal roles in electrophysiological homeostasis in human cardiomyocytes and the mutations in potassium current genes result in several human arrhythmic disorders. *KCNH2* encodes the a-subunit of the *I*Kr channel, and membrane depolarization induced by strong inward currents produces a sequence of conformation changes within the channel that allows permeation of potassium ions. As a clinical phenotype, LQT2 is likely to result in cardiac events during exercise or emotional stress in more than half cases and during rest or sleep in some cases. More specifically, an auditory stimulus (telephone, alarm clock, ambulance siren, etc) can be a specific trigger in LQT2[10]. β-blocker use significantly reduces the risk of cardiac arrhythmic events in LQT2. And maintenance of the extracellular potassium concentration by long-term oral potassium supplementation is also reported to be effective because it shortens the QT interval in LQT2 patients. Besides those therapies, we cannot fully prevent sudden cardiac death in LQT2 patients. So we have to carry on the drug development for LQT2 by using LQT2 disease model.

In this study we showed the generation of iPS cells from a patient with *KCNH2* G603D mutation. These patient-specific iPS cells may contribute to future analysis for disease pathogenesis and drug innovation.

## Materials and Methods

### Patient consent

All subjects provided informed consent for blood testing for genetic abnormalities associated with hereditary long QT syndrome. The isolation and use of patient somatic cells was approved by the Ethics Committee of Keio University (approval no. 20-92-5) and the Ethics Committee of Tokyo Medical and Dental University (approval no. 2009-27), and was performed only after the patient and the parent had provided written informed consent.

### Generation of human iPS cell

Human iPS cells were established from T lymphocytes as described previously[11,12]. Briefly, peripheral blood mononuclear cells (PBMCs) were separated by the centrifugation of heparinized whole blood sample obtained, using a Ficoll-Paque PREMIUM (GE Healthcae) gradient. The mononuclear cells were seeded on the anti-human CD3 antibody (BD Pharmingen)-coated 6-well plates in 2 mL GT-T502 (KOJIN BIO) medium per well, and incubated for 5 to 7 days until the activated T cells reached 80% to 90% confluent. Activated PBMCs were collected and transferred at $1.5 \times 10^6$ cells per well to a fresh anti-CD3 antibody coated 6-well plate, and incubated for an additional 24 hours. Then, the solution which contained sendai virus vectors individually carrying each of *OCT3/4*, *SOX2*, *KLF4*, and *c-MYC* were added at 10 MOI. After 24 hours of infection, the medium was changed to fresh GT-T502 medium, and the cells were collected and split at $5 \times 10^4$ cells into 10 cm-plates pre-seeded with mouse embryonic fibroblasts (MEFs) at more 24 hours after infection. After an additional 24 hours of incubation, the medium was changed to human iPS cell medium supplemented with 4 ng/mL of bFGF. The cells were cultured for another 20 days. On day 25, ES cell-like colonies were dissociated mechanically and transferred to a 24 well plate on the MEF feeder cells.

### iPS cell culture

Human iPS cells were maintained on irradiated MEF feeder cells in human iPS cell culture medium, consisting of 80% DMEM/F12 (Sigma-Aldrich), 20% KO Serum Replacement (Invitrogen), 4 ng/mL basic fibroblast growth factor (bFGF; WAKO), 2 mmol/L L-glutamine (Invitrogen), 0.1 mmol/L non-essential amino acids (Sigma-Aldrich), 0.1 mmol/L 2-mercaptoethanol, 100 U/mL penicillin, and 100 μg/mL streptomycin

(Invitrogen). The human iPS cell medium was changed every 2 days and the cells were passaged using 1 mg/mL collagenase IV (Invitrogen) every 5-7 days. 293FT cells were cultured in DMEM supplemented with 10% FBS (Nichirei Bioscience), 1 × $10^{-4}$ M non-essential amino acids (NEAA; Sigma-Aldrich), 2 mmol/L L-glutamine (Invitrogen), 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen).

### Immunocytochemistry

Immunostaining was used to analyze the expression of pluripotency markers. Cells were placed on a 35 mm glass-bottomed dish (IWAKI) before being fixed with 4% paraformaldehyde for 30 min at 4℃. The cells were then rinsed three times with phosphate-buffered saline (PBS) and permeabilized with 0.2% Triton-X 100 in PBS. The cells were then washed and blocked with Immunoblock (DS Pharma) three times for 5 min each time. Samples were incubated overnight at 4℃ with each of the primary antibodies: anti-NANOG (1:200 dilution; ab21624; Abcam), anti-OCT3/4 (1:100 dilution; sc-5279; Santa Cruz). Following incubation with primary antibodies, samples were incubated at room temperature for 1 h with the following secondary antibodies: Alexa Fluor 488 chicken anti-mouse IgG (1:200 dilution; A21200; Invitrogen), and Alexa Fluor 594 goat anti-rabbit IgG (1:200 dilution; A11037; Invitrogen). After cells had been washed by PBS, samples were mounted using Vectashield Hard Set Mounting Medium with 4′,6′-diamidino-2-phenylindole (DAPI) (Vector Laboratories) for nuclear staining. Images were obtained using a × 10 objective lens (NA = 0.45) on a fluorescence microscope (BZ-9000; Keyence).

### Genome sequencing

DNA sequencing was used to confirm the presence of the LQT2 mutation in patient-derived iPSCs. Genomic DNA was isolated using a Gentra Puregene Cell Kit (Qiagen) and the region encoding *KCNH2*, including the mutation, was amplified using polymerase chain reaction (PCR) with the following primer set: 5′-TAGCCTGCATCTGGTACGC-3′ (forward) and 5′-GCCCGCCCCTGGGCACACTCA-3′ (reverse). The PCR product (277 bp) was electrophoresed on a 1% agarose gel and purified using a Wizard SV Gel and PCR Clean-Up System (Promega). The purified PCR product was sequenced with original primers.

## Results

### Novel *KCNH2* mutation

A 10-year-old man was given surgery for funnel chest, without any symptoms. Before operation, routine surface electrocardiogram (ECG) was recorded (Figure 1A). At that time, QT interval prolongation at ECG was firstly pointed out. The patient had no history of previous syncopal episode, palpitation or other cardiac symptoms. But his mother showed repetitive syncopal episodes at rest, triggered by sudden loud noises such as alarm clock and telephone call. Exercise testing shortened the QT interval and epinephrine challenge induced the QT interval prolongation and the form of polymorphic ventricular tachycardia called torsades de pointes. She underwent the genetic test which showed the novel *KCNH2* G603D (G1808A) mutation. Therefore he also underwent the genotype analysis which also showed the novel *KCNH2* G603D (G1808A) mutation (Figure 1B).

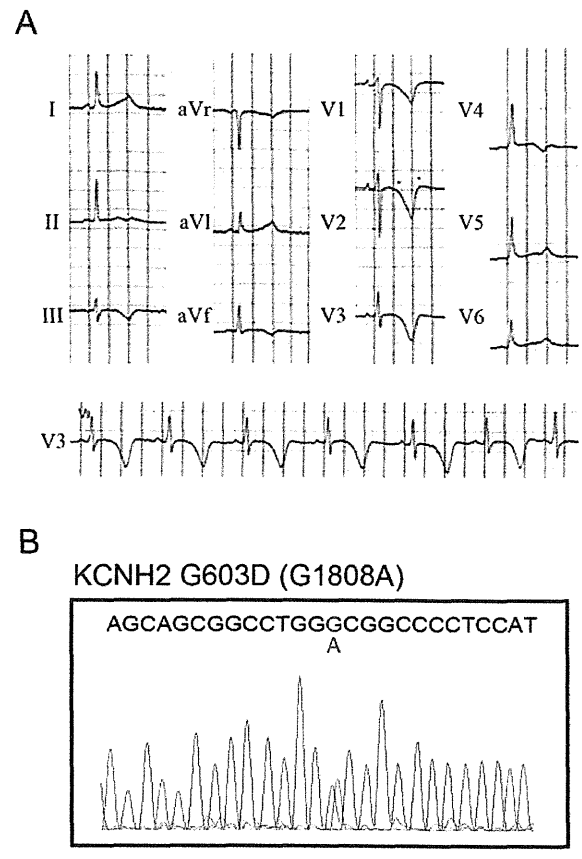


**Figure 1. Novel *KCNH2* mutation in the patient.**
**A.** Electrocardiogram from the patient during sinus rhythm. **B.** Sequence analysis of genomic *KCNH2* in the patient. The novel *KCNH2* G603D (G1808A) mutation.

### iPS cell generation from a patient with *KCNH2* mutation

To generate iPS cells, we used peripheral blood cells as donor somatic cells from the patient. Separated peripheral mononuclear cells were stimulated by CD3 antibody and IL-2 to activate T lymphocytes. And activated T lymphocytes were reprogrammed by using Sendai virus carrying *SOX2*, *OCT3/4* (also known as *POU5F1*), *KLF4*, and *MYC*. Several clones were generated, expanded and stored. All iPS cell lines showed typical iPS cell morphology and expressed human pluripotency markers (Fig. 2a and b). These iPS cells were moved to petri-dishes and formed embryoid bodies with spontaneous beating, which indicated that these patient-specific iPS cells properly differentiated into beating cardiomyocytes *in vitro*.

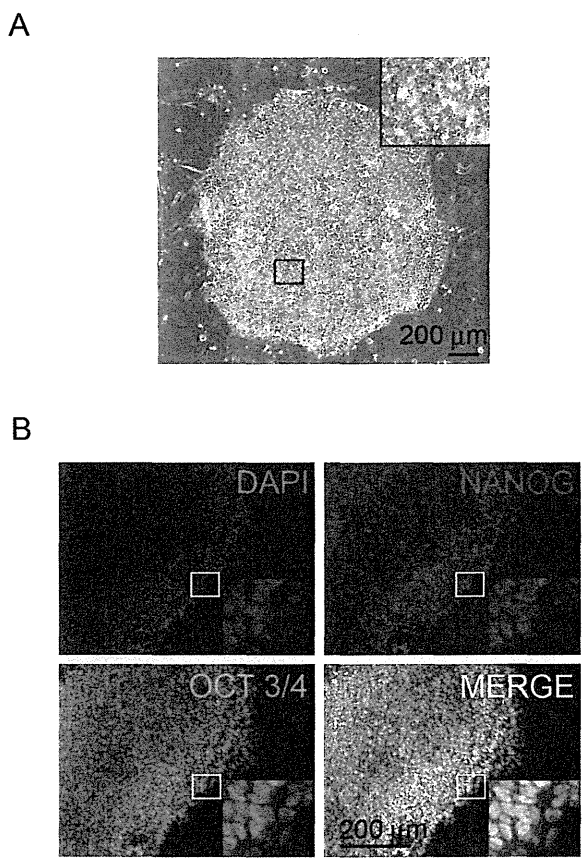


**Figure 2. Generation of iPS cells from the patient with *KCNH2* G603D (G1808A) mutation.**
**A.** Representative phase-contrast image of patient-specific iPS cell colony. Black box in figure is shown at a higher magnification in the inset. **B.** Immunofluorescence staining for stem cell markers (OCT3/4, NANOG and DAPI) in the patient-specific iPS cell colony. White boxes in each figure are shown at a higher magnification in the inset.

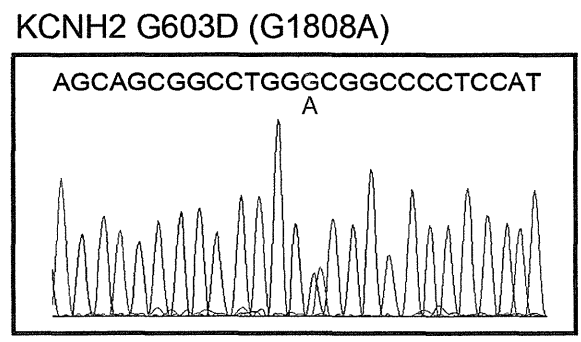


**Figure 3. Novel *KCNH2* mutation in the patient-specific iPS cell colony.**
Sequence analysis of genomic *KCNH2* in the patient-specific iPS cell colony. The novel *KCNH2* G603D (G1808A) mutation.

### KCNH2 mutation in iPS cells derived from a patient with *KCNH2* mutation

To confirm that the generated iPS cells have a same mutation as the patient has, the genotype analysis was performed. It revealed the *KCNH2* G603D (G1808A) mutation was taken over (Figure 3).

## Discussion

In the present study, we successfully generated iPS cells from a patient with the *KCNH2* G603D mutation who didn't exhibit any symptoms but showed prolonged QT interval at ECG. This patient is still young and may exhibit the cardiac symptom in the future. In real clinical setting, it is very important to know whether patients with genetic mutation will develop severe diseases or not. If we can predict the severity in the future disease manifestation, we can easily determine to do those patients, e.g., intensive care, exercise limitation, no medication and so on. So it is valuable to establish patient-specific disease model and develop the systems to evaluate the characteristics of patient-specific diseases. Patient-specific iPS cells may contribute to these concepts.

In terms of disease modeling using iPS cells, LQT2 is firstly noticed[13-15] because LQT2 is one of the most common genetic variants in long QT syndrome and there is no definitive therapy for LQT2. And drug discovery often failed at the expense of immense cost, due to the side effects related to HERG which is LQT2 associated gene product, following QT prolongation and lethal arrhythmia. First report showed the generation of LQT2 patient-specific iPS cells harboring A614V missense mutation in the *KCNH2* gene, which was previously shown to lead to a significant reduction of

IKr which is responsible for LQT2[15]. Detailed whole-cell patch-clamp and multi-electrode array (MEA) recordings revealed significant prolongation of the action potential duration in LQT2 iPS cell-derived cardiomyocytes. Voltage-clamp studies confirmed a significant reduction of the cardiac potassium current IKr. LQT2 iPS cell-derived cardiomyocytes also showed marked arrhythmogenicity, characterized by early-after depolarizations (EAD) and triggered arrhythmias. And calcium-channel blockers, $K_{ATP}$-channel openers and late sodium channel blockers ameliorate the disease phenotype in LQT2 iPS cell-derived cardiomyocyte. Second report showed the generation of LQT2 patient-specific iPS cells harboring G1681A missense mutation in the *KCNH2* gene, which was also previously shown to lead to a significant reduction of IKr[14]. MEA and patch-clamp recording showed prolonged field/action potential duration in LQT2 iPS cell-derived cardiomyocytes. LQT2 iPS cell-derived cardiomyocytes developed EADs when challenged with the E4031 (IKr blocker) and isoprenaline. Action potential duration and EAD were ameliorated by propranolol, nadolol, nicorandil and an activator of hERG, PD118057. The other report showed the generation of LQT2 patient-specific iPS cells harboring R176W missense mutation in the *KCNH2* gene[13]. The *KCNH2* R176W mutation is relatively common variant and was reported to have the frequency of 0.5% in apparently healthy individuals. Although there were some reports showed that this mutation was related to long QT syndrome, the majority of these individuals were completely asymptomatic and unaware of their carrier status, as is the case with this patient. In heterologous expression system, R176W reduced hERG tail current density by ~75%, but upon coexpression with wild type the difference in current densities was nullified. But the action potential duration of LQT2 iPS cell-derived cardiomyocytes was significantly longer than that of control, and IKr density of the LQT2 iPS cell-derived cardiomyocytes was significantly reduced. Consistent with clinical observations, the LQT2 iPS cell-derived cardiomyocytes demonstrated a more pronounced inverse correlation between the beating rate and repolarization time compared with control cells. Additionally, LQT2 iPS cell-derived cardiomyocytes were more sensitive than controls to potentially arrhythmogenic drugs, including sotalol, and demonstrated arrhythmogenic electrical activity.

In this study we chose a patient with a novel mutation in the *KCNH2* G603D. Patient showed QT interval prolongation but never showed any symptoms. To treat properly and prevent cardiac lethal arrhythmia, we believe it is valuable to generate experimental methods to predict how susceptible to lethal arrhythmia in various stimulations in those patients. Actually, many genomic variations such as many SNPs in each patient's genome affect disease manifestation even in the patients with major functional mutation and may be the cause of low penetrance for long QT syndrome[16]. So it is difficult to accurately predict disease susceptibility only by genomic information such as patient's mutation and SNPs. Patient-specific iPS cells have all genomic information encoded in patient's genome including mutation and all SNPs, and can be ideal disease models for the patients. Actually, each patient shows different disease phenotype and drug response, which is also partly due to patient genomic variation. In terms of personalized medicine, we can also try many notorious and beneficial drugs on patient-specific iPS cell-derived cardiomyocyte and predict disease susceptibility before the patient will use those drugs. To generate patient-specific disease models using iPS cells, we established the patient-specific iPS cells and confirmed the patient-specific iPS cells had the same mutation as the patient.

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ORIGINAL ARTICLE

# Anatomical variations affect radial artery spasm and procedural achievement of transradial cardiac catheterization

**Yohei Numasawa · Akio Kawamura · Shun Kohsaka · Masashi Takahashi · Ayaka Endo · Takahide Arai · Yohei Ohno · Shinsuke Yuasa · Yuichiro Maekawa · Keiichi Fukuda**

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**Abstract** Transradial cardiac catheterization (TRCC) has unique technical challenges such as access difficulty related to anatomical variations and/or radial artery (RA) spasm. We sought to evaluate the incidence of anatomical variations of the RA and whether they would affect RA spasm and procedural achievement of TRCC. A total of 744 consecutive patients who underwent TRCC were analyzed by routine radial arteriography. Anatomical variations were defined as abnormal origin of the RA and/or radioulnar loop and/or tortuous configuration. RA spasm was defined as >75 % stenosis at first radial arteriography. Overall, anatomical variations were noted in 68 patients (9.1 %), including 39 cases of abnormal origin (5.2 %), 11 cases of radioulnar loop (1.5 %), and 42 cases of tortuous configuration (5.6 %). Transradial procedures failed in 26 patients (3.5 %), and more frequently in patients with anatomical variation than in those with normal anatomy (23.5 % vs 1.5 %, $P < 0.001$). Importantly, on multivariate analysis the presence of anatomical variation was a distinct predictor of transradial procedure failure (odds ratio (OR) 17.80; 95 % CI 7.55–43.73; $P < 0.001$). RA spasm was observed in 83 patients (11.2 %), and more frequently in patients with anatomical variation than in those with normal anatomy (35.3 % vs 8.7 %, $P < 0.001$). Anatomical variation (OR 4.74; 95 % CI 2.61–8.47; $P < 0.001$) and female gender (OR 2.23; 95 % CI 1.01–4.73; $P = 0.041$) were distinct predictors of RA spasm. Anatomical variations were observed in 9.1 % of the patients, and strongly correlated with RA spasm and procedural achievement of TRCC.

**Keywords** Radial artery · Transradial approach · Vascular access · Spasm

## Introduction

Cardiac catheterization is an essential diagnostic and therapeutic method in evaluating cardiac disease. In recent years, the radial artery (RA) has been considered a safe and useful vascular access site for cardiac catheterization in comparison with the conventional transfemoral approach [1–4]. In general, access-site complications are significantly lower in the transradial approach than in the transfemoral approach. Furthermore, transradial cardiac catheterization (TRCC) shortens hospital stay and improves postprocedure quality of care [5, 6]. The RA has also been considered a reasonable alternative to the saphenous vein graft in coronary artery bypass surgery [7, 8].

However, TRCC also has unique technical challenges, such as access difficulty related to anatomical variations [9–11], RA spasm [12–15], and RA occlusion [16, 17]. Anatomical variations of the RA, such as abnormal RA origin, radioulnar loop, tortuous configuration, and severe RA spasm, occasionally lead to procedural failure. Some previous studies reported that the frequency of anatomical variations of the RA may differ between Asian and Western populations [11, 18]. Furthermore, there is a paucity of data on the relationship between RA spasm and anatomical variations. Thus, we investigated the incidence of anatomical variations of the RA and whether they would affect RA spasm and procedural achievement of TRCC.

Y. Numasawa (✉) · A. Kawamura · S. Kohsaka · M. Takahashi · A. Endo · T. Arai · Y. Ohno · S. Yuasa · Y. Maekawa · K. Fukuda
Department of Cardiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
e-mail: numasawa@cpnet.med.keio.ac.jp

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## Patients and methods

### Study population

This was a single-center, prospective study in Japan. This study included 756 consecutive patients with normal Allen test results who underwent TRCC from January 2009 to April 2011. Exclusion criteria were absence of a radial pulse, abnormal Allen test results, and patients undergoing hemodialysis. Patients' characteristics, RA anatomy, RA diameter, and procedural outcomes including spasm were assessed.

### Transradial procedure

After local subcutaneous anesthesia with 2 % lidocaine, the RA was punctured with a 20-gauge needle, and a 16-cm hydrophilic-coated sheath (Terumo, Japan) was inserted with gentle manipulation. The size of the sheath was selected at the discretion of the operator. Basically, a 4-F or 5-F sheath was used for diagnostic coronary angiography, and a 6-F sheath was used for coronary intervention. After sheath insertion, 1 mg of isosorbide dinitrate and 5000 IU of heparin were administered through the sheath to prevent RA spasm and thrombotic complications. After administration of isosorbide dinitrate and heparin, retrograde radial arteriography around the elbow joint via a 16-cm sheath was performed in anteroposterior projection to evaluate the RA anatomy from the edge of the sheath to the mid portion of the brachial artery and the RA diameter. We injected half-diluted contrast to prevent contrast-induced spasm. When the transradial procedure failed, it was at the discretion of the operator to attempt the contralateral RA approach, transbrachial approach, or transfemoral approach. Additional doses of heparin were provided based on an ACT level of >300 s during percutaneous coronary intervention (PCI). After the procedure, the arterial sheath was removed immediately and a TR band (Terumo, Tokyo, Japan) was applied for hemostasis in all patients.

### Definition of anatomical variations

In this study, anatomical variations of the RA were defined as abnormal origin of the RA and/or radioulnar loop and/or tortuous configuration [9–11, 16, 19]. The RA normally originated from the brachial artery at the level of the

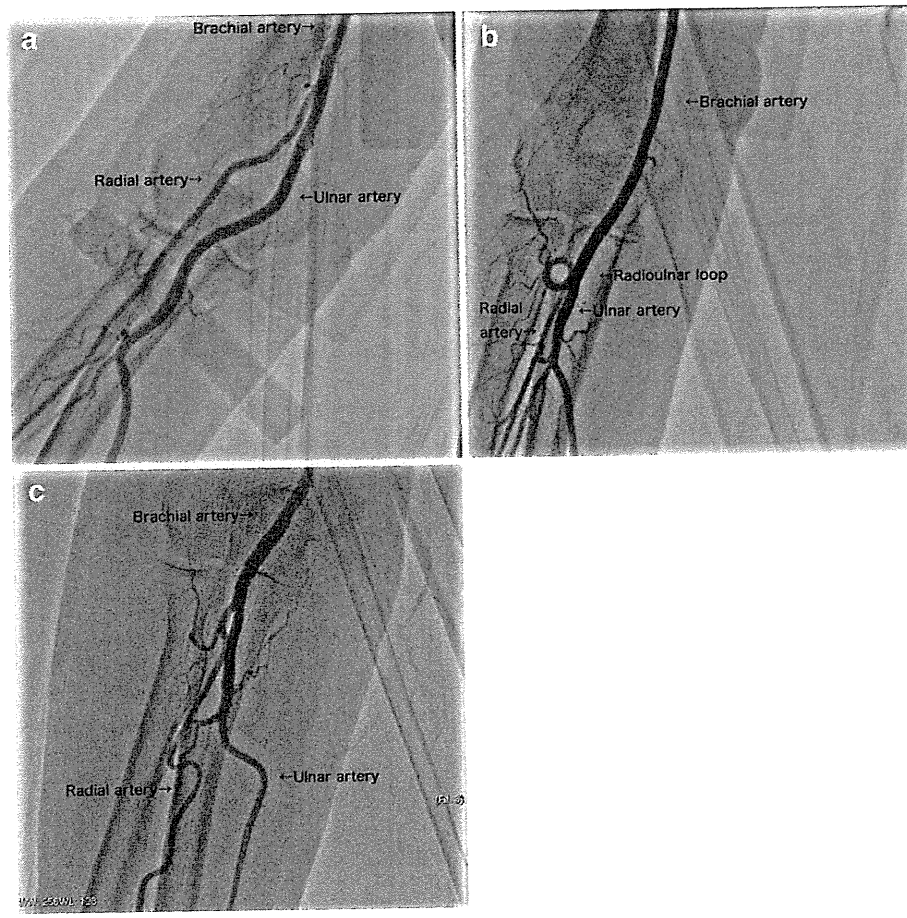


**Fig. 1** Anatomical variations of the radial artery, defined as an abnormal origin of the radial artery (**a**) and/or radioulnar loop (**b**) and/or a tortuous configuration of >90° (**c**)

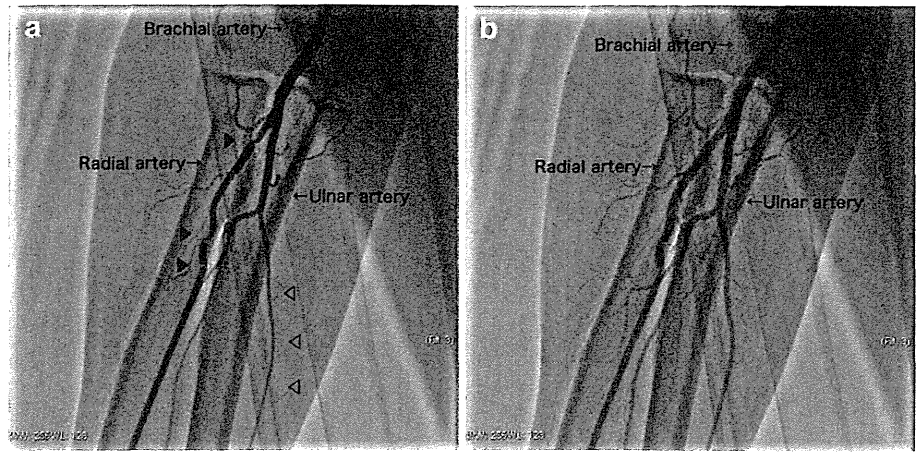


**Fig. 2** Radial artery spasm, defined as >75 % stenosis (*black arrowheads*) at first radial arteriography after administration of isosorbide dinitrate and heparin (**a**). The ulnar artery was also diffusely spastic in this case (*white arrowheads*). Arterial spasm was ameliorated after additional administration of isosorbide dinitrate (**b**)

antecubital fossa. Forearm arterial patterns were classified by Uglietta and Kadir [20]. The site of abnormal origin was determined relative to the intercondylar line of the humerus. This line represented the proximal border of the antecubital fossa. Bifurcation of the brachial artery proximal to this line was considered to be the abnormal origin of the RA (Fig. 1a). A radioulnar loop was defined as the presence of a full 360° loop of the RA distal to the bifurcation of the brachial artery (Fig. 1b). Tortuous configuration of the RA was defined as the presence of maximum angulation of >90° (Fig. 1c).

### Definition of RA spasm

Radial artery spasm was defined as >75 % stenosis at first radial arteriography after administration of isosorbide dinitrate and heparin [12] (Fig. 2a, b).

### Measurement of RA diameter

Proximal RA diameters of the patients were measured at a point 2 cm proximal from the sheath edge. Radial arteriograms were quantitated using the computer-based quantitative coronary angiography (QCA) system Centricity CA1000 (GE Healthcare, Burlington, VT, USA). The outer diameter of the arterial sheath was used for calibration purposes. The outer diameters of the 4-, 5-, and 6-F sheaths (Terumo, Japan) were 1.86, 2.20, and 2.48 mm, respectively. The ratio of sheath outer diameter to RA inner diameter was calculated to measure the RA diameter.

### Data analysis

JMP version 8.0 (SAS Institute, Cary, NC, USA) was used for statistical analysis of the data. Continuous variables were expressed as mean and standard deviation (SD). Categorical variables were expressed as a percentage. Continuous variables were compared using Student's *t* test, and the differences between categorical variables were examined using the Chi-square test. Multivariate logistic regression was performed to evaluate the distinct predictors of transradial procedure failure and RA spasm. A *P* value of less than 0.05 was considered statistically significant.

## Results

### Study population and clinical characteristics

The baseline clinical characteristics of the 756 patients are expressed in Table 1. Mean age was 67.6 ± 11.5 years, and 632 cases (83.6 %) were male. RA puncture was unsuccessful in 12 of 756 cases (1.6 %). Routine retrograde radial arteriography was obtained in 744 cases. PCI was performed in 202 patients (26.7 %). A total of 93.4 % of procedures were attempted via the right RA, and 54.4 % of the procedures were performed using a 5-F sheath.

### Anatomical variations of the RA

Overall, anatomical variations of the RA were noted in 68 patients (9.1 %). There were 39 cases of abnormal origin of the RA (5.2 %), 11 cases of radioulnar loop (1.5 %), and 42 cases of tortuous configuration (5.6 %). Patients with abnormal origin of the RA or radioulnar loop frequently had tortuous configuration (33.3 % and 72.7 %, respectively). Six cases had both abnormal origin of the RA and radioulnar loop. The baseline clinical characteristics of patients with anatomical variation and patients with normal anatomy are expressed in Table 2. Patients with anatomical variation were slightly older (70.8 ± 10.7 vs 67.3 ± 11.4 years, $P = 0.017$), shorter (161.1 ± 9.0 vs 164.4 ± 8.1 cm, $P = 0.002$), and lighter (61.8 ± 11.9 vs 65.6 ± 12.4 kg, $P = 0.015$) than patients with normal anatomy. There were

**Table 1** Baseline clinical and procedural characteristics of the 756 patients

| | |
|---|---|
| Age (years) | 67.6 ± 11.5 |
| Male | 632 (83.6) |
| Height (cm) | 164.0 ± 8.3 |
| Body weight (kg) | 65.2 ± 12.4 |
| Body mass index (kg/m$^2$) | 24.1 ± 3.6 |
| Risk factors | |
| Hypertension | 558 (73.8) |
| Hyperlipidemia | 495 (65.5) |
| Diabetes mellitus | 271 (35.8) |
| Smoking | 172 (22.8) |
| Family history of CAD | 119 (15.7) |
| Serum creatinine (mg/dl) | 0.96 ± 0.28 |
| Initial approach | |
| Right RA | 706 (93.4) |
| Left RA | 50 (6.6) |
| RA puncture failure | 12 (1.6) |
| PCI | 202 (26.7) |
| Sheath size | |
| 4 F | 69 (9.1) |
| 5 F | 411 (54.4) |
| 6 F | 276 (36.5) |
| Change approach site | 38 (5.0) |
| RA to FA | 22 (2.9) |
| RA to BA | 12 (1.6) |
| RA to contralateral RA | 4 (0.5) |

Values are presented as *n* (%) or mean ± SD unless otherwise noted
*CAD* coronary artery disease, *RA* radial artery, *PCI* percutaneous coronary intervention, *FA* femoral artery, *BA* brachial artery

**Table 2** Clinical characteristics of the patients with anatomical variation of the radial artery and patients with normal anatomy

| | Anatomical variation (+), *n* = 68 | Anatomical variation (−), *n* = 676 | *P* value |
|---|---|---|---|
| Age (years) | 70.8 ± 10.7 | 67.3 ± 11.4 | 0.017 |
| Male | 43 (63.2) | 582 (86.1) | <0.001 |
| Height (cm) | 161.1 ± 9.0 | 164.4 ± 8.1 | 0.002 |
| Body weight (kg) | 61.8 ± 11.9 | 65.6 ± 12.4 | 0.015 |
| Body mass index (kg/m$^2$) | 23.7 ± 3.6 | 24.2 ± 3.6 | 0.305 |
| Hypertension | 46 (67.7) | 503 (74.4) | 0.247 |
| Hyperlipidemia | 43 (63.2) | 445 (65.8) | 0.689 |
| Diabetes mellitus | 22 (32.4) | 246 (36.4) | 0.596 |
| Smoking | 15 (22.1) | 155 (22.9) | 1.000 |
| Family history of CAD | 12 (17.7) | 105 (15.5) | 0.604 |
| Serum creatinine (mg/dl) | 0.99 ± 0.32 | 0.96 ± 0.27 | 0.407 |
| Transradial procedure failure | 16 (23.5) | 10 (1.5) | <0.001 |
| RA spasm | 24 (35.3) | 59 (8.7) | <0.001 |

Values are presented as *n* (%) or mean ± SD unless otherwise noted
*CAD* coronary artery disease, *RA* radial artery

no statistically significant differences in coronary risk factors between patients with anatomical variation and patients with normal anatomy. Details of anatomical variations between males and females are expressed in Table 3. Females had a higher frequency of anatomical variations compared with males (21.0 % vs 6.9 %, $P < 0.001$), especially those with radioulnar loop (5.9 % vs 0.6 %, $P < 0.001$) and tortuous configuration (15.1 % vs 3.8 %, $P < 0.001$). Anatomical variations were frequently related to the transradial procedure failure rate (16 of 68 cases, 23.5 %) and RA spasm (24 of 68 cases, 35.3 %).

## Transradial procedure failure

Transradial procedure failure was observed in 26 of 744 cases (3.5 %), and more frequently in patients with anatomical variation of the RA than in patients with normal anatomy (23.5 % vs 1.5 %, $P < 0.001$). The baseline clinical characteristics of these patients are expressed in Table 4. The approach in 18 cases was changed to the femoral artery, and the approach in 8 cases was changed to the brachial artery because of failure of the initial RA approach. The reason for transradial procedure failure included inability to advance the guide wire or catheter in 21 cases (2.8 %) and necessity for a larger sheath or catheter >6 F for PCI in 5 cases (0.7 %). Multivariate logistic regression analysis revealed that the presence of anatomical variation of the RA was the distinct predictor of transradial procedure failure (odds ratio [OR], 17.80; 95 % confidence interval [CI], 7.55–43.73; $P < 0.001$), while age, gender, and short stature (height <155 cm) were not statistically independent predictors after adjustment (Table 5). The procedure failure rate with each of the individual anatomical variations is expressed in Table 6. Each of the variations was associated with transradial procedure failure. Of the anatomical variations, the presence of a radioulnar loop was strongly associated with the highest rate of procedure failure in 8 of the 11 cases (72.7 %, $P < 0.001$).

## RA spasm

The baseline clinical characteristics of patients with and without RA spasm are expressed in Table 7. Although routine radial arteriography was performed after administration of isosorbide dinitrate and heparin, RA spasm was

**Table 3** Details of anatomical variations of the radial artery between males and females

| | Total ($n$ = 744) | Male ($n$ = 625) | Female ($n$ = 119) | $P$ value |
|---|---|---|---|---|
| Anatomical variation (+) | 68 (9.1) | 43 (6.9) | 25 (21.0) | <0.001 |
| Abnormal origin of the RA | 39 (5.2) | 27 (4.3) | 12 (10.1) | 0.021 |
| Radioulnar loop | 11 (1.5) | 4 (0.6) | 7 (5.9) | <0.001 |
| Tortuous configuration | 42 (5.6) | 24 (3.8) | 18 (15.1) | <0.001 |

Values are presented as $n$ (%)

*RA* radial artery

**Table 4** Clinical characteristics of patients with transradial procedure failure and procedure success

| | Procedure failure, $n$ = 26 | Procedure success, $n$ = 718 | $P$ value |
|---|---|---|---|
| Age (years) | 71.5 ± 12.6 | 67.5 ± 11.4 | 0.075 |
| Male | 17 (65.4) | 608 (84.7) | 0.024 |
| Height (cm) | 159.8 ± 6.9 | 164.2 ± 8.2 | 0.008 |
| Body weight (kg) | 59.1 ± 12.7 | 65.5 ± 12.3 | 0.010 |
| Body mass index (kg/m$^2$) | 23.0 ± 3.9 | 24.2 ± 3.6 | 0.113 |
| Hypertension | 18 (69.2) | 531 (74.0) | 0.650 |
| Hyperlipidemia | 18 (69.2) | 470 (65.5) | 0.835 |
| Diabetes mellitus | 9 (34.6) | 259 (36.1) | 1.000 |
| Smoking | 4 (15.4) | 166 (23.1) | 0.478 |
| Family history of CAD | 3 (11.5) | 114 (15.9) | 0.784 |
| Serum creatinine (mg/dl) | 1.00 ± 0.27 | 0.96 ± 0.28 | 0.466 |
| Anatomical variation | 16 (61.5) | 52 (7.2) | <0.001 |
| RA spasm | 10 (38.5) | 73 (10.2) | <0.001 |

Values are presented as $n$ (%) or mean ± SD unless otherwise noted

*CAD* coronary artery disease, *RA* radial artery

**Table 5** Multivariate logistic regression analysis on transradial procedure failure

| | OR | 95 % CI | $P$ value |
|---|---|---|---|
| Anatomical variation (+) | 17.80 | 7.55–43.73 | <0.001 |
| Age >75 years | 2.04 | 0.83–4.97 | 0.117 |
| Female gender | 1.28 | 0.34–4.25 | 0.703 |
| Height <155 cm | 1.11 | 0.27–4.41 | 0.879 |

*OR* odds ratio, *CI* confidence interval

observed in 83 cases (11.2 %), and more frequently in patients with anatomical variation of the RA than in patients with normal anatomy (35.3 % vs 8.7 %, $P$ < 0.001). Females had a higher frequency of RA spasm compared with males (24.4 % vs 8.6 %, $P$ < 0.001). Multivariate logistic regression analysis revealed that the presence of anatomical variation (OR 4.74; 95 % CI 2.61–8.47; $P$ < 0.001) and female gender (OR 2.23; 95 % CI 1.01–4.73; $P$ = 0.041) were the distinct predictors of RA spasm, while use of a 6-F sheath and short stature (height <155 cm) were not statistically independent predictors after adjustment (Table 8).

**Table 6** Transradial procedure failure rate in patients with each of the anatomical variations of the radial artery

| | $n$ | Procedure failure rate, $n$ (%) | $P$ value |
|---|---|---|---|
| Total | 744 | 26 (3.5) | |
| Anatomical variation (+) | 68 | 16 (23.5) | <0.001 |
| Abnormal origin of the RA | 39 | 8 (20.5) | <0.001 |
| Radioulnar loop | 11 | 8 (72.7) | <0.001 |
| Tortuous configuration | 42 | 11 (26.2) | <0.001 |

*RA* radial artery

### RA diameter

Radial artery diameter was analyzed in 701 patients by an angiographic method. Of 744 cases, 43 were excluded from the analysis using the QCA system because of poor angiographic image quality. Mean RA diameter was 2.69 ± 0.37 mm, and was significantly correlated with gender and presence of anatomical variation of the RA. Females had smaller RA diameters than did males (2.40 ± 0.26 vs 2.75 ± 0.36 mm, $P$ < 0.001) (Fig. 3a). Furthermore, patients with anatomical variation of the RA had smaller diameters than did patients with normal anatomy in both males (2.51 ± 0.31 vs 2.77 ± 0.36 mm, $P$ < 0.001) (Fig. 3b) and females (2.24 ± 0.16 vs 2.44 ± 0.26 mm, $P$ < 0.001) (Fig. 3c).

## Discussion

The major findings from this study show that anatomical variations of the RA were strongly correlated with RA spasm and procedural achievement of TRCC. It was reported that the procedure success rate of the transradial approach is lower compared with that of the conventional

**Table 7** Clinical characteristics of patients with and without radial artery spasm

| | Radial artery spasm (+), $n = 83$ | Radial artery spasm (−), $n = 661$ | $P$ value |
|---|---|---|---|
| Age (years) | 68.5 ± 11.5 | 67.5 ± 11.4 | 0.436 |
| Male | 54 (65.1) | 571 (86.4) | <0.001 |
| Height (cm) | 160.8 ± 9.9 | 164.5 ± 7.9 | <0.001 |
| Body weight (kg) | 62.2 ± 14.4 | 65.6 ± 12.1 | 0.018 |
| Body mass index ($kg/m^2$) | 23.8 ± 3.8 | 24.2 ± 3.5 | 0.426 |
| Hypertension | 59 (71.1) | 490 (74.1) | 0.596 |
| Hyperlipidemia | 51 (61.5) | 437 (66.1) | 0.394 |
| Diabetes mellitus | 23 (27.7) | 245 (37.1) | 0.114 |
| Smoking | 20 (24.1) | 150 (22.7) | 0.782 |
| Family history of CAD | 14 (16.9) | 103 (15.6) | 0.750 |
| Serum creatinine (mg/dl) | 0.92 ± 0.27 | 0.97 ± 0.28 | 0.145 |
| Transradial procedure failure | 10 (12.1) | 16 (2.4) | <0.001 |
| Anatomical variation | 24 (28.9) | 44 (6.7) | <0.001 |
| Use of 6-F sheath | 32 (38.6) | 241 (36.5) | 0.718 |

Values are presented as $n$ (%) or mean ± SD unless otherwise noted

*CAD* coronary artery disease

transfemoral approach [3]. Thus, it is necessary to elucidate what caused the failure of the transradial approach and identify predictors for a successful transradial approach.

## Anatomical variations of the RA and transradial procedure failure

In this study, the incidence of anatomical variations of the RA was 9.1 % (68 of 744 cases), including 39 cases of abnormal origin of the RA (5.2 %), 11 cases of radioulnar loop (1.5 %), and 42 cases of tortuous configuration (5.6 %). The incidence of abnormal origin of the RA, radioulnar loop, and tortuous configuration has been reported to range from 2.4 % to 8.3 %, 0.8 % to 2.3 %, and 2.0 % to 5.2 %, respectively [9–11, 16, 19]. We obtained similar results in the present study. Based on previous reports and the results of our study, it should be recognized that anatomical variations of the RA are not rare beyond those associated with race.

More importantly, in 16 of the 68 patients (23.5 %) with anatomical variations, we failed to perform TRCC as the initial approach. As a result, the procedure success rate was significantly lower in patients with anatomical variation than in patients with normal anatomy (76.5 % vs 98.5 %, $P < 0.001$). Multivariate logistic regression analysis revealed that the presence of anatomical variation was the

**Table 8** Multivariate logistic regression analysis on radial artery spasm

| | OR | 95 % CI | $P$ value |
|---|---|---|---|
| Anatomical variation (+) | 4.74 | 2.61–8.47 | <0.001 |
| Use of 6-F sheath | 1.18 | 0.71–1.93 | 0.518 |
| Female gender | 2.23 | 1.01–4.73 | 0.041 |
| Height <155 cm | 1.37 | 0.59–3.14 | 0.463 |

*OR* odds ratio, *CI* confidence interval

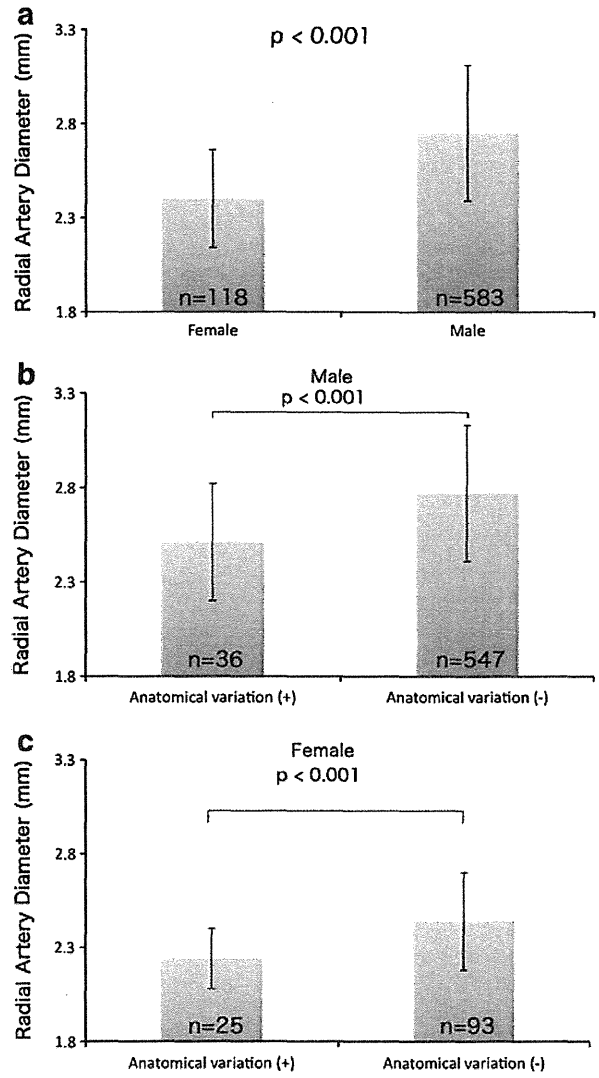


**Fig. 3** Differences in radial artery diameter. Proximal radial artery diameter was analyzed in 701 patients by an angiographic method. Females had a smaller radial artery than did males (2.40 ± 0.26 vs 2.75 ± 0.36 mm, $P < 0.001$) (**a**). Patients with anatomical variation of the RA had smaller diameters than did patients with normal anatomy in both males (2.51 ± 0.31 vs 2.77 ± 0.36 mm, $P < 0.001$) (**b**) and females (2.24 ± 0.16 vs 2.44 ± 0.26 mm, $P < 0.001$) (**c**)

distinct predictor of transradial procedure failure. Of the anatomical variations, radioulnar loop was strongly associated with the highest procedural failure rate in 8 of the 11 cases (72.7 %, $P < 0.001$). The transradial procedure failure rate in patients with anatomical variation has been reported to range from 6.9 % to 14.2 % [9, 10, 16]. In our study, it was higher than those reported previously. These differences might be due to differences in definitions of anatomical variations, populations' clinical backgrounds and race, and operators' tenacity in performing the transradial procedure. In any case, the transradial procedure failure rate was higher in patients with anatomical variations. Dehghani et al. [21] reported that an age of >75 years, prior coronary artery bypass graft surgery, and short stature are independent predictors of transradial PCI failure. Although the transradial procedure failure rate tended to be higher in older and shorter patients, an age of >75 years and short stature (height <155 cm) were not statistically independent predictors in our study. Furthermore, the presence of an anatomical variation was much more strongly correlated with transradial procedure failure than was age and height. It is not contraindicated to perform TRCC in patients with anatomical variation of the RA. However, we should recognize that different anatomical variations are associated with different transradial procedure failure rates. Some previous studies reported that the presence of a radioulnar loop was the most common cause of transradial procedure failure [9, 10, 22]. We were sometimes able to pass the radioulnar loop with a hydrophilic guide wire, but the procedure could cause vasospasm and pain. According to previous reports and the results of our study, the presence of a radioulnar loop was not suited to the performance of the transradial procedure.

In this study, radial arteriography was performed immediately after sheath insertion and administration of isosorbide dinitrate and heparin. When we had to change the approach site owing to anatomical variation or severe spasm, especially from the RA to the femoral artery or brachial artery, heparinization might have been associated with bleeding complications. From the standpoint of preventing bleeding complications, we should have performed radial arteriography before the administration of heparin.

Fukuda et al. [12] reported that the proximal RA diameter in a Japanese population was 2.72 ± 0.77 mm in males and 2.49 ± 0.36 mm in females. In our study, the proximal RA diameter measured by the QCA system was similar to those reported previously. Interestingly, both male and female patients with anatomical variation of the RA had smaller diameters than did those with normal anatomy. Smaller RA diameter might contribute to puncture failure and severe vasospasm [12, 13]. In general, the RA is easier to palpate at the wrist than is the ulnar artery in most patients. However, in some patients the ulnar artery is particularly strong on palpation, and quite superficial. Some patients with anatomical variation of the RA had a significantly larger ulnar artery than RA (Fig. 1a). In some of these difficult transradial cases with anatomical variation, the transulnar approach might be a safe and useful alternative to the transradial procedure if the reverse Allen test result is normal [23–25].

It is difficult to detect anatomical variations of the RA by physical examination. Ultrasound examination of the RA is an effective and noninvasive method with which to evaluate RA anatomy before the transradial procedure [13, 19]. Yokoyama et al. [19] reported that preprocedural ultrasound examination could be helpful to measure the RA diameter and to exclude patients with inaccessible arteries including radioulnar loop and tortuous configuration, and those at high risk for access failure. In addition, postprocedural ultrasound examination could detect the patency of the RA [19, 23]. Yokoyama et al. [26] also reported that preprocedural ultrasound examination could be useful not only to detect anatomical variations, but also to assess the collateral supply to the hand instead of a modified Allen test. Thus, ultrasound examination of the RA should be considered before the transradial procedure.

## RA spasm

In our study, severe RA spasm (>75 % stenosis at first radial arteriography) was observed in 11.2 % of patients despite preadministration of isosorbide dinitrate. Furthermore, patients with anatomical variation tended to have a smaller RA diameter and more frequent RA spasm than did patients with normal anatomy (35.3 % vs 8.7 %, $P < 0.001$). Multivariate logistic regression analysis revealed that the presence of anatomical variation of the RA and female gender were the distinct predictors of RA spasm. The incidence of RA spasm has been reported to range from 3.8 % to >50 % [12–14, 27–29]. The incidence varied because there were differences in premedication and definition of RA spasm. A case report suggested a relationship between severe vasospasm and anatomical variation of the RA [30]; a patient with abnormal origin of the RA had severe vasospasm of the branching point of the distal axillary artery, resulting in entrapment of the catheter. Although there are some reports on RA spasm, there is a paucity of evidence about the relationship between RA spasm and anatomical variations.

Previous studies reported that female gender, younger age, small RA diameter, lower body mass index, diabetes mellitus, and unsuccessful access at first attempt were distinct predictors of RA spasm [13, 14, 29]. In addition, the presence of anatomical variation was a distinct predictor of RA spasm in our study. Ruiz-Salmeron et al. [31] also reported that anatomical variations of the RA were

strongly associated with RA spasm in multivariate analysis (OR 5.1; 95 % CI 2.2–11.4; $P < 0.001$). These results are in accordance with those in our present study. Use of a 6-F sheath was not a distinct predictor of RA spasm in comparison with use of a 4- or 5-F sheath, while the ratio of the RA diameter and sheath outer diameter was reportedly related to RA occlusion [19].

When preprocedural radial arteriography shows anatomical variation of the RA, especially with small diameter or severe vasospasm, good judgment is needed to potentially change the approach site to the contralateral RA, brachial artery, femoral artery, or even ulnar artery [23–25].

### Study limitations

A limitation of this study was the lack of follow-up information on RA patency. The lack of the strict criteria for the operator's experience, the lack of independent angiographic evaluation by a core laboratory, and the lack of ultrasound data on RA anatomy were other limitations of the study. Although radial arteriography was performed after administration of isosorbide dinitrate, pain and stimulation of the sheath insertion might have affected the RA spasm, and RA spasm might have affected RA diameter in this study. Ultrasound examination of the RA is an effective noninvasive method with which to evaluate RA anatomy. Radial arteriography seems to be more precise than ultrasound, but further studies are needed to compare these two methods in terms of accuracy.

## Conclusions

Anatomical variations of the RA were observed in 9.1 % of patients, and were strongly correlated with RA spasm and procedural achievement of TRCC. From the standpoint of preventing serious complications, preprocedural radial arteriography is a powerful tool with which to provide precise anatomical information. It requires only a very small amount of contrast media and radiation, and helps operators to safely perform transradial procedures.

**Conflict of interest** No financial support was provided for our study. We have no relationships with industry. We have no commercial or proprietary interest in the submitted article.

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Current Topics

## Stem Cell Research for Regenerative Medicine/Personalized Medicine

# Novel Insights into Disease Modeling Using Induced Pluripotent Stem Cells

Toru Egashira, Shinsuke Yuasa, and Keiichi Fukuda*

*Department of Cardiology, Keio University School of Medicine; 35 Shinanomachi, Shinjuku-ku, Tokyo 160–8582, Japan.*

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**Induced pluripotent stem cell (iPSC) technology has great potential to establish novel therapeutic approaches in regenerative medicine and disease analysis. Although cell therapy using iPSC-derived cells still has many hurdles to overcome before clinical applications, disease analysis using patient-specific iPSCs may be of practical use in the near future. There are several reports that patient-specific iPSC-derived cells have recapitulated the apparent cellular phenotypes of a wide variety of diseases. Moreover, some studies revealed that it could be possible to discover effective new drugs and to clarify disease pathogenesis by examination of patient-specific iPSC-derived cells *in vitro*. We have recently reported that iPSCs can be a diagnostic tool in a patient with a novel mutation. For definitive diagnosis in a patient with long QT syndrome who had an uncharacterized genetic mutation, we succeeded in clarifying the patient's cellular electrophysiologic characteristics and the molecular mechanism underlying the disease phenotype through the multifaceted analyses of patient-specific iPSC-derived cardiomyocytes. In this review, we focus on the conceptual and practical issues in disease modeling using patient-specific iPSCs and discuss future directions in this research field.**

**Key words** induced pluripotent stem cell; disease modeling; cardiovascular disease; long QT syndrome

## 1. INTRODUCTION

Induced pluripotent stem cells (iPSCs) are defined as artificial pluripotent stem cells that can be generated from somatic cells by introducing reprogramming factors (*e.g.*, *OCT3/4*, *SOX2*, *KLF4*, *c-MYC*, *NANOG*, and *LIN28*).[1,2] The methodology for generating iPSCs has markedly improved and now integration-free iPSCs, without transgene insertion in the host genome, can be obtained using several procedures.[3–7] iPSCs maintain the two essential stem cell characteristics of infinite self-renewal capability and pluripotency, meaning that they can give rise to all cell types of the three germ layers and differentiate in a fashion similar to normal embryogenesis.[8,9]

One of the expectations of iPSCs is the generation of human disease-specific pluripotent stem cells from patients. Such iPSCs, refered to as patient-specific iPSCs, can differentiate into any type of cell including a patient's diseased organ tissue, and the genetic information of patient-specific iPSCs is identical to that of the patient.[10] Therefore we can directly and repetitively analyze diseased cells using patient-specific iPSC-derived cells. Figure 1 shows the conceptual scheme for the utilization of patient-specific iPSCs in clinical practice. To date, many groups have reported that the apparent cellular phenotypes of genetic disorders can be recapitulated in patient-specific iPSC-derived cells *in vitro* (Table 1). Some reports also involved drug screening using iPSCs, resulting in the proposal of novel drug candidates.[11,12] We have recently reported that functional analyses of patient-specific iPSC-derived cardiomyocytes elucidated the molecular mechanism of the disease phenotype in a patient with undiagnosed sporadic long QT syndrome (LQTS).[13] This paper reviews current topics in disease modeling using patient-specific iPSCs and introduces our study as an actual example in this research field.

## 2. GENERATION OF PATIENT-SPECIFIC iPSCs

**Disease Selection** Although any type of disease can theoretically be reproduced by patient-specific iPSC-derived cells, in many diseases it appears difficult to recapitulate the phenotype using this technique because of problems related to both the properties of iPSCs and the disease causality. First, the differentiation efficiency of iPSCs into specific cells restricts the category of disease.[14] In terms of the maturity of iPSC-derived cells, it may be easier to to reproduce the phenotype of disease occurring in younger individuals because of the immaturity of iPSC-derived cells.[15] Disease mainly caused by the alteration of epigenetic status due to environmental parameters is not suitable for modeling using iPSCs because the cellular epigenetic information can be partly renewed during the process of reprogramming.[16,17] On the other hand, in disease directly caused by a genetic aberration that is clearly preserved in iPSCs, it is feasible to confirm whether patient-specific iPSC-derived cells can reproduce diseased cellular kinetics. In addition, apparent phenotypes can be determined even at the single-cell level because of the difficulty in organ formation from iPSCs.[18]

Considering those issues comprehensively, the first disease to be analyzed using patient-specific iPSCs should be a monogenetic disorder with severe phenotypes diagnosed in infancy and easily examined with simple methods at the single-cell level. Most studies using patient-specific iPSCs focus on diseases that satisfy such requirements. LQTS was selected by

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* To whom correspondence should be addressed. e-mail: kfukuda@a2.keio.jp

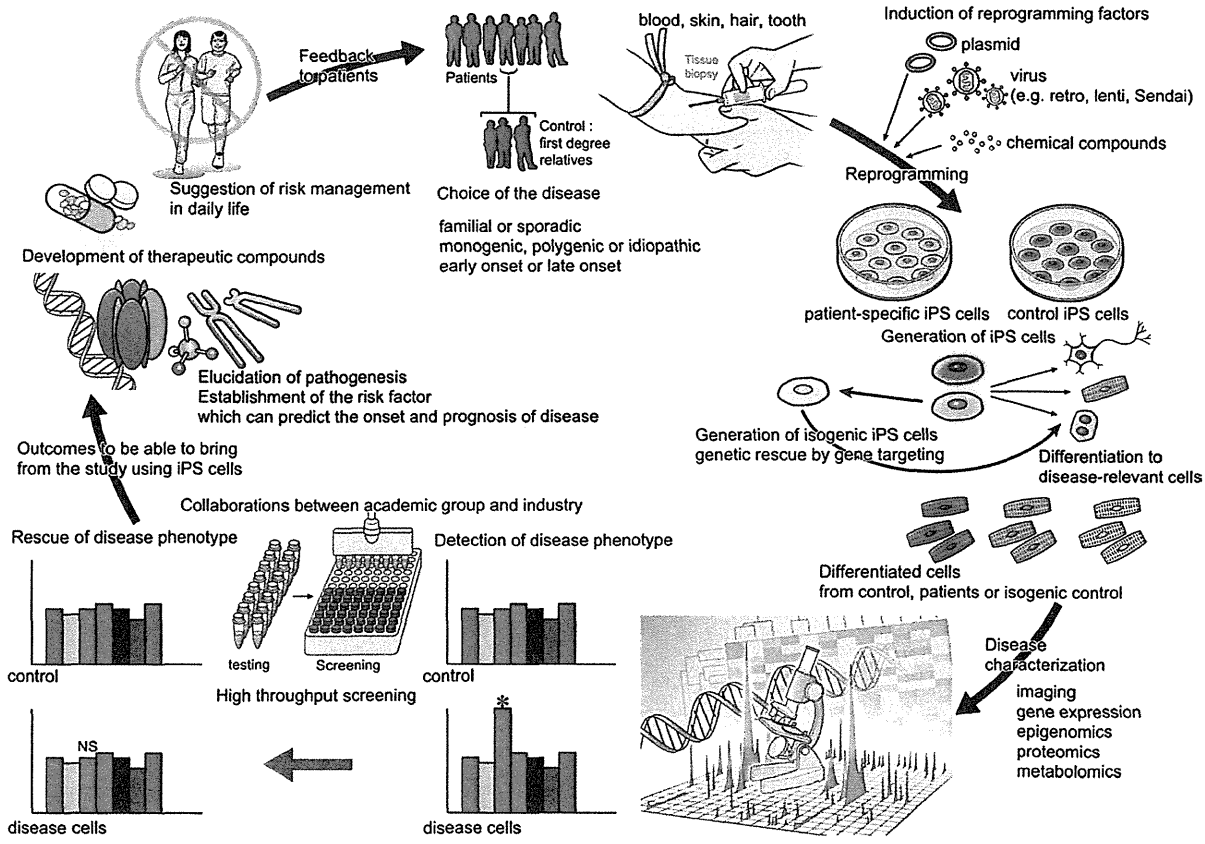


Fig. 1. Overview of Issues in Clinical Applications Using Patient-Specific iPSC Technology

This scheme shows the circle that is one model of patient-specific iPSC technology. The disease type was selected from among many conditions. Controls are often first-degree relatives of patients. Somatic cells are obtained from the blood, skin, hair, or teeth and then reprogramming factors are introduced using various methods (*e.g.*, plasmid, virus, or chemical compounds) and both patient-specific and control iPSCs are generated. If possible, isogenic control iPSCs could be generated through genetic rescue by gene targeting. After evaluating the quality of iPSCs generated, they are differentiated into disease-relevant cells. Then both types of iPSC-derived cell are characterized using various techniques (imaging, genomics, epigenomics, proteomics, metabolomics, *etc.*). Based on the results of pioneering studies, further examinations such as high-throughput screening using large chemical libraries are planned through collaborations between academic research groups and pharmaceutical companies. Expected results of studies on iPSCs are the development of novel therapeutic compounds, establishment of novel risk factors predicting the onset of disease and prognosis, and suggestions on appropriate lifestyles which can serve as feedback to patients.

our and other groups as a disease model using patient-specific iPSCs. LQTS is an inherited life-threatening disease caused by functional impairment of the cardiac ion channel with a monogenetic aberration and often causes sudden cardiac death due to ventricular tachyarrhythmia even in infancy.[19,20]

**Derivation and Characterization of Patient-Specific iPSCs** Originally, iPSCs were generated from dermal fibroblasts in a retroviral transduction system.[1,21] Subsequently, the methodology for generating iPSCs rapidly improved and became simpler and more efficient, enabling the generation of iPSCs using less patient-invasive methods. Moreover, using plasmid vectors, RNA viruses, and other methods, good-quality iPSCs can be obtained without the need for integrating reprogramming factors.[3–7] Integration-free iPSCs appear ideal because exogenous genes integrated in the host genome may affect the genetic properties of the iPSCs generated and modify the cellular phenotypes of patient-specific iPSC-derived cells.[22]

We previously reported that integration-free iPSCs can be efficiently, easily, and rapidly generated from terminally differentiated circulating T lymphocytes in peripheral blood using Sendai virus (RNA virus).[23] Our method makes it possible to generate iPSCs from any patient including infants, girls, and the very elderly *via* simple blood sampling, which is one of the least-invasive common clinical procedures. Such cumulative progress in generating iPSCs can accelerate the widespread application of patient-specific iPSC technology.

Before the utilization of generated iPSCs in disease modeling, their characteristics must be evaluated.[24] It should be determined whether problems occurred during iPSC reprogramming and maintenance, such as the occurrence of somatic coding mutations,[25] dynamic changes in the allelic copy number variation,[26] abnormality of X chromosome inactivation,[27] incomplete demethylation,[28] *etc.* These elements may affect the phenotype of iPSC-derived cells and skew the interpretation of the results of their assay. In addition, the most appropriate control group remains controversial. In most previous studies, the control groups comprised healthy volunteers without genetic mutations who were unrelated to or relatives of the patients involved. It remains unclear which controls are optimal in disease modeling using patient-specific iPSCs. To examine the unadulterated functions of mutated genes, it appears preferable to compare patients with family members who do not carry the mutation, although related