表 1　QT延長症候群(先天性・二次性)とBrugada症候群の診療に関するガイドラインにおけるBrugada症候群のICD植込み適応[25]

	Coved(Type 1)型(自然または薬物)						
失神	+	+	+	+	−	−	−
突然死家族歴	+	+	−	−	+	+	−
VF 誘発	+	−	+	−	+	−	+
クラス分類	IIa	IIa	IIa	IIb	IIa	IIb	IIb

クラスI：心停止・心蘇生例，自然停止する VF/多形性 VT が確認されている例.

クラスII：Brugada 型心電図を有する例(薬物負荷，1 肋間上の心電図記録例も含む)で，失神，家族歴，VF 誘発のうち，2 つ以上の指標があればIIa，1 つだとIIb とする.

療指針と思われるが，一方で，対象を Type 1 例に限定していることと，失神例の取扱いや EPS の意義に関して若干議論の余地を残している(表 1).

2. 薬物治療

VF 多発時の薬物治療としてはイソプロテレノールの持続点滴が有用である. VF 予防の経口薬としては，これまでβ刺激薬や，Ito チャネル遮断作用のある薬物(キニジン，ベプリジルなど)，Ca 電流を増加させるシロスタゾールが有効と報告されている. しかし，これらの薬剤は VF を完全に抑制するまでには至らないため，無症候群の一次予防には用いられていない.

一方，Brugada 症候群で AF や冠攣縮性狭心症，神経調節性失神を伴うことが少なくない. この際，Na チャネル遮断薬や Ca 拮抗薬，β遮断薬，向精神薬などが使用される可能性があるが，これらの薬剤では心筋のイオン電流を変化させて，ST を上昇させることが報告されている. このすべてが Brugada 症候群例に VF を発生させるわけではないが，治療の際にはその選択に十分な留意が必要となろう.

Brugada症候群と早期再分極症候群

特発性 VF では V_1-V_3誘導での ST 上昇はないが，II，III，aVF 誘導または V_3-V_6誘導で J 波が存在～増高していたり saddleback 型に近い ST 上昇のみられる例が報告されていた. また，J 波増高と Brugada 型(coved)心電図の両方が異なった時期の異なった誘導部位で認められる例もあり，それらは，Brugada 症候群の亜型であるか，または VF の基質が Brugada 症候群とは異なる部位にある可能性が示唆されていた. 近年，Haissaguerre らは V_1-V_3誘導を除く下側壁誘導(II，III，aVF，I，aVL，V_4-V_6)の 2 誘導以上で，1 mm 以上の J 波(notch や slur を伴う)増高を有する早期再分極症候群の臨床像を報告した[25]. 本症候群では VF による突然死が生じるが，約 30%の症例では病態・予後ともに Brugada 症候群に類似していることが指摘されている.

Haissaguerre らは対象から V_1-V_3誘導での Type 1 心電図例を除外しているため，本症候群は Brugada 症候群とは異なる疾患とみなされる傾向にある. 一方で，J 波の原因は Brugada 症候群と同様な心室再分極異常と考えられている. しかし，本症候群には非 Type 1 心電図を呈する Brugada 症候群が含まれている可能性を否定できない. 今後，Brugada 症候群との類似性を含めて真の早期再分極症候群の病態，および機序の解明が望まれる.

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g. 不整脈

④特殊な病態の不整脈を診る b. Brugada症候群

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Introduction

Brugada症候群とは，12誘導心電図で右脚ブロック様波形と，V_1～V_3誘導におけるcoved型またはsaddleback型のST上昇を呈し，主として若年～中年男性が夜間に心室細動（ventricular fibrillation；VF）を引き起こして突然死する疾患である。本症候群は器質的心疾患を伴わない特発性心室細動の1種として1992年にBrugada兄弟により初めて報告された[1]。本疾患にはVFや失神などの症状を伴う有症候群と，心電図異常を有するが症状のない無症候群があり，1998年以降，心筋のNaチャネル遺伝子変異，L型Caチャネル遺伝子変異など，これまでに計8つの原因遺伝子が報告されている。

1 Brugada症候群の機序

Brugada症候群では，遺伝子変異を背景として，右室流出路心外膜側で内向きのNa電流やCa電流などが減少する結果，I_{to}など相対的な外向き電流が増加して，活動電位第1相のnotchが大きくなり，心外膜－心内膜間に電位勾配が生じる。それによりJ波の増大に引き続いてST上昇が起こる。さらに相対的な内向き電流が減少すると，第2相のdome形成が遅延し，心内膜側より心外膜側で再分極が遅れて，ST上昇に加えT波の陰転が生じる。一方，相対的な内向き電流がさらに減少すると心外膜側でdomeが消失し，周囲との間に大きな電位勾配が生じるために，貫壁性および心外膜層内で再分極時間のばらつきが生じるとともに，domeの消失した心筋において再脱分極が起こる。これはphase2 reentryとよばれ，これからVFが発生する[2]。

これらは動物実験に基づくBrugada症候群の“再分極仮説”として永らく支持されてきたが，右室局所の伝導遅延に原因を求める“脱分極仮説”が近年では有力になりつつある。特にNademaneeらはVFを繰り返す例において，右室流出路の心外膜側だけに限局する，低電位で持続時間の長い（＞80msec）異常電位領域を発見し，それらを広範囲に焼灼することでBrugada波形が消失し，かつVFが消失したと報告している[3]。これらの例ではBrugada症候群に催不整脈性右室心筋症（arrhythmogenic right ventricular cardiomyopathy；ARVC）

を合併していた可能性を否定できないものの，流出路心外膜側の伝導異常が大きく関与する疾患ではないかと考えられるようになりつつある。

Brugada症候群のエビデンス

(1) Brugada症候群の予後

日本人のBrugada症候群の予後を解明するため，2001年から厚生労働省の循環器病委託研究と，特発性心室細動研究会によるJ-IVF研究が開始され，Brugada症候群の予後が前向きで検討された[4,5]。その結果，いずれの研究においても無症候群と失神群の予後は良好(心事故発生率0.5～1%/年)で，VF群の予後は不良であった(**図1，2**)。これらは2010年に報告された欧米でのFINGER研究の各群の予後ともほぼ一致していた[6]。委託研究では常に非Type1心電図(Type2，Type3，J点で1mm以上2mm未満のcovedまたはsaddleback型ST上昇)を有する症例の予後も検討された。それによると，非Type1群もType1群と同様な予後を示し，VF既往例では約10%/年の頻度で心事故を生じていた(**図2**)。

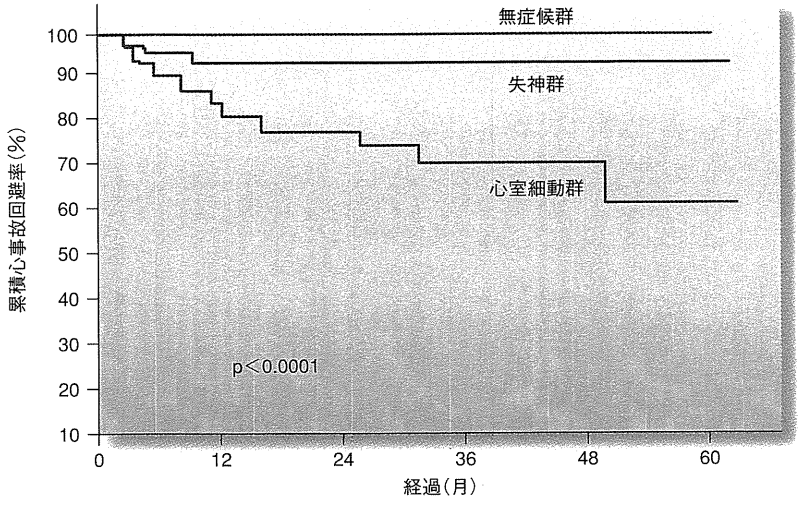
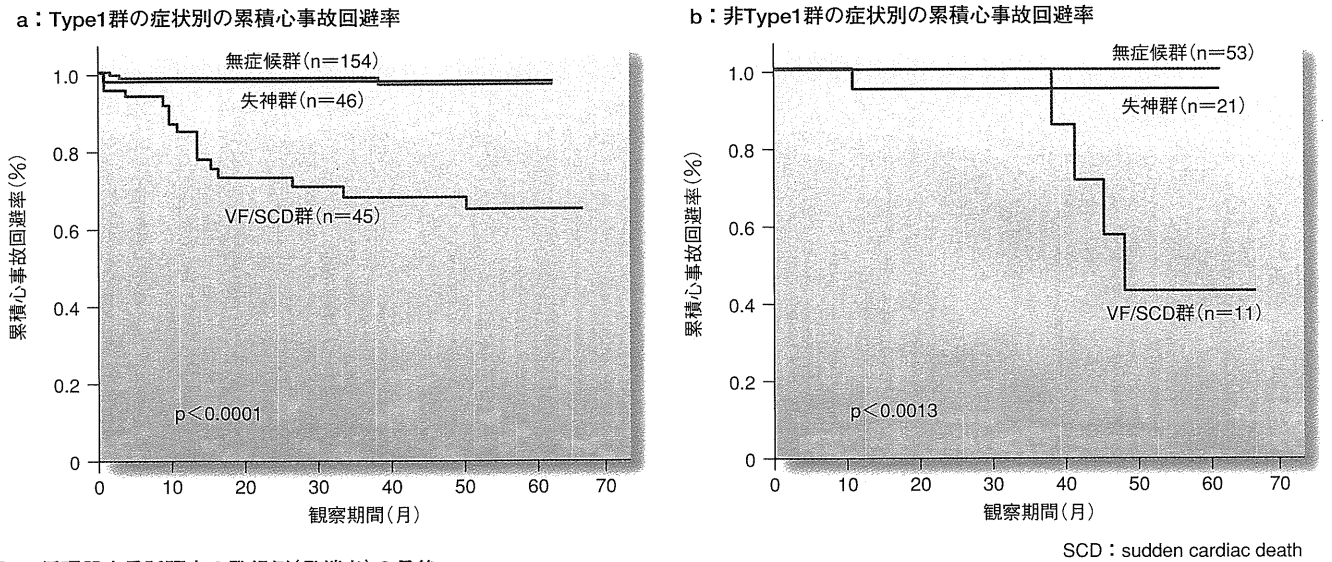


図1　J-IVF研究での登録症例全体の症状別の累積心事故回避率(文献5より引用)

(2) 予後予測因子

循環器病委託研究では，VFの既往，



SCD：sudden cardiac death

図2　循環器病委託研究の登録例(発端者)の予後

70歳未満での突然死の家族歴，下側壁誘導での早期再分極（J波）の合併が心事故の独立した予測指標であった。欧米の検討では，失神の既往，自然発生のType1心電図，電気生理学的検査（electrophysiologic study；EPS）でのVF誘発が不良な予後の予測指標として報告されているが，日本ではこれらの指標は必ずしも有用でなかった。一方，失神群，無症候群に限定すると，日本人の心事故の予測因子として有意であったのは突然死の家族歴だけであった。最近Makimotoらはトレッドミル運動負荷検査時の回復期でのST上昇（運動前に比較してV_1～V_3誘導で0.5mm以上）が失神群，無症候群の予後予測に有用と報告している[7]。今後，本法が無症候群の予後を診断する有用な検査法になるかもしれない（**図3**）。

Brugada症候群の診断

(1) 病歴聴取

Brugada症候群では，VFまたは失神の既往と，突然死の家族歴が予後予測に有用であるため，失神の有無，発生時間，失神時の体位，前駆症状の有無などを尋ねる。また3親等までの親族で，胸痛を伴わない睡眠中または安静時の突然死がないかを尋ねる。一般に，

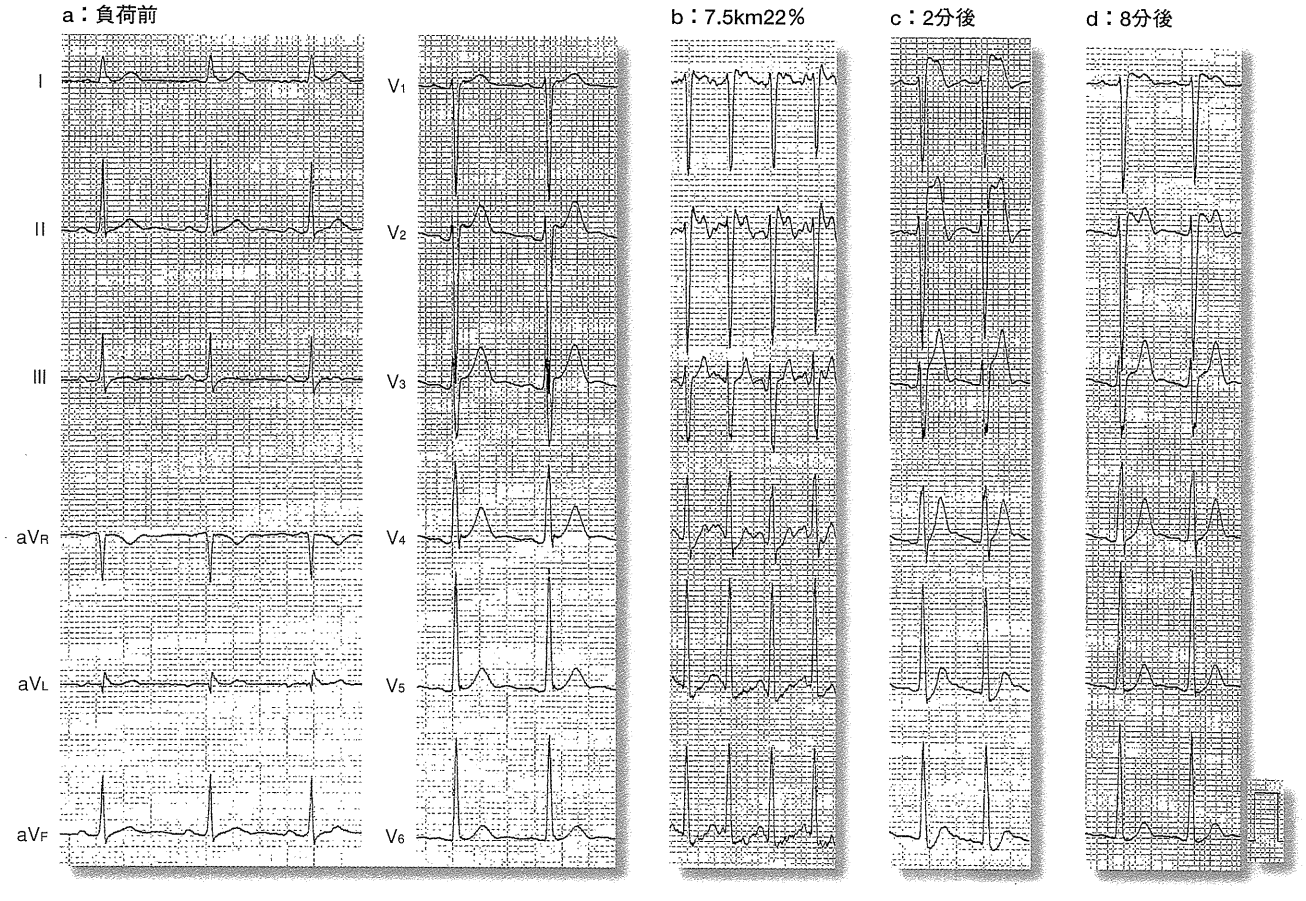


図3　トレッドミル運動負荷時のST変化（65歳男性）

負荷前にはV_2誘導でsaddleback型のST上昇（2mm）がみられ，運動中はST上昇が改善するが（1.5mm），運動後2分で著明にSTが再上昇し（10mm），V_1誘導はcoved型波形を呈す。

Brugada症候群は女性ではまれ(5%以下)であり，突然死は夜間～早朝の，睡眠時または安静時に発生するという特徴がある。血管迷走神経反射による失神をVF由来の失神と混同してはいけない。

(2)必要な検査

12誘導心電図では通常肋間(V_1～V_3)のほかに，高位肋間(第2，3肋間)での記録が必要である。このほかに加算平均心電図，遺伝子検査が予後推定に有用な可能性がある。満腹試験，ピル

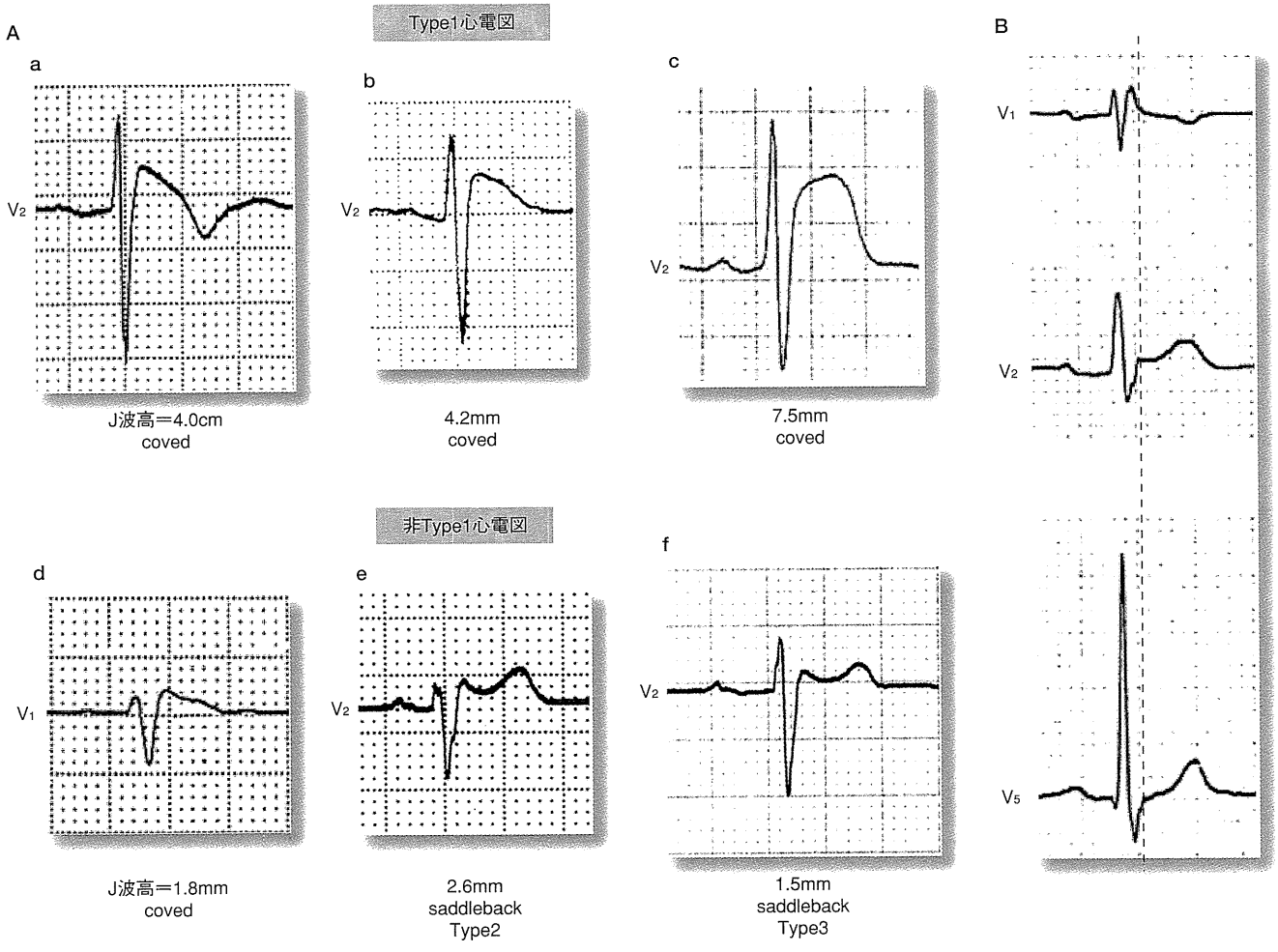


図4 Brugada症候群の心電図波形

A：Type1心電図(a～c)と非Type1心電図(d～f)。
aはcoved型でJ点の波高が2mm以上あり，典型的なType1心電図である。
b，cはT波の陰転はないがType1とすべき波形である。
dはJ点で1.8mmしかなく，Type1とはいえない。
eはType2，fはType3に相当する。
B：J点の求め方。
V_5誘導の終末点で時相をあわせて，V_1，V_2誘導の終末点(J点)を求め，波高を計算する。V_1誘導のJ点波高は0.5mmであり，V_2誘導はsaddleback型ではないため，本例はBrugada症候群またはBrugada心電図ではなく，不完全右脚ブロックと診断する。

ジカイニドなどのNaチャネル遮断薬による薬剤負荷試験も診断確定に有用である。EPSは突然死の家族歴や原因不明の失神を有する例で必要となる。

(3)心電図診断

通常記録に限らず，高位肋間記録や負荷試験時にType1心電図(J点で2mm以上のcoved型ST上昇)が認められればBrugada症候群と考えてよい。一方，Type2，Type3心電図(J点で2mm以上のsaddleback型ST上昇)に留まる場合はBrugada型心電図例と判定する(**図4a**)。

この際，Brugada症候群のJ波を通常の不完全右脚ブロック，または完全右脚ブロックのlate r'またはR'波と混同してはいけない。このため，心電図の時相を一致させて，V_1～V_3誘導のQRS波後半部分と，V_5またはV_6誘導のQRS終末点とを比較し，V_1～V_3誘導のQRS終末点(J点)の波高が2mm以上あることを確認する(**図4b**)。

Brugada症候群の治療

(1)ICD適応

日本循環器学会の診療ガイドライン[8]では，Brugada症候群でVFの既往がある場合は，心電図波形に関係なくクラスⅠの植込み型除細動器(implantable cardioverter defibrillator；ICD)植込み適応としている。また失神群，無症候群においては，Type1心電図が確認され，かつ，

①原因不明の失神

②突然死家族歴

③EPSでのVF誘発

のうち，2つ以上の指標を満たす場合をクラス2aとし，1つだけの場合は2bの適応としている。

本ガイドラインは現時点で最も適切な治療指針と思われるが，Deliseらも同様の基準によるリスク層別が妥当であることを報告している[9]。

(2)薬物療法

一方，薬剤はVFを完全に抑制できないため，本症候群の一次予防には用いられていない。しかしながらVF多発時の薬物治療としてはイソプロテレノールの持続点滴が有用である。VF既往例で再発予防の経口薬としては，I_{to}チャネル遮断作用のある薬物(キニジン，ベプリジルなど)，Ca電流を増加させるシロスタゾール，β刺激薬などが有効と報告されている。

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早期再分極（J波）症候群

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はじめに

下側壁誘導における早期再分極（early repolarization）症候群またはJ波症候群は，Haïssaguerreらが2008年にNew Engl J Med誌に発表して以来[1]，急速に注目を集めている突然死疾患である。本疾患は器質的心疾患を伴わない特発性心室細動（VF）のBrugada症候群に類似しているが，Brugada症候群がV_1～V_3誘導でのcoved型，またはsaddleback型という特異的なST上昇を示すのに対し，Ⅱ，Ⅲ，aV_F誘導またはⅠ，aV_L，V_4～V_6誘導での1mm以上の波高のJ波（notch or slur）とそれに続くST上昇を特徴とする。一方，従来からV_4～V_6誘導での早期再分極波形が若年男性に多く存在することも知られており，これらは予後が良好と報告されていた。現時点で，下側壁の早期再分極症候群はその有病率，発症率，後ろ向き予後が判明しつつある段階であるが，発症機序，遺伝的背景は解明途中であり，前向きの予後，予後予測因子は未解明である。本稿では，現時点で判明している早期再分極症候群の病態について述べる。

心電図所見

Haïssaguerreの定義では，下側壁の早期再分極症候群とは前述の如く，側壁（V_4～V_6），高位側壁（Ⅰ，aV_L），または下壁（Ⅱ，Ⅲ，aV_F）誘導のうち，2誘導以上で1 mm以上の高さのJ波とST上昇が認められる症例をさす。一方，定義はされていないが，その考えを普遍化すると前壁（V_1～V_3）誘導の早期再分極症候群も存在してよい。この場合，slurなJ波とそれに続くST上昇を示す症例が相当するが，それらはとりもなおさずsaddleback型のBrugada症候群を意味する。ただ，Haïssaguerreらが指摘するように，典型的なBrugada症候群，すなわちType1のBrugada症候群に移行する症例は除外する必要がある。したがって早期再分極の必要条件をJ点波高≧1 mmと定義するなら，Type2およびType3のBrugada波形例[2]，またはJ点が1 mm～2 mmのsaddleback型ST上昇を右前胸部誘導で伴う例で，かつ薬剤負荷等でType1に変化しない症例が前壁の早期再分極症候群ということになる（図1）。これらの見解は未だ認知されているわけではないが，これにほぼ匹敵する波形例の臨床病態と前向き予後は，非Type1のBrugada症候群として循環器病委託研究の中で我々が報告している[3]。

一方，J波はBrugada波形と同様に，記録時期により形状や高さが異なる，または消失することもあるので，できるだけ多くの心電図で評価する必要がある。

J波と心室細動発生機序

J波の類似波形は低体温時に見られるOsborn波として知られているが[4]，それ以前の1930年代から，J波は正常亜型の波形として健常成人の数%，特に若年男女に見られると報告されていた。一方，J波の存在する例でVFが生じることは1984年頃から報告されはじめ，AizawaやTakagiらが下壁誘導や側壁誘導でJ波の存在する複数例を報告している[5,6]。

このJ波とVFの機序に関しては，Antzelevitchらが動脈灌流心筋切片を用いたモデルで，心内膜側と心外膜側間に生じる貫壁性電位勾配の差で説明している[7]。すなわち，活動電位第1相において，主として心室心外膜側に生じる一過性外向き電流（I_{to}）により，貫壁性電位勾配

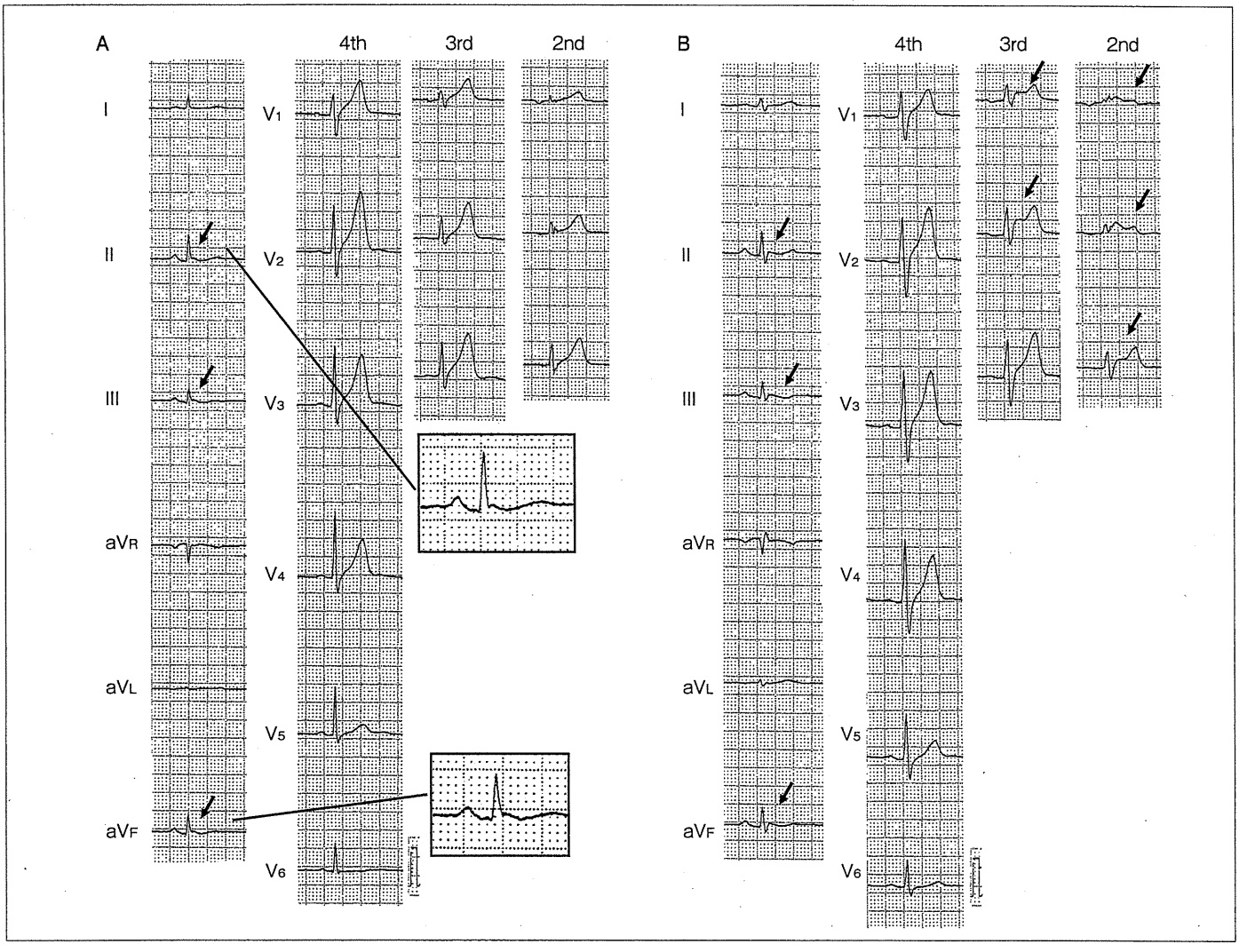

図1　VF既往のある下側壁早期再分極症候群例の標準12誘導ならびに高位肋間心電図
A：control時，B：ピルジカイニド50 mg静注後。矢印はJ波を示す。
Control時にはⅡ，Ⅲ，aVF誘導でのみJ波が認められるが（図A），ピルジカイニド負荷後には同部位と，高位肋間（第2，3肋間）のV_1～V_3誘導でJ波（saddleback型ST上昇）が認められる（図B）。本例は下壁早期再分極＋前壁早期再分極例と思われる。

が生じてJ波が出現するが，遺伝子異常等により，内向きのNa電流やCa電流などが減少すると，I_{to}を含む相対的な外向き電流が増加して，活動電位第1相のnotchが大きくなり，心外膜－心内膜間に大きな電位勾配が生じる。それによりJ波の増大に引き続いてST上昇が起こる。さらに相対的な外向き電流が増加すると，心外膜側で第2相のdome形成が遅延または消失し，貫壁性および心外膜層内で再分極時間のばらつきが生じると共に，domeの消失した心筋において再脱分極が起こる。これはphase2 reentryと呼ばれ，これからVFが発生するとされている[8]。

この理論はBrugada症候群におけるJ波とVFの発生機序でもあり，このためAntzelevitchらは早期再分極症候群とBrugada症候群が一連の疾患であるとして，早期再分極症候群を4つのタイプに分類している。すなわち側壁誘導（Ⅰ，V_4～V_6）にのみJ波が存在するものをType1，下壁（Ⅱ，Ⅲ，aV_F）または下側壁誘導に存在するものをType2，下壁，側壁と右前胸部のすべての誘導に広範囲に存在するものをType3，そしてBrugada症候群をType4とし，早期再分極症候群では1から3の順で危険性が増すとしている[9]。

一方，J波が真に再分極の波形なのかを疑問視する報告もある。Surawiczらは，種々の論文で引用された早期再分極波形からJ波の時相を検討すると，J波はR波の一部にすぎない可能性があり，再分極の波形とは断言できないと述べている[10]。我々も体表面電位図や心磁図の検討から，少なくともJ波notchのpeakはQRS内にあることを確認している。この他，J波は急性心筋虚血，肥大型心筋症，左室乳頭筋の肥大例，運動選手，QT短縮症候群など

に認められると報告されており[11]，J波の成因に関しては，今後多方面からのアプローチが必要と考えられる。

原因遺伝子

これまで早期再分極症候群では，KATP電流を増加させるKCNJ8遺伝子，L型Ca電流を低下させるCACNA1C, CACNB2b, CACNA2D1遺伝子，Na電流を低下させるSCN5A遺伝子の変異が報告されている[12,13]。しかしながらそれらはいずれもBrugada症候群の原因遺伝子でもあり，報告された例の心電図を見るとBrugada症候群と紛らわしいものも少なくない。このため，これらが軽症型のBrugada症候群である可能性を否定できない。

心電図陽性率，発症率

これまでに，1 mm以上のJ波は，1回の心電図記録で数%から11%に認められるが，運動選手では陽性率が25%から44%に上昇すると報告されている。男性には女性の2～3倍多く存在し，その他に若年者，黒人，徐脈，左室肥大例で出現頻度が高くなる。運動選手には側壁の早期再分極が多いが，成人全体で見ると下壁の早期再分極の方が側壁よりも多い[14-18]。Harutaらは，原爆の被爆者を46年間経過観察した結果，J波の発症率は年間0.7%で，Brugada症候群の約50倍に相当し，心電図陽性率は初回の10.9%から加齢，記録回数と共に上昇して最終的には23.9%に達すると報告している[18]。

一方，VFの既往のある例では，J波の頻度は約30%に上り，下壁，高位側壁，側壁誘導の順に頻度が低下すると報告されている[19]。またVF既往の運動選手ではST上昇を伴わないJ波が健常人に比し有意に多いことも指摘されている[20]。

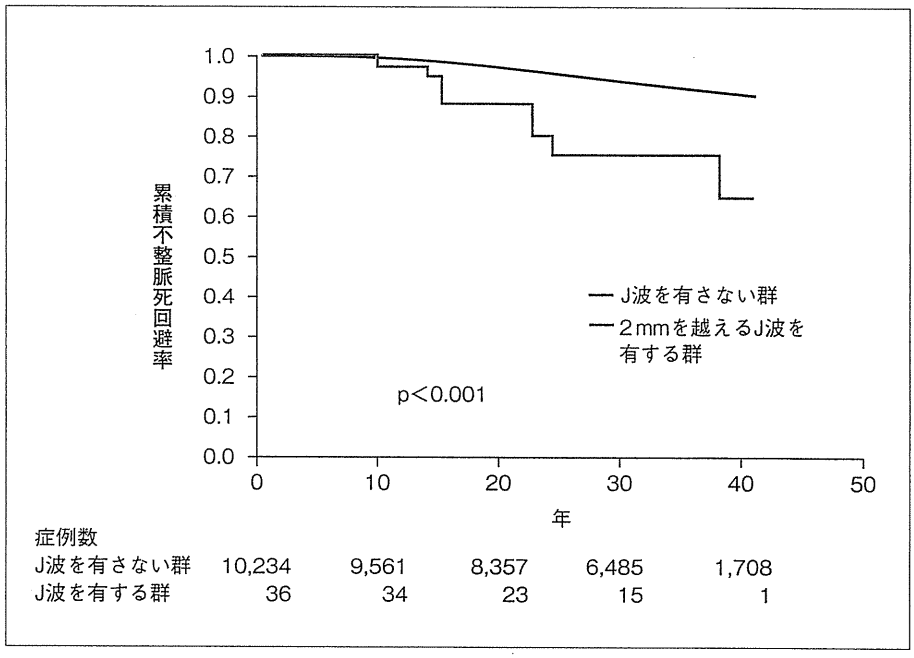

図2　2mmを越える高さのJ波を有する下側壁早期再分極症候群の後ろ向き予後
図はJ波のある群と，ない群の累積不整脈死回避率を示す。
(文献14より引用)

病態と予後

1．下側壁早期再分極症候群

Haïssaguerreらは VFを合併した下側壁早期再分極症候群の一連の報告の中で，VFは睡眠中に20%が起こり，その連結期は260～400 msecと短く，起源の2/3は左室である。電気生理学検査（EPS）でのVF誘発率は34%で，経過観察中27%が複数回VFを生じる。VF直前にはJ波が増高するが，β遮断薬でも増高する。一方，イソプロテレノールではJ波は減高し，ストーム出現例ではイソプロテレノールによりVFが消失する。また予防にキニジンが有効であると述べている[1,21]。これらの特徴はBrugada症候群に似ているが，睡眠中のVF発作率，EPSでのVF誘発率等は頻度が少なく，VFの1/3だけが右室から生じる点もやや異なっている。

VFの既往のない早期再分極例の予後に関しては，フィンランドのTikkanenらが10,864人の成人を約30年間後ろ向きに経過観察している[14]。それによると，側壁誘導のJ波は予後に関係しないが，下壁にJ波のある例は心臓死，不整脈死が有意に多い。またJ波の大きい例の予後が悪く，下壁誘導に2 mmを越えるJ波を有する例では，30年間で心臓死が約35%，不整脈死が約25%に生じたと報告している。この他に彼らは，下壁誘導のJ波例の中でもJ波に続くST部がascending/upslopingの例の予後はよいが，horizontal/descendingの例は有意に不整脈死が多いとも述べている[15]。Tikkanenらは死亡原因を死亡診断書から推測しており，虚血性心疾患の多い北欧では不整脈死の一定数が虚血で生じた可能性がある。しかしながら2 mm以上のJ波をもつ無症候

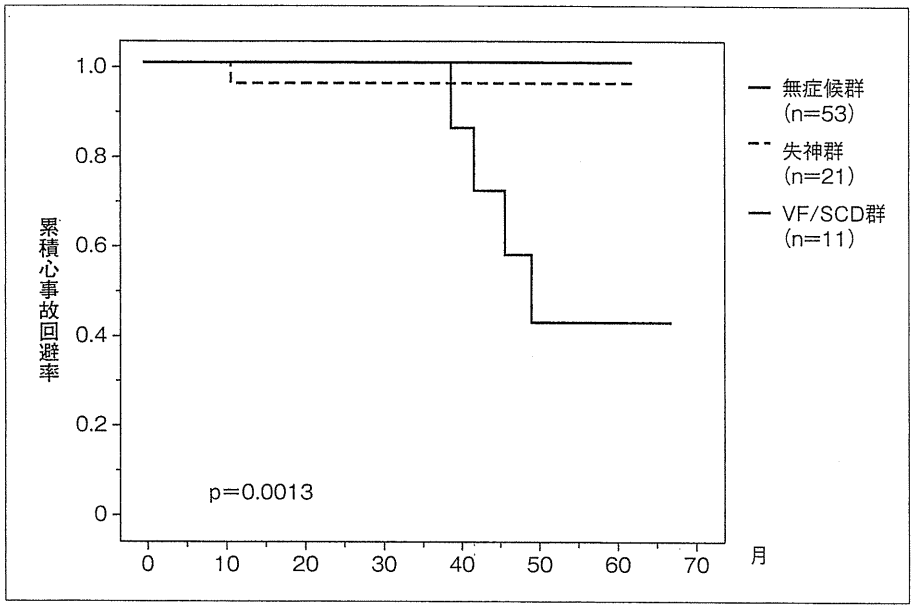

図3　循環器病委託研究における前壁早期再分極（非Type1 Brugada症候群）例の前向き予後
図は症状別の累積心事故回避率を示す。

性早期再分極症候群の年間不整脈死率は約0.8％程度であり，この結果から下側壁早期再分極症候群の予後は，日本人の無症候性Brugada症候群の予後と同等かそれ以下と考えられる（**図2**）。

一方，Harutaらも下側壁に早期再分極を伴う被爆者の予後を調査しており，それによると早期再分極があると不測死が有意に多く（HR：1.8），特に下壁および側壁にJ波のある人，J波にslurとnotchの両方がある人に不測死が多いと報告している[18]。

2. 前壁早期再分極症候群

われわれはBrugada症候群の前向き予後調査（循環器病委託研究）の中で，非Type1 Brugada症候群の病態と予後を報告している[3]。報告当時は前壁の早期再分極という概念がなかったので，この群をBrugada症候群の一部と考えたが，用いた非Type1 Brugada例の心電図基準は，はからずも前壁の早期再分極の基準にほぼ一致するものであった。約5年間の経過観察の結果，前壁の早期再分極例はType1 Brugada症候群とほぼ同一の病態，予後を示していた（**図3**）。

Haïssaguerreらは下側壁早期再分極症候群の定義の中で，Type1 Brugada症候群を除外しているが，この定義ではV_1～V_3誘導のsaddleback型心電図例，すなわち前壁の早期再分極例が下側壁早期再分極症候群に含まれる可能性がある。我々はVFを伴う早期再分極症候群23例にNaチャネル遮断薬による薬物負荷を高位肋間心電図記録下で行い，下側壁早期再分極症候群を，純粋な下側壁早期再分極例と，前壁＋下側壁早期再分極例に分類して病態と予後を調査した。その結果，前者は体動時にVFが生じ，かつほとんどの例で再発を生じなかったが，後者は主として睡眠中にVFが生じ，高率に再発，ストームが生じて，VFを合併するType1 Brugada症候群と同様な臨床所見を示した[22]。後者は早期再分極症候群全体の約30％を占めたが，これはくしくもHaïssaguerreらの報告において，30％近い症例でVFが再発し，それらにBrugada症候群の治療が有効であった事実に一致していた。このため，下側壁早期再分極症候群でVFを繰り返す例は前壁＋下側壁の早期再分極合併例である可能性が示唆された。早期再分極症候群の病態・予後に関しては種々の方面からの解析が必要と思われるが，今後は早期再分極の部位を前壁，下側壁に厳密に区分した上で病態・治療を再検討する必要があると考えられた。

治療

前述の如く，下側壁早期再分極症候群の一部ではイソプロテレノール，キニジンなどBrugada症候群の治療が有効と報告されている。特に前壁早期再分極症候群を合併したVF既往例はVFが再発する危険性が極めて高いので，ICD植込みが必須と考えられる。一方，それ以外の早期再分極症候群に関しては前向きの予後が未だ不明である。後ろ向き調査の結果からは予後が良好と思われるが，一般人におけるJ波の陽性例が多いことから，機序の解明を含めた総合的な診断・治療指針の確立が急務と考えられる。

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A novel gain-of-function *KCNJ2* mutation associated with short-QT syndrome impairs inward rectification of Kir2.1 currents

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Aims Short-QT syndrome (SQTS) is a recently recognized disorder associated with atrial fibrillation (AF) and sudden death due to ventricular arrhythmias. Mutations in several ion channel genes have been linked to SQTS; however, the mechanism remains unclear. This study describes a novel heterozygous gain-of-function mutation in the inward rectifier potassium channel gene, *KCNJ2*, identified in SQTS.

Methods and results We studied an 8-year-old girl with a markedly short-QT interval (QT = 172 ms, QTc = 194 ms) who suffered from paroxysmal AF. Mutational analysis identified a novel heterozygous *KCNJ2* mutation, M301K. Functional assays displayed no Kir2.1 currents when M301K channels were expressed alone. However, co-expression of wild-type (WT) with M301K resulted in larger outward currents than the WT at more than −30 mV. These results suggest a gain-of-function type modulation due to decreased inward rectification. Furthermore, we analysed the functional significance of the amino acid charge at M301 (neutral) by changing the residue. As with M301K, in M301R (positive), the homozygous channels were non-functional, whereas the heterozygous channels demonstrated decreased inward rectification. Meanwhile, the currents recorded in M301A (neutral) showed normal inward rectification under both homo- and heterozygous conditions. Heterozygous overexpression of WT and M301K in neonatal rat ventricular myocytes exhibited markedly shorter action potential durations than the WT alone.

Conclusion In this study, we identified a novel *KCNJ2* gain-of-function mutation, M301K, associated with SQTS. Functional assays revealed no functional currents in the homozygous channels, whereas impaired inward rectification demonstrated under the heterozygous condition resulted in larger outward currents, which is a novel mechanism predisposing SQTS.

Keywords Arrhythmia (mechanisms) • Short-QT syndrome • K-channel • Atrial fibrillation • Inward rectification

1. Introduction

Short-QT syndrome (SQTS) is a recently recognized disorder, characterized by a shortened QT interval in the electrocardiogram (ECG), and associated with a high incidence of atrial fibrillation (AF), syncope, and sudden death due to ventricular tachyarrhythmias without structural cardiac abnormalities. The syndrome was first described by Gussak *et al.*[1] in 2000 within the context of a familial AF case associated with short-QT interval. SQTS is a genetically heterogeneous disease, and five ion channel genes (SQT1-6) have been identified as causative genes thus far: *KCNH2* encoding the α-subunit of the rapidly activating delayed rectifier potassium channels, I_{Kr} (SQT1)[2]; *KCNQ1* encoding the α-subunit of the slowly activating delayed rectifier potassium channels, I_{Ks} (SQT2)[3]; *KCNJ2* encoding

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the Kir2.1 channels that underlie the inward rectifier potassium currents, I_{K1} (SQT3)[4]; *CACNA1C*, *CACNB2b*, and *CACNA2D1*, which encode the α1C, β2b, and α2δ-1-subunits of cardiac L-type calcium channels (SQT4, SQT5,[5] and SQT6[6]), respectively. SQT4 and SQT5 are considered clinical entities with the combined phenotypic characteristics of SQTS and Brugada syndrome, manifesting in a J point and ST-segment elevation in the right precordial ECG leads.

Regardless of the extensive genetic screening carried out on SQTS patients, genetic mutations have been identified in a small number of cases.[2–5,7,8] In 2005, Priori *et al.*[4] first reported that a *KCNJ2* mutation was responsible for SQTS (SQT3); however, no additional SQT3 variants have been reported thus far. This lack of progress has significantly hindered our advances in understanding the mechanisms underlying this disease. In the present study, we describe a novel *KCNJ2* mutation which impaired the inward rectification of Kir2.1 currents. This is a novel *KCNJ2* gain-of-function mechanism leading to SQTS.

2. Methods

2.1 Genetic analysis

Genetic analysis was performed after written informed consent in accordance with the study protocol approved by the Kyoto University ethical committee. The investigation conforms to the principles outlined in the Declaration of Helsinki. Genomic DNA was isolated from blood lymphocytes, and screened for the entire open-reading frames of *KCNQ1*, *KCNH2*, *KCNE1-3*, *KCNJ2*, *CACNA1C*, and *SCN5A* by denaturing high-performance liquid chromatography using a WAVE System Model 3500 (Transgenomic, Omaha, NE, USA). Abnormal conformers were amplified by polymerase chain reaction and sequencing was performed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and compared with 400 Japanese control alleles.

2.2 Neonatal rat ventricular myocyte isolation

This investigation was performed in accordance with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996), and was approved by the Kyoto University Animal Experimentation Committee. A standard trypsin dissociation method was used to prepare neonatal rat ventricular myocytes (NRVMs).[9] The hearts were removed from 1- to 2-day-old Wistar rats euthanized by decapitation. The ventricles were minced, and the myocytes were dissociated with trypsin. Dispersed cells were preplated on 100 mm culture dishes for 1 h at 37°C in 5% CO_2 to remove fibroblasts. Non-attached, viable myocytes were collected, and placed on 35 mm culture dishes.

2.3 Mutagenesis and transient transfection of *KCNJ2* plasmids

The entire coding region of the *KCNJ2* was subcloned into the pCMS-EGFP vector (Clontech, Palo Alto, CA, USA) using methods previously described.[10] The mutation was introduced by site-directed mutagenesis using the QuikChange Mutagenesis Kit (Stratagene, La Jolla, CA, USA). We sequenced the entire plasmid to confirm the presence of the mutation and the absence of any unwanted variations. To assess the functional modulation of mutant channels, human embryonic kidney (HEK) 293 cells were transiently transfected with *KCNJ2* WT and/or mutant plasmids using FuGENE 6 (Roche, Indianapolis, IN, USA) as directed in the manufacturer's instructions. In order to investigate the mutant's effects on myocyte action potentials, plasmids were transfected 1 day after plating NRVMs, using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).[11]

2.4 Cell surface expression of *KCNJ2*

Immunofluorescence microscopy was used to detect the presence of KCNJ2 channels on the plasma membrane of HEK 293 cells. A haemagglutinin (HA) epitope (YPYDVPDYA) was introduced into the pCMS-EGFP-KCNJ2 [wild-type (WT) and mutant] construct between residues Ala-115 and Ser-116 (extracellular loop between TM1 and TM2).[10,12] HEK 293 cells were transfected with 1.0 μg of WT or mutant plasmids, or 0.5 μg of each WT and mutant plasmids to assess a heterozygous condition in 35 mm glass-bottom dishes. Two days later, the cells were fixed with 4% paraformaldehyde solution, and images were taken at ×40 magnification on an LSM 510 confocal microscope (Carl Zeiss, Jena, Germany).

2.5 Electrophysiological analysis

For voltage-clamp experiments, a total of 0.75 μg of WT and/or mutant *KCNJ2* plasmids were transfected in HEK 293 cells; 48–72 h after transfection, functional assays were conducted on GFP-positive cells by a conventional whole-cell configuration of patch-clamp techniques at 37°C, using an Axopatch 200A patch clamp amplifier and a Digidata 1322A digitizer (Axon Instruments, Foster City, CA, USA).[10] Pipettes were filled with a solution (in mM): 140 KCl, 2 $MgCl_2$, 1 EGTA, and 10 HEPES (pH 7.3 with KOH). The bath solution was composed of (in mM): 135 NaCl, 5 KCl, 1 $MgCl_2$, 10 glucose, and 10 HEPES (pH 7.4 with NaOH).

In order to record action potentials on NRVMs, 3 μg of WT, or a mixture of 1.5 μg WT and 1.5 μg mutant *KCNJ2* plasmids, were transfected; 48–72 h after transfection, functional assays were conducted on non-transfected or transfected cells that were recognized by their obvious green fluorescence, using a whole-cell patch-clamp technique at 37°C with the same devices. Action potentials were evoked by 2 ms supra-threshold current pulses at 10 Hz in a current-clamp mode. The pipette solution contained (in mM): KCl 140, $MgCl_2$ 1, MgATP 4, NaCl 10, and HEPES 10 (pH 7.2 with KOH). Tyrode solution contained (in mM): NaCl 140, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, and glucose 10 (pH 7.4 with NaOH). Action potential duration (APD) was measured as the time from the overshoot to 90% repolarization (APD_{90}).

2.6 Statistics

All the data are shown as mean ± standard error of the mean. For mean value and comparisons between two sample groups, an unpaired Student's *t*-test was used to evaluate statistical significance. For comparisons between multiple groups, we applied a Steel–Dwass test. For either evaluation, a *P*-value <0.05 was considered significant.

3. Results

3.1 Clinical features

An 8-year-old girl with a markedly shortened QT interval (QT = 172 ms, QTc = 194 ms; *Figure 1A*) had been suffering from multiple disorders, such as severe mental retardation, abnormal proliferation of oesophageal blood vessels, epilepsy, and Kawasaki disease. Upon presentation during a routine check-up, her treating physician noticed an irregular heart rhythm. Her 12-lead ECG showed AF (*Figure 1B*), and she underwent external electrical cardioversion because intravenous infusion of procainamide (15 mg/kg) failed to recover sinus rhythm. The echocardiography revealed no significant abnormality. During further evaluation with right-heart catheterization, the Swan–Ganz catheter induced supra-ventricular tachycardia when it was inserted in the right atrium, and ventricular fibrillation occurred at the position of the right ventricular outflow tract, which suggested the presence of increased myocardial irritability.

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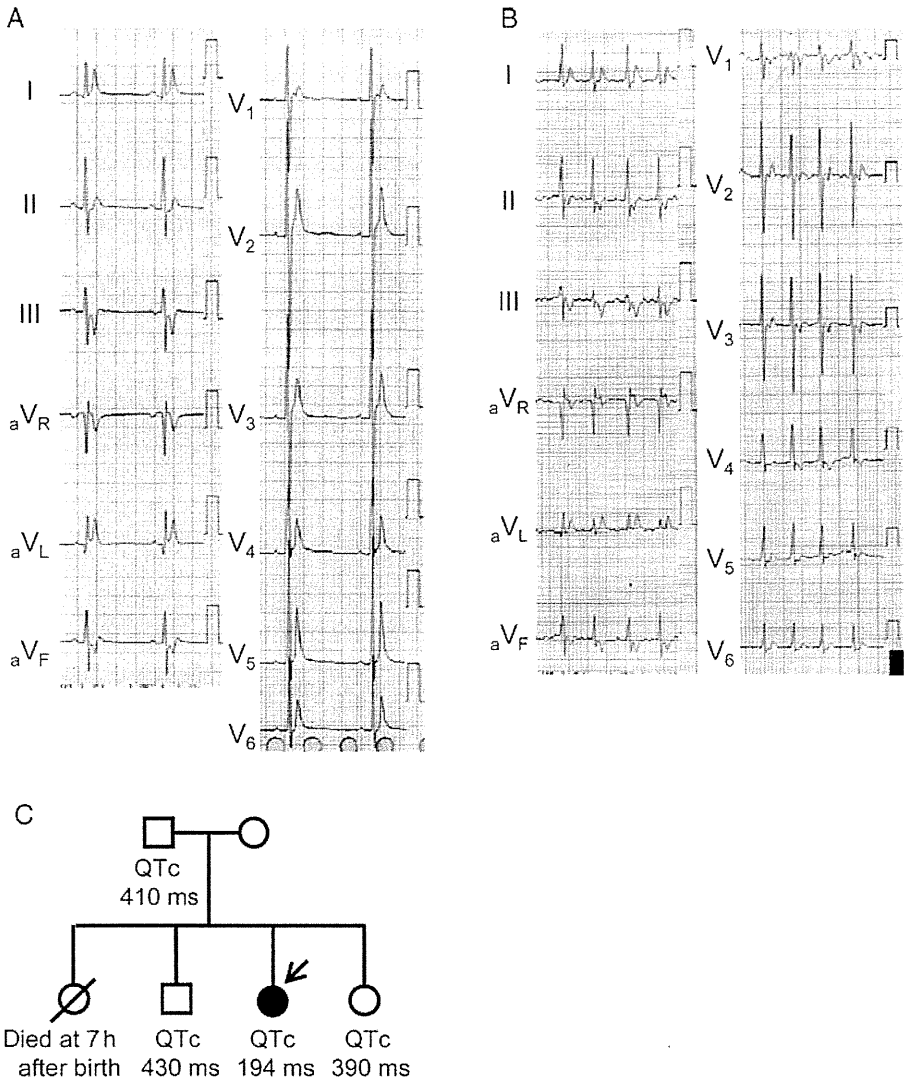

Figure 1 ECG of the proband and family pedigree. ECG shows sinus rhythm (*A*) and AF (*B*). The QT and QTc intervals were 172 and 194 ms, respectively. (*C*) Family pedigree. Arrow indicates the proband; a filled symbol indicates clinically and genetically affected individual.

She was diagnosed with SQTS from these clinical features (i.e. a markedly shortened QT interval, paroxysmal AF, and VF inducibility).

The proband had a family history of perinatal death in her elder sister (*Figure 1C*), but her family did not undergo genetic investigation or further clinical evaluation with the exception of ECGs taken for her father, elder brother, and younger sister. Genetic investigations could not be carried out due to a lack of informed consent. The ECGs for the family members displayed normal QTc intervals (410, 430, and 390 ms, respectively; *Figure 1C*).

3.2 Genetic analysis

In this patient, we screened for candidate cardiac ion channel genes (*KCNQ1*, *KCNH2*, *KCNE1-3*, *KCNJ2*, *CACNA1C*, and *SCN5A*). As a result of the genetic analysis, we identified a novel heterozygous mutation, a single-base substitution at nucleotide 902 (c.902T>A) in the *KCNJ2* gene, resulting in an amino acid change from methionine to lysine at 301 in the Kir2.1 potassium channel (*Figure 2A*). Met-301 is located in the C-terminal cytoplasmic domain of the channel (*Figure 2B*).[13] The amino acid at codon 301 (methionine) is highly conserved among different species (*Figure 2C*). Furthermore, this mutation was absent in 400 Japanese control alleles. We failed to identify mutations in any other candidate genes.

3.3 Cell surface expression of KCNJ2 mutants

In order to investigate whether the M301K mutations affect intracellular Kir2.1 trafficking, we introduced an HA epitope into the extracellular domain of KCNJ2, and examined the subcellular distribution of channels in transfected HEK 293 cells using confocal microscopy[10] (*Figure 2D*). *Figure 2D* illustrates the typical results of confocal imaging. HEK 293 cells were successfully transfected with either HA-*KCNJ2 WT*, *KCNJ2* WT/HA-M301K, or HA-M301K (*Figure 2D*, upper panels). All types of HA-tagged Kir2.1 proteins exhibited red fluorescence at the plasma membrane (*Figure 2D*, middle and lower panels), indicating that both homo- and heterozygous mutant channels were trafficking-competent.

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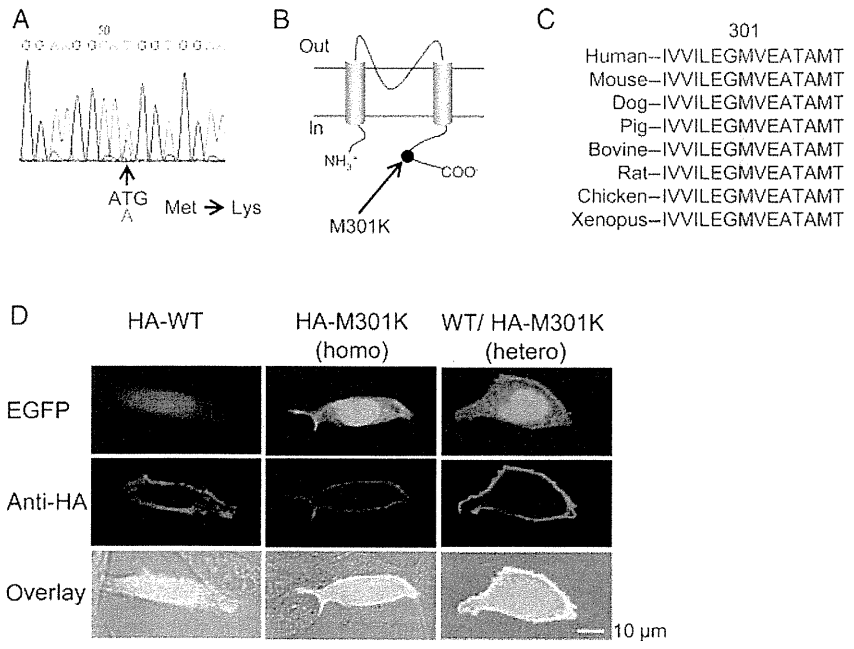

Figure 2 DNA sequence, topology, and homology. (*A*) Mutated DNA sequences derived from patient's genomic DNA. The trace shows a heterozygous substitution of thymine to adenine resulting in the amino acid change M301K. (*B*) Topology of the Kir2.1 channel showing localization of M301. (*C*) Amino acid sequence alignment of Kir2.1 channels from various species in the region surrounding codon 301 (highlighted). (*D*) Cellular localization of WT and mutant Kir2.1 channels. HA-WT indicates HA-tagged KCNJ2-WT, HA-M301K;HA-tagged KCNJ2-M301K, and WT/HA-M301K;KCNJ2-WT without HA-tagging and HA-tagged KCNJ2-M301K. The upper panel shows GFP, the middle panel shows the red fluorescence of the secondary anti-HA antibody, and the bottom panel is a mergence of the green fluorescence, red fluorescence, and transmission.

3.4 Cellular electrophysiology

We performed a functional characterization of the mutant channels in HEK 293 cells. *Figure 3A* shows representative current traces from cells expressing *KCNJ2* WT, M301K, or WT/M301K, elicited by voltage-clamp steps (duration 400 ms) from −120 to +100 mV (10 mV step), applied from a holding potential of −60 mV. The currents were normalized to cell capacitance and were plotted as a function of test potentials (*Figure 3B*). As previously reported, expression of the *KCNJ2* WT in HEK 293 cells resulted in normal inward rectifying potassium currents (*Figure 3A* left panel and blue symbols in *Figure 3B*). When M301K mutant channels were expressed alone, they were entirely non-functional (*Figure 3A* middle panel and green symbols in *Figure 3B*). In contrast, when cells were co-transfected with both equimolar WT and M301K, ample potassium currents showing a very weak inward rectification could be recorded (*Figure 3A* right panel and red symbols in *Figure 3B*). Average current densities were significantly smaller than those of WT Kir2.1 channels at potentials between −120 and −90 mV ($P < 0.05$), and significantly larger at potentials between −30 and +100 mV ($P < 0.05$).

3.5 Contribution of amino acid charge at residue 301 to Kir2.1 currents

Methionine at 301 is located within the G-loop that forms the narrowest segment of the cytoplasmic pathway,[13,14] and negatively charged amino acids on the inner wall of the cytoplasmic pore, where the G-loop is located, are known to be important for the strength of the inward rectification.[13–15] We therefore speculated that the amino acid charge at this position may be crucial for the inward rectification of Kir2.1 channels, and that its change from methionine (neutrally charged) to lysine (positively charged) may result in functional changes in Kir2.1 currents. In order to analyse the contribution of the amino acid charge at 301 to inward rectification, we changed the amino acid at M301 to another positively charged amino acid, arginine, and to another neutral amino acid, alanine, for comparison. *Figure 4A* illustrates the whole-cell Kir2.1 currents in homo- and heterozygous mutant conditions for M301R (left panel) and M301A (right panel). Homozygous M301R mutant channels displayed no functional currents, whereas WT/M301R attenuated the inward rectification (*Figure 4A* left panel). These observations suggest that the currents through the M301R channels are similar to those of the M301K channels (*Figure 3*) under both homo- and heterozygous conditions. On the other hand, in the M301A channels—in which the residual charge remained neutral—the currents showed normal inward rectification in both homo- and heterozygous conditions similar to those produced by WT Kir2.1 channels (*Figure 4A* right panel). In order to evaluate the intensity of inward rectifying properties, we assessed the rectification index, along with the ratio of the current amplitudes at 0 and −100 mV.[15] *Figure 4B* shows the rectification indexes obtained from WT, M301A (0.10 ± 0.02, $n = 10$), WT/M301A (0.073 ± 0.015, $n = 11$), WT/M301K (1.12 ± 0.16, $n = 11$), and WT/M301R (0.99 ± 0.14, $n = 11$). Although the rectification indexes for WT/301A and M301A showed no significant difference, the indexes for both WT/M301K and WT/M301R were significantly increased in comparison with WT (0.061 ± 0.01, $n = 15$, $P < 0.001$, left-most bar in *Figure 4B*).

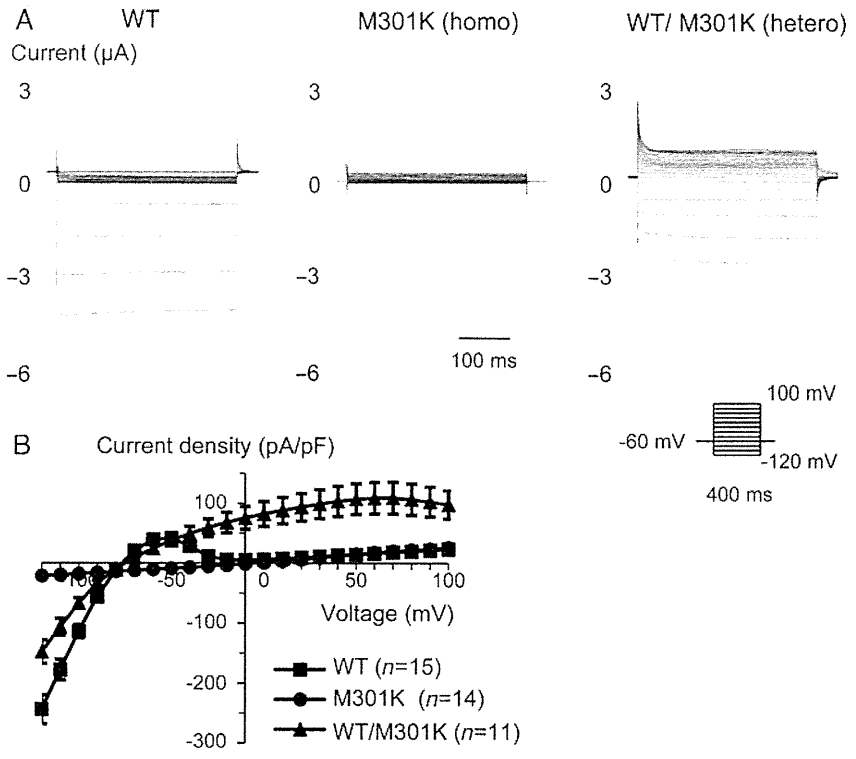

Figure 3 Voltage-clamp recordings from transfected HEK 293 cells. (*A*) Representative current traces of WT, M301K, and WT/M301K. Currents were elicited by 400 ms depolarizing voltage steps from −120 to +100 mV and from a holding potential of −60 mV. (*B*) Current–voltage relationships are plotted as the current. Current density was calculated by dividing the whole-cell current amplitude by cell capacitance. No functional currents were recorded in the homozygous M301K channels. On the other hand, the mean current densities of the WT/M301K channels are significantly larger than the WT ($P < 0.05$) at each voltage from −30 to +100 mV, and smaller at each voltage from −120 to −90 mV ($P < 0.05$).

3.6 Action potentials recording in *KCNJ2*-M301K-transfected NRVMs

We investigated the impacts of M301K mutant Kir2.1 channels on NRVMs' action potentials using a transient transfection method. *Figure 5A* shows typical action potentials recorded for non-transfected (control) NRVMs (*Figure 5A*, left panel), and NRVMs transfected with *KCNJ2* WT or WT/M301K (*Figure 5A* middle and right panels, respectively). Phase 3 repolarization was accelerated in the *KCNJ2* WT- and WT/M301K-overexpressed groups (*Figure 5A* middle and right panels, respectively) and we could further note that the dome is nearly lost in the WT/M301K group. APD_{90} was significantly abbreviated in the *KCNJ2* WT-overexpressed group (28.2 ± 3.4 ms, $n = 10$, $P < 0.001$, *Figure 5A*, middle panel) in comparison with the control group (123.3 ± 12.2 ms, $n = 11$, *Figure 5A*, left panel; bar graphs in *Figure 5B*). Additionally, APD90 was significantly shorter in the WT/M301K mutant-overexpressed group (9.4 ± 2.1 ms, $n = 16$, $P < 0.001$, *Figure 5A*, right panel; bar graph in *Figure 5B*) than in the WT-overexpressed group.

4. Discussion

4.1 Major findings

In the present study, we identified a novel heterozygous *KCNJ2* mutation, M301K, in a patient with a markedly shortened QT interval. The QT interval, 172 ms, of this patient is the shortest among previous SQTS reports,[2–7,16] to our knowledge. The methionine at position 301 is located in the C-terminus of Kir2.1 channel, and is considered to form a pore-facing loop region.[13] Functional assays using a heterologous expression system revealed that homozygous M301K Kir2.1 channels carried no currents with preserved plasma membrane expression; however, heterozygous WT/M301K Kir2.1 channels attenuated inward rectifying properties, which resulted in increased outward currents for positive voltages and negative voltages down to −30 mV. Significant increases in outward currents within the voltage range of the action potentials shortened APD by accelerating membrane repolarization as shown in *Figure 5*, which is implicated in increased cardiac vulnerability.

4.2 Impaired inward rectification of Kir2.1 currents: a novel mechanism predisposing SQTS

In 2005, Priori *et al.*[4] first reported a heterozygous gain-of-function *KCNJ2* mutation, D172N, in a patient with SQTS. In the report, homozygous D172N Kir2.1 channels displayed larger outward currents compared with WT Kir2.1 alone, and heterozygous channels yielded intermediate results. In both homozygous and heterozygous D172N mutant channels, the inward rectification properties of Kir2.1 currents were preserved. In heterozygous M301K mutant channels identified in our patient, however, the inward rectification was significantly reduced, allowing ample outward potassium currents at positive potentials. In addition, it should be emphasized that the homozygous M301K mutant channels were non-functional. These functional changes, such as the impaired inward rectification of the

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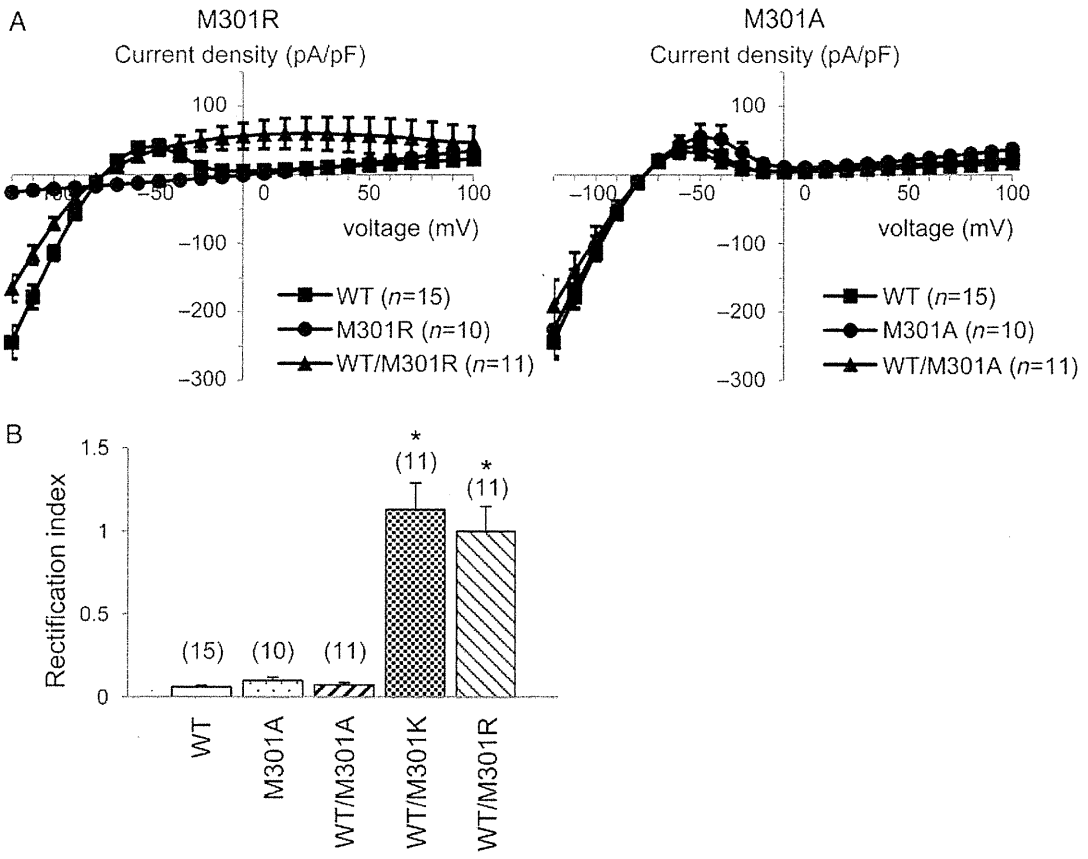

Figure 4 Comparison of macroscopic currents through WT Kir2.1 and mutants. (*A*) Current–voltage relationships for WT, M301R, and M301A are shown. M301R mutant channels displayed no functional currents and WT/M301R mutant channels displayed decreased inward rectification. On the other hand, the currents recorded in the homozygous M301A and heterozygous WT/M301A mutant channels showed no significant difference from WT. (*B*) Rectification index for WT ($n = 15$), M301A ($n = 10$), WT/M301A ($n = 11$), WT/M301K ($n = 11$), and WT/M301R ($n = 11$) channels. The rectification index was calculated by dividing the value of the outward currents measured at 0 mV by the absolute value of the inward currents measured at −100 mV. $^*P < 0.001$.

Kir 2.1 currents resulting in increased outward currents, are a novel *KCNJ2* gain-of-function mechanism predisposing SQTS.

The phenotypic characteristics of our index patient somewhat differ from those of the *KCNJ2*-D172N mutation carriers.[4] No apparent arrhythmias were recorded with D172N mutation carriers. On the other hand, our M301K patient showed paroxysmal AF and multiple disorders. Additionally, mechanical stimulation by a Swan–Ganz catheter induced paroxysmal supraventricular tachycardia and VF. Moreover, the QTc interval in our patient was much shorter (QTc = 194 ms, *Figure 1*) than that of the D172N carriers (QTc = 315 and 320 ms).[4] Another gain-of-function *KCNJ2* mutation, V93I, was reported in a familial AF case.[17] Their functional analysis showed a similar result with D172N, but the affected members had normal QT intervals. These diverse clinical manifestations may be related to the extent and the different gain-of-function mechanisms of the Kir2.1 currents.

4.3 Relationship between impaired inward rectification and charged amino acid residues at 301

Kir currents exhibit strong inward rectification, which is thought to be due to pore blocking induced by multivalent ions from intracellular Mg^{2+}.[18–20] Channel blockade by physiological concentrations of Mg^{2+} is influenced by the electrostatic negativity within the cytoplasmic pore.[15] Negative charges on the inner wall of the cytoplasmic pore are therefore key determinants of the strength of the inward rectification. Many amino acid residues inside the pore demonstrate interactions with the ion over long distances, suggesting that mutations potentially affect ion or blocker energetics over the entire pore profile.[14,21] The M301K mutation causes the change of the amino acid residue at 301 from a non-charged amino acid residue, methionine, to a positively charged residue, lysine. In order to evaluate the importance of the charge at 301, additional whole-cell patch-clamp recordings were carried out on M301A (remained neutral) and M301R (neutral to positive) (*Figure 4*). Inward rectification of Kir2.1 currents was well preserved in both homozygous and heterozygous M301A channels. Heterozygous M301R channels, however, attenuated inward rectification, and homozygous M301R channels were non-functional similar to that of the M301K channels. These electrophysiological results indicate that the neutral amino acid residue at 301 plays an important role in generating Kir2.1 inward rectification. The decrease in the net negative charge within the cytoplasmic pore may facilitate the reduction in both the susceptibility of the channel to Mg^{2+} block and the voltage dependence of the blockade. It

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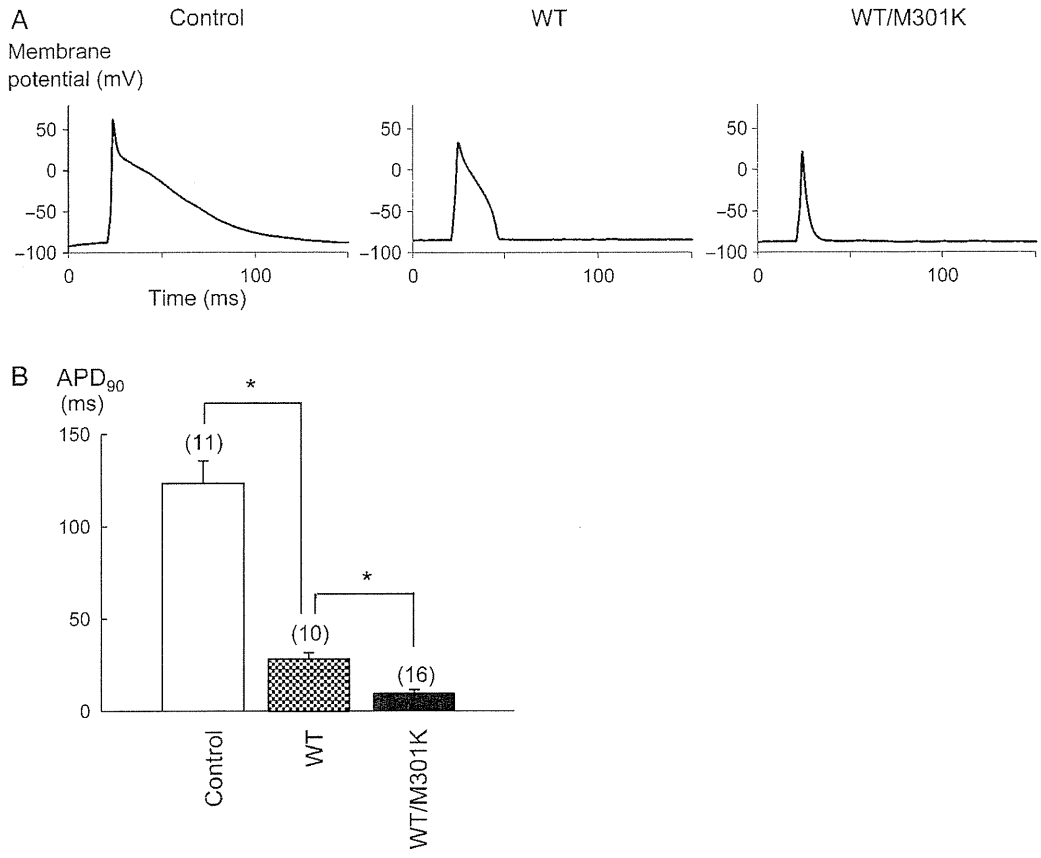

Figure 5 Effects of the M301K mutation on NRVM action potentials. Typical action potentials were demonstrated in a non-transfected cell (*A*), in a WT-overexpressed cell (*B*), and in a heterozygous overexpressed cell (*C*). Graphs show APD at 90% repolarization from the overshoot (*D*). In WT-overexpressed NRVMs, the plateau phase of the cardiac AP was markedly abbreviated, resulting in short repolarization. Under the heterozygous overexpressed condition, the results exhibited virtually no plateau phase, and the mean APD_{90} was significantly shorter in comparison with WT overexpressed alone. $^*P < 0.001$.

remains unknown why only tentative hetero-multimers of WT and M301K are active and lose their inward rectification properties. In homozygous M301K channels, all of the tetrameric subunits must have a positively charged lysine at 301, which may impair potassium ion permeation due to a conformational change in the near-pore region.

4.4 Heterozygous *KCNJ2*-WT/M301K overexpression shortened APD in NRVMs

In cardiomyocytes, Kir2.1, Kir2.2, and Kir2.3 channels are supposed to be able to co-assemble in order to modulate their channel properties.[22] Thus, there can be a multitude of Kir2.x heteromultimers, and to date a wide range of single-channel conductances of inward rectifier channels have been reported in studies conducted on various mammalian myocytes, including human.[23–25] This variety at the individual channel level may contribute to the different stoichiometry of the tetrameric channels.[26] Because Kir2.1 is a major component of IK1 in the myocardium, we overexpressed the *KCNJ2* M301K mutant channels in NRVMs to examine the effects of the mutation on APD. Overexpression with WT alone resulted in shorter APD in comparison with non-transfected myocytes (*Figure 5B*). These results are consistent with a previously published report.[27] Notably, heterozygous overexpression with WT and M301K further amplified the shortened APD (*Figure 5C*). These results were compatible with the electrophysiological changes assessed in HEK 293 cells, because the heterozygous WT/M301K channels showed a larger outward current than WT Kir2.1 channels under the physiological range of membrane potentials (*Figure 3*). Weak inward rectification observed in the heterozygous WT/M301K channels suggests that potassium ion can get through Kir2.1 channel at depolarized potential, probably resulting in loss of the action potential dome recorded in the *KCNJ2* WT/M301K-overexpressed group. The experiments were performed using a transient overexpression system that was different from the patient's heart, and the amount of overexpressed channels was difficult to be estimated accurately. But, these results are beneficial in understanding that the heterozygous *KCNJ2* M301K mutation could abbreviate APD and cause an extremely short-QT interval in the patient's ECG.

4.5 Clinical features of the index patient with *KCNJ2*-M301K

Regarding the clinical criteria for the diagnosis of SQTS, they have yet to be defined. However, we should consider SQTS in a patient presenting with a QTc <340 ms and other factors suggestive of arrhythmia (such as syncope or family history of sudden death).[28] A prominent clinical manifestation of SQTS is arrhythmias, such as AF

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and VF.[1–5,7] In this patient, however, additional medical histories not limited to arrhythmias, such as severe mental retardation, abnormal proliferation of the oesophageal blood vessels, epilepsy, and Kawasaki' disease, were also documented. Because *KCNJ2* is known to be expressed in a variety of tissues, such as cardiac and skeletal muscle, the brain, arterial smooth muscle cells and developing bony structures of the craniofacial region, extremities, and vertebrae,[29–31] some of her compound disorders may be attributed to the *KCNJ2* mutation. In fact, loss-of-function mutations in *KCNJ2* cause Andersen–Tawil syndrome, which is characterized by prolonged repolarization, dysmorphic features, and periodic paralysis.[10,32] In the family of our female patient, we could not perform extensive genetic testing. We cannot exclude the possibility of the presence of other affected genes. Further analyses using knock-in mice or induced pluripotent stem cells would culminate monumental insight into the relationship between the *KCNJ2* M301K mutation and the patient's extra-cardiac phenotypes.

4.6 Conclusions

We described a novel *KCNJ2* gain-of-function mutation, M301K, in a patient with SQTS. Functional assays revealed no functional currents in the homozygous channels, whereas impaired inward rectification in the heterozygous channels manifested in larger outward currents, which is a novel mechanism predisposing SQTS.

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Conflict of interest: none declared.

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