Functional and clinical characterization of *KCNJ2* mutations associated with LQT7 (Andersen syndrome)

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Andersen syndrome (AS) is a rare, inherited disorder characterized by periodic paralysis, long QT (LQT) with ventricular arrhythmias, and skeletal developmental abnormalities. We recently established that AS is caused by mutations in *KCNJ2*, which encodes the inward rectifier K^+ channel Kir2.1. In this report, we characterized the functional consequences of three novel and seven previously described *KCNJ2* mutations using a two-microelectrode voltage-clamp technique and correlated the findings with the clinical phenotype. All mutations resulted in loss of function and dominant-negative suppression of Kir2.1 channel function. In mutation carriers, the frequency of periodic paralysis was 64% and dysmorphic features 78%. LQT was the primary cardiac manifestation, present in 71% of *KCNJ2* mutation carriers, with ventricular arrhythmias present in 64%. While arrhythmias were common, none of our subjects suffered sudden cardiac death. To gain insight into the mechanism of arrhythmia susceptibility, we simulated the effect of reduced Kir2.1 using a ventricular myocyte model. A reduction in Kir2.1 prolonged the terminal phase of the cardiac action potential, and in the setting of reduced extracellular K^+, induced Na^+/Ca^{2+} exchanger–dependent delayed afterdepolarizations and spontaneous arrhythmias. These findings suggest that the substrate for arrhythmia susceptibility in AS is distinct from the other forms of inherited LQT syndrome.

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Introduction

Andersen syndrome (AS) is a rare disorder with clinical manifestations that include periodic paralysis, prolongation of the QT interval with ventricular arrhythmias, and characteristic physical features including low-set ears, micrognathia, and clinodactyly (1–4). AS is inherited in an autosomal dominant fashion, although many cases are sporadic. Penetrance is extremely variable, with some patients manifesting only some characteristics of the syndrome (3–6). Cardiac arrhythmias can occur with any form of periodic paralysis, secondary to extreme fluctuations in serum K^+ levels. However, Klein et al. first recognized that cardiac arrhythmias are a primary manifestation in a subset of individuals with periodic paralysis (4). This subset was later established as having AS and defined by long QT (LQT) periodic paralysis, and dysmorphic features (3).

AS is unique among ion channelopathies due to the combination of both a skeletal and a cardiac muscle phenotype. The co-occurrence of periodic paralysis and LQT in the same individual suggested a shared ion channel defect as the cause of AS. Other forms of periodic paralysis are known to be due to mutations in skeletal muscle–specific Na^+, Ca^{2+}, and K^+ channels (7). Similarly, the congenital forms of the LQT syndrome (LQTS) are caused by mutations in cardiac muscle Na^+ and K^+ channel genes (8). Earlier studies excluded the possibility that AS is an allelic disorder of the two common forms of periodic paralysis or LQT1, LQT2, LQT3, or LQT4 (2, 3). Recently, linkage to chromosome 17q23 was established in a multigenerational AS kindred, leading to the identification of a mutation in the K^+ channel gene *KCNJ2* that cosegregated with the disease (6). *KCNJ2* encodes the inward rectifier K^+ channel Kir2.1, which is expressed in skeletal and cardiac muscle (9).

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Nonstandard abbreviations used: Andersen syndrome (AS); long QT (LQT); long QT syndrome (LQTS); delayed afterdepolarization (DAD); corrected QT interval (QTc); electrocardiogram (ECG); WT (wild-type); milliequivalent (meq); milliseconds (ms); premature ventricular contraction (PVC); ventricular tachycardia (VT); early afterdepolarization (EAD).

Kir2.1 is an important contributor to the inward rectifier K^+ current, I_{K1} (10). Nine mutations were subsequently identified from a total of 13 unrelated kindreds (6). Confirming the pathogenic role of these mutations, in vitro electrophysiological analysis revealed that two of the mutations (D71V and R218W) have a dominant-negative effect on Kir2.1 channel function. Several kindreds do not exhibit mutations in *KCNJ2*, indicating locus heterogeneity in AS (6).

In this report, we identify three novel AS-associated mutations in *KCNJ2* and present extensive clinical and in vitro electrophysiological studies on a total of 17 kindreds with 10 different mutations. All AS-associated mutations in *KCNJ2* caused dominant-negative suppression of Kir2.1 channel function. There was significant overlap in the extent of dominant-negative effect as well as overlap in clinical phenotype. LQT and ventricular arrhythmias were common in mutation carriers. Unlike other forms of inherited LQTS, sudden death has not been reported in AS subjects. To gain insight into the mechanism of arrhythmia susceptibility, we used a ventricular myocyte model (11) to simulate the effects of reduced Kir2.1. A reduction in Kir2.1 prolonged the terminal phase of the cardiac action potential, and in the setting of reduced extracellular K^+, induced delayed afterdepolarizations (DADs) and spontaneous arrhythmias. These findings suggest that the substrate for arrhythmia susceptibility in AS is distinct from the other forms of inherited LQTS.

Methods

The studies described below were performed in accordance with the institutional guidelines for human and animal research of the University of Utah and the University of Rochester.

Identification and evaluation of AS kindreds. Individuals were classified as affected with AS if two of the three following features were present: episodic muscle weakness, cardiac manifestations, and dysmorphology. The presence of periodic paralysis was based on standard criteria (12). Cardiac involvement was determined by the presence of ventricular arrhythmias or prolongation of the corrected QT interval (QTc). Subjects were classified as having LQT if the QTc exceeded 440 milliseconds (ms) for males and 460 ms for females, in accordance with standard criteria (13). Dysmorphology was noted by the presence of two or more of the following: (a) low-set ears, (b) hypertelorism, (c) small mandible, (d) clinodactyly, and (e) syndactyly (persistent webbing between fingers or toes). Individuals fulfilling none of the criteria were classified as unaffected. One of the authors (R. Tawil), who was blinded to the results of the mutational analysis, reviewed the clinical information on each subject and confirmed the subjects' diagnostic classification. The QTc of all available electrocardiograms (ECGs) was calculated in a blinded fashion by two investigators (R. Tawil and M. Tristani-Firouzi).

Mutational analysis. The entire coding region of Kir2.1 (about 1.6 kb) was amplified from the genomic DNA in all individuals sampled from each kindred, as previously described (6). PCR primer sequences were F1, 5′-CCAAAGCAGAAGCACTGGAG-3′ and R1, 5′-AATCAAATACCCAACCAAGGC-3′. PCR was performed using 50 μl of reaction mixture containing 100 ng of genomic DNA and 20 pmol of F1 and R1 as described previously (6). The products were sequenced using the F1 and R1 primers and two additional primers, F2 (5′-GTGTTTGATGATGTGGCGAGTGG-3′) and R2 (5′-ATTCCACTGTCAAACCCAAC-3′). Sequencing was performed by the DNA Sequencing core facility at the University of Utah. Identified mutations were screened against 100 unaffected, unrelated individuals by single-strand conformation polymorphism (SSCP) or mutation-specific (MSP) analysis.

Heterologous expression of KCNJ2 in oocytes. Human cardiac *KCNJ2* was obtained as a kind gift from Carol Vandenberg (University of California, Santa Barbara, Santa Barbara, California, USA) (14). Site-directed mutagenesis of *KCNJ2* and in vitro transcription were performed as described (6). cRNA was quantified using UV spectroscopy, gel electrophoresis, and the RiboGreen RNA Quantitation Kit (Molecular Probes Inc., Eugene, Oregon, USA). Isolation, maintenance, and injection of stage IV and stage V *Xenopus* oocytes with wild-type (WT) or mutant cRNA were performed as described (15). Whole-cell currents were recorded 2–3 days after cRNA injection using standard two-microelectrode voltage-clamp techniques (16). Oocytes were bathed at room temperature (22–25°C) in a modified ND96 solution containing 96 mM NaCl, 4 mM KCl, 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, and 5 mM HEPES (pH 7.6). Currents were elicited by 200-ms pulses applied in 20 mV increments to potentials ranging from –150 to –10 mV from a holding potential of –70 mV. Linear leak at each potential was compensated for offline by subtracting the mean current obtained in H_2O-injected oocytes from the same batch of oocytes. Tiny currents, consistent with leak, were recorded in H_2O-injected oocytes (see Figure 1a).

Data analysis. Electrophysiological data are expressed as mean ± SEM (n = number of oocytes). Electrocardiographic data from AS subjects are expressed as mean ± SD.

Simulation of ventricular action potential. The Puglisi-Bers model of a rabbit ventricular myocyte (11) served as the basis for the simulations in this study. The Puglisi-Bers model and the LabHEART software required to run model simulations were obtained from the website http://www.meddean.luc.edu/templates/ssom/depts/physio/labheart.cfm. For baseline conditions, the model cell was paced at a basic cycle length of 1,000 ms. The normal extracellular K^+ concentration $[K^+]_O$ was defined as 4.5 milliequivalent [meq]/l.

Results

Three new AS-associated KCNJ2 mutations identified. We identified three novel missense mutations in *KCNJ2*: P186L (C785T), V302M (G1132A), and N216H (A874C). All three mutations are located in putative functional

domains within the C terminus. P186L alters an amino acid of a PKKR motif (amino acids 186–189) implicated in binding the signaling phospholipid PIP_2 (17). N216H is located in a region (amino acids 207–246) thought to be involved in PIP_2 interactions (18), and V302M lies in a region that may be responsible for channel subunit assembly (19). These mutations were not found in unaffected family members or in 100 controls.

Functional characterization of mutations in KCNJ2. In preliminary experiments, current magnitude increased as a function of injected cRNA and saturated following injections of ≥ 1.6 ng *KCNJ2* cRNA per oocyte. Coexpression experiments were therefore performed using 1.6 ng cRNA per oocyte. The ability of mutant Kir2.1 subunits to form functional homomultimeric channels was assessed by comparing oocytes injected with WT or mutant *KCNJ2* cRNA (1.6 ng/oocyte). Injection of WT *KCNJ2* induced K^+ currents that activated nearly instantaneously and exhibited strong inward rectification at potentials positive to –70 mV (Figure 1a), consistent with prior reports (9, 14). Small outward currents were detected at potentials between –70 mV and –30 mV, with peak outward current measured at –50 mV (see Figure 1b, inset). While the physiological voltage range for Kir2.1 channel function is ≥ –90 mV, we extended the voltage range of study to –150 mV to assess channel function in the absence of rectification. In contrast to oocytes injected with WT *KCNJ2*, oocytes injected with G144S cRNA were no different from H_2O-injected oocytes, indicating that the G144S subunits were unable to form functional homomultimeric channels (Figure 1a). Similar to G144S, none of the *KCNJ2* mutant subunits formed functional channels when expressed alone (data not shown). Thus, all ten AS-associated *KCNJ2* mutations caused complete loss of channel function.

AS is an autosomal dominant disorder, and affected individuals possess one normal and one mutant *KCNJ2* allele. To assess the ability of mutant Kir2.1 subunits to form functional heteromultimeric channels with WT subunits, we coinjected oocytes with mutant and WT *KCNJ2* cRNA (0.8 ng/oocyte of each cRNA) and compared currents with those induced by injection of WT *KCNJ2* cRNA alone (0.8 ng/oocyte). If mutant Kir2.1 subunits did not coassemble with WT subunits (a haploinsufficiency effect), then current in the coinjection group would be similar to that in the WT group, as both groups were injected with equal amounts (0.8 ng/oocyte) of WT cRNA. Coexpression of WT and G144S *KCNJ2* induced an inwardly rectifying K^+ current whose current amplitude was reduced relative to that induced by expression of WT *KCNJ2*, consistent with a dominant-negative mechanism (Figure 1 and Figure 2). Current magnitude was reduced at potentials where rectification is absent (e.g., –150 mV) as well as at physiological voltages (e.g., –50 mV). Similar to G144S, all AS-associated mutations caused dominant-negative suppression of Kir2.1 channel function, with significant overlap in the degree of this effect (Figure 1 and Figure 2).

Clinical characteristics of AS kindreds with KCNJ2 mutations. A total of 25 kindreds fulfilling the diagnostic criteria of AS were identified. Mutations in *KCNJ2* were identified in 17 (68%) of these kindreds, and the mode of inheritance was autosomal dominant in six kindreds. In five kindreds, de novo mutations were identified. In the remaining six kindreds, the mode of inheritance could not be determined (i.e., parental DNA and/or clinical information was not available). A total of 36 individuals within these kindreds had *KNCJ2* mutations. Expressivity of the AS features was variable within the individual kindreds. Overall, 21 of 36 individuals (58%) carrying a *KCNJ2* mutation demonstrated the full clinical triad, and 29 of 36 (81%) had at least two-thirds of the major characteristics of AS. Two individuals with a confirmed mutation appeared to be nonpenetrant.

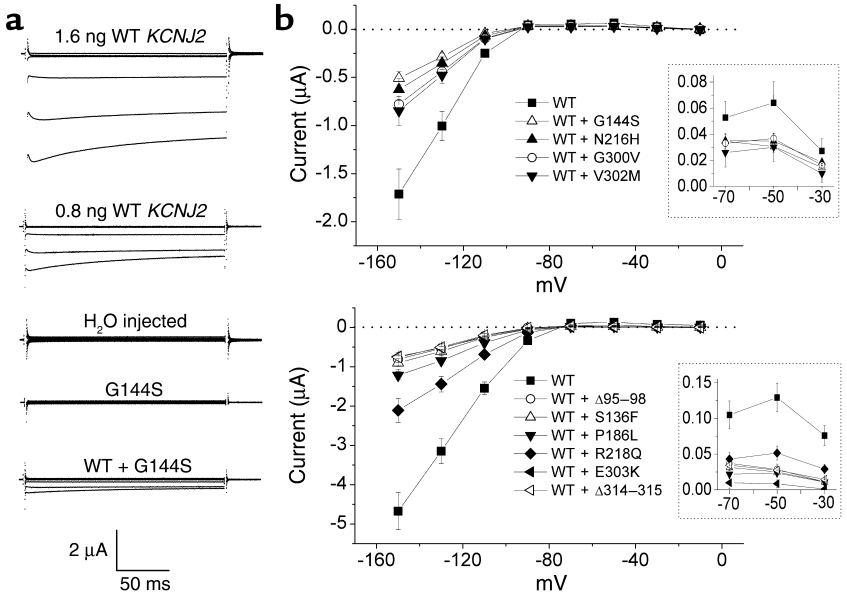

Figure 1
Functional consequences of AS-associated mutations in *KCNJ2*. (**a**) Whole-cell currents in *Xenopus* oocytes induced by injection of WT *KCNJ2*, H_2O, G144S and co-expressed WT and G144S *KCNJ2*. Currents were elicited by 200-ms pulses applied in 20-mV increments to potentials ranging from –150 to –10 mV from a holding potential of –70 mV. G144S mutant subunits failed to form functional channels when expressed alone. Coexpression of WT and G144S *KCNJ2* induced small, inwardly rectifying currents. (**b**) Current voltage relationships for WT *KCNJ2* (0.8 ng/oocyte) and coexpressed WT and mutant *KCNJ2* (0.8 ng/oocyte of each cRNA). *n* = 10–18 oocytes/group. Inset, mean current between –70 and –30 mV shown on an expanded scale.

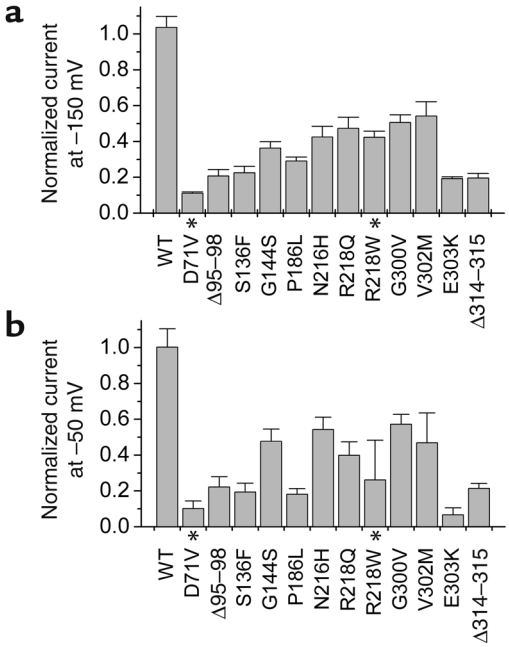

Figure 2
AS-associated mutations in *KCNJ2* cause dominant-negative suppression of Kir2.1 channel function. (**a**) Peak current induced by coexpression of mutant and WT *KCNJ2* (0.8 ng/oocyte for each) at –150 mV was normalized to currents induced by expression of WT *KCNJ2* (0.8 ng/oocyte). Current magnitude is reported at –150 mV because this gives a measure of conductance in the absence of rectification. *KCNJ2* mutations caused variable degrees of dominant-negative suppression of channel function. Note that a haploinsufficiency effect would result in current magnitude equal to that of the WT group. (**b**) Normalized current at –50 mV. *KCNJ2* mutations also reduce magnitude of outward current in a dominant-negative manner. *D71V and R218W data were obtained from ref. 6.

Cardiac manifestations of individuals with mutations in KCNJ2. The cardiac manifestations in AS individuals with mutations in *KCNJ2* included LQT, premature ventricular contractions (PVCs), complex ventricular ectopy (bigeminy, two or more consecutive PVCs, or multifocal PVCs), polymorphic ventricular tachycardia (VT), and bidirectional VT (Table 1 and Figure 3). LQT was present in 14 of 15 probands (93%). Two probands were excluded from analysis due to the presence of complete right bundle branch block. The mean QTc was 479 ± 42 ms for male probands (range, 416–525 ms) and 493 ± 27 ms for female probands (range, 470–560 ms). When family members with the gene defect were included in the analysis, 22 of 31 individuals (71%) had LQT. Three family members were excluded from analysis because excessive ventricular ectopy precluded accurate analysis of the QTc interval. Other ECG parameters, such as PR interval, QRS duration, P wave axis, and QRS axis were normal in most mutation carriers (Table 2). Complete right bundle branch block was identified in two subjects, and first-degree atrioventricular heart block was present in one subject (PR = 303 ms). Thus, *KNCJ2* mutations primarily affect cardiac repolarization, and not indices of conduction.

Ventricular arrhythmias were common in AS subjects (Figure 3, b and c). Nonsustained VT was present in 11 of 17 probands (65%) and 14 of 36 mutation carriers (39%). Bidirectional VT was identified in 3 of 17 probands (18%) but was not found in other affected family members. Ventricular arrhythmias (defined as PVCs, complex ventricular ectopy, or any form of VT) were present in 15 of 17 probands (88%) and 23 of 36 mutation carriers (64%). In 6 of 17 probands, ventricular ectopy was exacerbated in the setting of hypokalemia and was documented to improve with normalization of the serum K^+ (Figure 4).

Despite the presence of frequent ventricular arrhythmias, the incidence of syncope and cardiac arrest was relatively low in the AS group. Two patients experienced a nonfatal cardiac arrest, one of whom presented with the distinctive polymorphic VT torsades de pointes. Torsades de pointes was also documented in a second subject. Four additional subjects experienced episodes of syncope. There were no cases of sudden cardiac death, nor was a family history of sudden cardiac death reported.

An interesting electrocardiographic finding was the presence of a prominent U wave, primarily in the anterior precordial leads, in a significant number of affected individuals (Figure 3d). A prominent U wave was

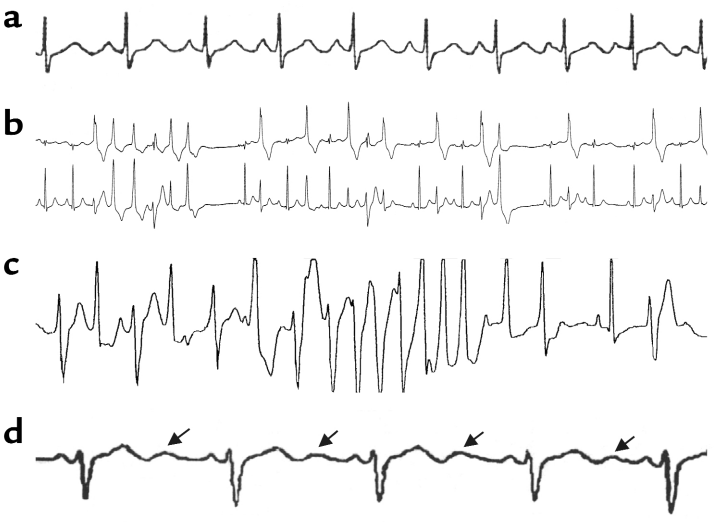

Figure 3
Representative ECGs from AS subjects. (**a**) ECG demonstrating prolongation of the QT interval. (**b**) ECG traces demonstrating a short run of nonsustained polymorphic VT followed by bigeminy (normal QRS complex alternating with a premature ventricular complex). Ventricular arrhythmias dominated the rhythm of this subject throughout the day. (**c**) Bidirectional VT (note alternating QRS axis polarity) degenerating into a brief run of polymorphic VT. (**d**) ECG trace demonstrating prominent U wave (indicated by arrows).

Table 1
Cardiac manifestations of AS probands with *KCNJ2* mutations

Mutation	Kindred	Gender	HR (bpm)	QTc (ms)	Arrhythmia	Age of onset (yr)	Current age (yr)	Ref.
D71V	4415	F	100	513	None	N/A	11	(5)
Δ95–98	3328	F	83	475	Bigeminy, polymorphic VT	12	26	
S136F	6634	F	68	500	Bigeminy	N/A	45	
G144S	3856	F	83	480	Bidirectional VT	N/A	23	
P186L	7246	M	94	RBBB	Polymorphic VT	N/A	18	
N216H	3442	F	65	470	Bidirectional VT and non-fatal cardiac arrest	4	21	(3)
R218W	2401	F	110	560	Bigeminy, torsades, non-fatal cardiac arrest, first-degree AV block	10	35	(3)
R218W	2679	M	68	510	Bigeminy, polymorphic VT	7	24	(3)
R218 W	2681	F	75	488	Bigeminy, polymorphic VT	13	29	
R218W	7480	M	94	525	Bigeminy	N/A	10	
R218W	6515	M	52	416	Polymorphic VT, bigeminy	2.5	17	
R218Q	6562	M	70	469	None	15	21	
G300V	3387	M	88	474	Bidirectional VT, frequent PVCs	9	19	(36)
G300V	3677	F	100	480	Bigeminy, polymorphic VT	19	34	
V302M	2682	M	79	RBBB	Bigeminy	N/A	48	
E303K	2281	F	85	495	Frequent PVCs	N/A	16	(2)
Δ314–315	5768	F	115	471	Torsades, monomorphic VT	4	6	

HR, heart rate; bpm, beats per minute; N/A, not available; RBBB, right bundle branch block (precludes accurate measurement of QTc); torsades, torsades de pointes; AV, atrioventricular.

identified in 13 of 17 probands (76%) and 17 of 36 gene carriers (47%). A U wave is often discernible in normal individuals at slow heart rates (20). However, the mean heart rate in the probands with a U wave was 84 ± 17 beats per minute (range 52–115), suggesting that the U wave represents a manifestation of the disease. Hypokalemia is also known to enhance the magnitude of the U wave (20). Because we do not have simultaneous serum K^+ levels in AS subjects with a prominent U wave, we cannot exclude a role for hypokalemia in the genesis of the U wave.

We attempted to correlate the functional consequences of the individual AS mutations with the severity of the clinical phenotype as assessed by QTc prolongation, arrhythmia, or symptoms. For many mutations, the degree of dominant-negative suppression overlapped, as did the severity of the clinical phenotype. The clinical severity of illness in individuals with the strongest dominant-negative mutations (D71V, E303K, Δ314–315) was not appreciably different from that of subjects with weaker dominant-negative mutations (N216H, R218W, G300V, and V302M).

In summary, LQT was the most common individual ECG finding in *KCNJ2*-associated AS cases. Ventricular arrhythmias occurred in a majority of mutation carriers. The clinical phenotype did not correlate directly with the degree of dominant-negative suppression of Kir2.1 channel function. Variable expressivity within affected family members and small sample size may have hindered our ability to correlate the functional consequences of individual mutations with the corresponding clinical phenotype.

Periodic paralysis and dysmorphic features. Periodic paralysis was present in 23 of 36 (64%) individuals with *KNCJ2* mutations. Rest following physical exertion was a common trigger, as in the classic forms of periodic paralysis. The episodes of periodic paralysis were associated with hypokalemia (serum $K^+ \leq 3.4$ meq/l) in a majority of subjects (55%), whereas paralysis was associated with hyperkalemia or normokalemia in 22% and 10% of subjects, respectively. Unlike hypokalemic periodic paralysis, in which attacks are precipitated by carbohydrate ingestion, no consistent trigger could be identified for AS subjects. Muscle biopsy performed in 12 individuals showed either minimal myopathic changes or tubular aggregates, which are common findings in other forms of periodic paralysis. Carbonic anhydrase inhibitors, which constitute the mainstay of treatment of other forms of periodic paralysis (21, 22), were effective in reducing attack frequency and severity in AS. Similar to the cardiac phenotype, no direct correlation was identified between the degree of Kir2.1 channel dysfunction or a specific mutation and the severity of neuromuscular symptoms.

Table 2
Electrocardiographic parameters of *KCNJ2* mutation carriers

PR (ms)	QRS (ms)	P axis	QRS axis	Heart rate (bpm)	QTc female (ms)	QTc male (ms)
148 ± 36	84 ± 19	52 ± 18	45 ± 29	82 ± 24	484 ± 28	461 ± 35
(110–210)	(<120)	(30–75)	(–30 to +90)	(60–100)	(<460)	(<440)

Data expressed as mean ± SD. Bottom row (values in parentheses) shows measurements from normal individuals obtained from refs. 37, 38.

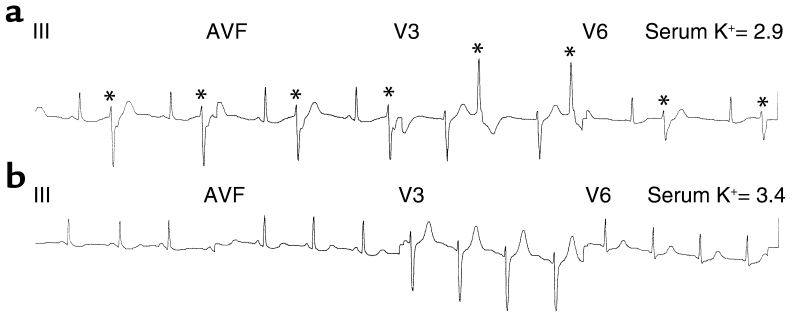

Figure 4
ECG demonstrating bigeminy (sinus beat alternating with a PVC) in a 16-year-old female AS subject with hypokalemia. PVCs are indicated by asterisks. (**b**): Following an increase in serum K^+, the bigeminy rhythm resolved. Six of 17 probands were documented to have exacerbation of ventricular ectopy in the setting of hypokalemia.

Twenty-eight of 36 *KCNJ2* mutation carriers (78%) had at least two dysmorphic features. Low-set ears were present in 14 of 36 (39%), hypertelorism in 13 of 36 (36%), small mandible in 16 of 36 (44%), clinodactyly in 23 of 36 (64%), and syndactyly in 4 of 36 (11%) carriers. Cleft palate was identified in 3 of 36 AS subjects (8%) and scoliosis in 4 of 36 (11%). The dysmorphic features were most often mild and nondisfiguring, and were easily overlooked on routine physical examination. This is relevant given the fact that in individuals with cardiac involvement, one-sixth demonstrated mild dysmorphic features as the only other clue to the diagnosis of AS.

Clinical characteristics of AS kindreds without mutations in KCNJ2. Eight kindreds fulfilling the diagnostic criteria of AS did not have a mutation in *KCNJ2*. These AS individuals were clinically indistinguishable from *KCNJ2*-associated AS subjects. Six of 8 probands were noted to have LQT (mean QTc, 488 ± 26 ms), and 6 of 8 had the dysmorphic features typical of AS. In addition, all 8 probands experienced episodic weakness. These kindreds likely represent a genetically heterogeneous group.

Cardiac action potential simulation. The effects of reduced I_{K1} on action potential configuration were simulated using a theoretical model of a rabbit ventricular myocyte. While the heterologous expression data demonstrated that *KCNJ2* mutations result in a greater than 50% reduction in Kir2.1 current magnitude, the contribution of Kir2.1 to total I_{K1} is not known. For the purpose of the simulation, we conservatively chose a 50% reduction in I_{K1} to mimic the consequence of a mutation in *KCNJ2*. Using the Puglisi-Bers model, a 50% reduction in I_{K1} prolonged action potential duration (APD_{95}) from 227 ms to 319 ms (Figure 5a), with a minimal change in resting membrane potential (E_m) from –90 mV to –87 mV. These data are consistent with the ECG findings of prolonged QTc in AS subjects with normal PR intervals and QRS durations. Because symptoms in some AS patients are elicited in the setting of hypokalemia, we simulated the effect of reduced $[K^+]_O$. Reducing $[K^+]_O$ from 4.5 to 2.9 meq/l in the presence of a 50% reduction in I_{K1} resulted in the appearance of a secondary depolarization following the terminal phase of repolarization (Figure 5a). We use the term terminal-phase early afterdepolarization (EAD) to distinguish this from the typical EADs of LQTS that arise from the plateau or early repolarization phases of the action potential. A reduction in $[K^+]_O$ and I_{K1} also resulted in the generation of spontaneous action potentials and DADs (Figure 5b), which were associated with oscillations in intracellular Ca^{2+} (data not shown). The spontaneous action potentials, DADs, and terminal-phase EADs were dependent upon the Na^+/Ca^{2+} exchanger. A reduction in the amplitude of the Na^+/Ca^{2+} exchanger current eliminated the spontaneous generation of action potentials, DADs, and EADs (Figure 5c).

Discussion

AS is a rare autosomal dominant or sporadic disorder characterized by periodic paralysis, prolongation of the QT interval, cardiac arrhythmias, and mildly dysmorphic features. In the current study, we report three novel *KCNJ2* mutations, characterize the functional consequences of ten AS-associated mutations in *KCNJ2*, correlate these results with the clinical phenotype, and simulate the effects of reduced Kir2.1 channel function on the cardiac action potential. Our findings support the notion that AS is a disorder of myocellular repolarization, and as such, we propose that AS be classified as LQT7.

To date, five genes that cause the inherited forms of LQTS have been identified: *KCNQ1* (LQT1), *HERG* (*KCNH2*, LQT2), *SCN5A* (LQT3), *KCNE1* (LQT5), and *KCNE2* (LQT6) (8). Similar to the other forms of inherited LQTS, the primary cardiac manifestation of AS was LQT, identified in 71% of all gene carriers. The mean QTc of male and female probands with AS was 479 and 493 ms, respectively, compared with 497 and 510 ms for males and females with other forms of LQTS (23).

While LQT was present in most *KCNJ2* mutation carriers, the ventricular arrhythmias manifested by these individuals were clearly distinct from the other forms of inherited LQTS. Complex ventricular ectopy and polymorphic VT were common in AS subjects (Figure 3 and Table 1). Fourteen AS subjects had frequent episodes of nonsustained VT documented on Holter monitor recordings. Although ventricular arrhythmias were common in AS subjects, only rarely did these arrhythmias degenerate into a hemodynamically compromising rhythm, such as torsades de pointes or ventricular fibrillation. Sudden cardiac death was not reported in any AS subject. Likewise, the incidence of symptoms such as syncope or cardiac arrest was lower

in AS subjects (19%) compared with LQT1 and LQT2, in which cardiac events were reported to be 63% and 46%, respectively (24). Thus, the distinct ventricular arrhythmias and the absence of sudden death suggest that the substrate for arrhythmia susceptibility in LQT7 is distinct from the other forms of LQTS.

To investigate the ionic basis of arrhythmia susceptibility in AS, we simulated the effects of reduced Kir2.1 channel function. I_{K1} contributes no repolarizing current during the plateau phase of the cardiac action potential but provides substantial current during the repolarization phase (25). Based on this, one would predict that a reduction in I_{K1} might have unique effects on action potential configuration and arrhythmia susceptibility. Indeed, the Puglisi-Bers model predicted that a reduction in I_{K1} would prolong the terminal repolarization phase of the cardiac action potential. Reductions in I_{K1} and $[K^+]_O$ caused terminal-phase EADs that triggered spontaneous action potentials, mirroring the frequent ventricular ectopy seen in a number of AS subjects. These EADs, DADs, and spontaneous action potentials were dependent upon depolarizing current through the Na^+/Ca^{2+} exchanger. In contrast to LQT7, mutations affecting I_{Ks}, I_{Kr}, or I_{Na} prolong the plateau phase of the cardiac action potential. Prolongation of the plateau phase allows for recovery from inactivation and reactivation of L-type Ca^{2+} channels, which trigger EADs arising from the plateau or early repolarization phases (26). Thus, the unique consequences of a reduction in repolarizing current through Kir2.1 channels, as opposed to reduced I_{Kr} and I_{Ks} or increased sustained I_{Na}, may underlie the differences in arrhythmia susceptibility and lethal cardiac events between LQT7 and other forms of LQTS.

Similar to our model of AS arrhythmias, Na^+/Ca^{2+} exchanger dependent triggered activity is the mechanism of arrhythmia associated with digitalis toxicity and DADs (27, 28). Bidirectional tachycardia is a rare form of VT typically associated with digitalis toxicity (29). This unique arrhythmia, characterized by alternating polarity of the QRS axis, was previously reported in association with AS (30–32) and was found in three AS subjects in our study. Bidirectional VT is also a characteristic arrhythmia seen in familial (catecholaminergic) polymorphic VT. Mutations in the gene encoding the human cardiac ryanodine receptor (*hRyR2*) were recently described as underlying this disorder of arrhythmia susceptibility in some families (33, 34). Familial (catecholaminergic) polymorphic VT also shares features of digitalis toxicity, but unlike AS, it is not associated with QTc prolongation (33, 34). Thus, LQT7 shares some features of inherited LQTS, such as QTc prolongation, but displays the arrhythmias of syndromes associated with Ca^{2+} overload, such as bidirectional VT and polymorphic VT.

There are clear limitations inherent in any ventricular myocyte model. While the simulated currents faithfully reproduce many properties of the cardiac action potential, numerous cellular processes are not incorporated into the model. Our computer simulations provide a working hypothesis that awaits further experimental testing. Within the context of the model, a reduction in $[K^+]_O$ was required to trigger spontaneous action potentials. Hypokalemia was documented to exacerbate ventricular ectopy in six of 17 probands, suggesting a role for serum K^+ in arrhythmia susceptibility in some AS subjects. However, ventricular ectopy also occurred in normokalemic subjects, indicating that factors other than serum $[K^+]$ are clearly important. The identification of a trigger in some but not all individuals has also been reported for the other forms of inherited LQTS (35).

In summary, we propose that AS should be considered a subtype of LQTS, namely LQT7. The differences in susceptibility to arrhythmia and lethal cardiac events between individuals with AS and other forms of LQTS are likely attributable to the consequences of a reduction in repolarizing current through Kir2.1 channels, as opposed to reduced I_{Kr} and I_{Ks} or increased sustained I_{Na}. Reduced Kir2.1 function in the setting of hypokalemia results in triggered activity resembling that observed with digitalis toxicity and Ca^{2+} overload. Thus, AS shares features of both LQTS and familial (catecholaminergic) polymorphic VT. The role of reduced Kir2.1 channel function in the predisposition to periodic paralysis and abnormal skeletal morphogenesis remains to be determined.

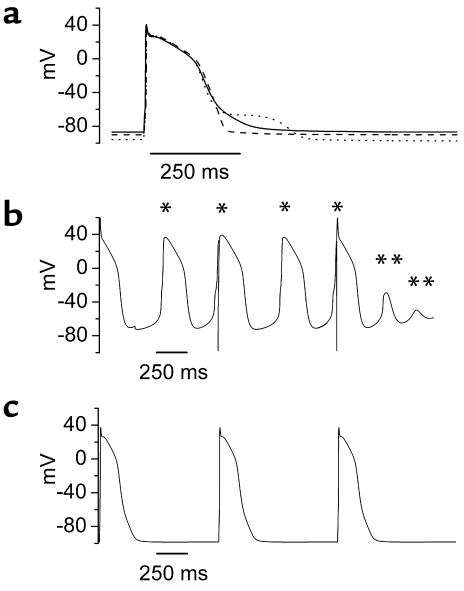

Figure 5
Simulated effect of reduced I_{K1} on cardiac action potentials. (**a**) Model of rabbit ventricular action potential under baseline conditions ($[K^+]_O$ 4.5 meq/l, dashed line); 50% reduction in I_{K1} conductance (solid line); and 50% reduction in I_{K1} conductance and $[K^+]_O$ = 2.9 meq/l (dotted line). (**b**) Reduction in $[K^+]_O$ and I_{K1} conductance resulted in spontaneous action potentials (*) and DADs (**) that did not reach threshold for action potential generation. Basic cycle length of stimulation was 1,000 ms. (**c**) Spontaneous action potentials and DADs seen in **b** were eliminated by a reduction in the amplitude of the Na^+/Ca^{2+} exchanger.

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