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Molecular Genetic and Functional Association of Brugada and Early Repolarization Syndromes with S422L Missense Mutation in *KCNJ8*

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Abstract

Background—ATP-sensitive potassium (K_{ATP}) cardiac channels consist of inward rectifying channel subunits Kir6.1 or Kir6.2 (encoded by *KCNJ8* or *KCNJ11*) and the sulfonylurea receptor subunits *SUR2A* (encoded by *ABCC9*).

Objective—To examine the association of mutations in *KCNJ8* with Brugada (BrS) and early repolarization (ERS) syndromes and elucidate the mechanism underlying the gain of function of K_{ATP} channel current ($I_{K\text{-}ATP}$).

Methods—Direct sequencing of *KCNJ8* and other candidate genes was performed on 204 BrS and ERS probands and family members. Whole-cell and inside-out patch clamp methods were used to study mutated channels expressed in TSA201 cells.

Results—The same missense mutation, p.Ser422Leu (c.1265C>T) in *KCNJ8*, was identified in 3 BrS and 1 ERS proband, but was absent in 430 alleles from ethnically-matched healthy controls. Additional genetic variants included *CACNB2b*-D601E. Whole cell patch clamp studies showed a two-fold gain of function of glibenclamide-sensitive $I_{K\text{-}ATP}$ when *KCNJ8*-S422L was co-

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expressed with *SUR2A*-wild type. Inside-out patch clamp evaluation yielded a significantly greater IC_{50} for ATP in the mutant channels (785.5±2 vs. 38.4±3 μM, n=5; p<0.01) pointing to incomplete closing of the K_{ATP} channels under normoxic conditions. Patients with a *CACNB2b*-D601E polymorphism displayed longer QT/QTc intervals, likely due to their effect to induce an increase in $I_{Ca\text{-}L}$.

Conclusion—Our results support the hypothesis that *KCNJ8* is a susceptibility gene for Brugada and early repolarization syndromes and point to S422L as a possible hotspot mutation. Our findings suggest that the S422L-induced gain of function in $I_{K\text{-}ATP}$ is due to reduced sensitivity to intracellular ATP.

Keywords

Cardiac Arrhythmias; Sudden Cardiac Death; Genetics; Hotspot mutation; Electrophysiology; J wave syndrome; ATP-sensitive potassium channel

Introduction

An early repolarization (ER) pattern in the electrocardiogram (ECG), consisting of a J point elevation, a notch or slur on the QRS (J wave), is found in approximately 2% of healthy young males and has traditionally been regarded as totally benign.[1] The observation by our group in 2000 that an ER pattern in the coronary-perfused wedge preparation can easily convert to one in which phase 2 reentry gives rise to polymorphic ventricular tachycardia/ventricular fibrillation (VT/VF), prompted the suggestion that ER may in some cases predispose to more malignant arrhythmias in the clinic.[2, 3] A number of case reports and experimental studies have long suggested a critical role for the J wave in the pathogenesis of idiopathic VF (IVF).[4, 5] A definitive association between ER and IVF has been presented in more recent reports.[6–9]

Based on available data pointing to an association of risk with spatial localization of the ER pattern, we recently proposed a classification scheme in which Type 1 early repolarization syndrome (ERS) is associated with ER pattern predominantly in the lateral precordial leads; this form is very prevalent among healthy male athletes and is thought to be largely benign. Type 2, displaying an ER pattern predominantly in the inferior or infero-lateral leads, is associated with a moderate level of risk and Type 3 ERS, displaying an ER pattern globally in the inferior, lateral and right precordial leads, appears to be associated with the highest level of risk and is often associated with electrical storms.[3] Brugada syndrome (BrS) represents a fourth variant in which ER is limited to the right precordial leads. ECG changes in both BrS and ERS are dynamic[10–12] with the most prominent ECG changes appearing just before the onset of VT/VF.[4, 5, 11–13] The amplitude of J waves, barely noticeable during sinus rhythm, may become progressively augmented with increased vagal tone and bradycardia and still further accentuated following successive extrasystoles and compensatory pauses giving rise to short long short sequences.

In light of the similarity in ECG characteristics, clinical outcomes, risk factors and because they share a common arrhythmic platform related to amplification of I_{to}-mediated J waves, these inherited and acquired syndromes have been grouped under the heading of J wave syndromes.[3]

Information relative to the genetic and molecular basis for the J wave syndromes is relatively limited. BrS has been associated with mutations in eight different genes including *SCN5A, GPD1L, CACNAC1C, CACNb2b, SCN1B, KCNE3, SCN3B and CACNA2D1*, whereas ERS has been associated with mutations in *KCNJ8, CACNA1C and CACNB2*.[3, 14, 15] Haissaguerre and co-workers first associated *KCNJ8* with ERS in the case

of a young female with frequent (>100) recurrences of VF, which were unresponsive to beta-blockers, lidocaine/mexiletine, verapamil, and amiodarone. Recurrences of VF were associated with marked accentuation of the early repolarization pattern at times mimicking acute myocardial ischemia, although coronary angiography during one of episodes was normal.[14] However, functional expression data for the S422L missense mutation in *KCNJ8* was not available to substantiate the association of the genotype with the phenotype.[14] A recent report by Medeiros-Domingo and co-workers[16] provided, for the first time, functional expression studies demonstrating a gain of function of ATP-sensitive potassium (K_{ATP}) channel current ($I_{K\text{-}ATP}$) with this mutation. These investigators genetically screened 87 probands with BrS and 14 with ERS and found one BrS and one ERS proband with a S422L-*KCNJ8* (Kir6.1) mutation; the variation was absent in 600 controls. The authors co-expressed the *KCNJ8* mutation with ATP regulatory subunit *SUR2A* in COS-1 cells and recorded $I_{K\text{-}ATP}$ using whole cell patch clamp techniques. They reported a significantly larger $I_{K\text{-}ATP}$ for the mutant vs. wild type (WT) in response to a high concentration of pinacidil (100 μM).

The present study is designed to further examine the association of mutations in *KCNJ8* with BrS and ERS and to elucidate the mechanism underlying the gain of function of K_{ATP} channel current ($I_{K\text{-}ATP}$) induced by the S422L mutation. The gold standard for demonstrating a change in sensitivity to ATP involves the study of inside-out patches with the internal patch membranes exposed to different ATP concentrations.

We report the identification of an S422L-*KCNJ8* mutation in 3 BrS and 1 ERS probands among 204 J wave syndrome probands screened. Functional characterization of the mutation using inside-out patch clamp techniques reveals for the first time an increase in the IC_{50} for ATP block of mutant vs. WT K_{ATP} channels. Our results therefore provide further evidence in support of the hypothesis that *KCNJ8* is a susceptibility gene for both BrS and ERS and suggest that S422L may be a hotspot mutation capable of causing activation of $I_{K\text{-}ATP}$ under normoxic conditions by reducing the sensitivity of the channel to intracellular levels of ATP.

Methods

Clinical subjects

The study was approved by the Regional Institutional Review Board. All members of the immediate family underwent clinical and genetic evaluation following informed consent. A total of 204 patients diagnosed with J wave syndromes according to accepted clinical criteria were genetically screened.[3]

Molecular genetic analysis

Genomic DNA was extracted and *KCNJ8* and others genes were amplified and direct sequenced as detailed in the online supplement.

Site-directed mutagenesis, transfection, electrophysiology study and statistical analysis

The ion channel variants were cloned by site-directed mutagenesis, expressed in TSA201 cells was studied using whole cell patch clamp techniques as described in the online supplement.

Results

Clinical evidence of association of J wave syndrome with *KCNJ8*

We identified an S422L-*KCNJ8* mutation in 4 cases among the 204 J wave syndrome probands screened. Three were diagnosed with BrS and one with early ERS. All were

symptomatic presenting with arrhythmias ranging from AF to VT/VF (Table 1). The 3 males and 1 female had a mean age of 42.6±7.2 years of age (range: 23 to 66). The first BrS case (MMRL#171, Figure 1A) was a 46 year-old male with ST segment elevation unmasked with ajmaline. Family history was significant for sudden cardiac death (SCD) of two maternal aunts. The second BrS case (MMRL#747, Figure 1B) was a 45-year-old male diagnosed with BrS and presenting with atrial fibrillation (AF). His mother also displayed a BrS phenotype and had AF. There was no family history of SCD. Echocardiogram revealed no structural abnormalities. The third BrS patient (MMRL#485, Figure 1D) was a 43 year-old male with sinus bradycardia, a positive ajmaline test and inducible polymorphic VT. The patient had no known family history of SCD or syncope. The patient experienced localized chest pain lasting 1–2 minutes. Echocardiogram showed normal ejection fraction of 56%. The patient was a tri-athlete competing in 6 highly competitive events per year.

The ERS Patient (MMRL#446, Figure 1C) was a young female who presented with a significant J point elevation in the infero-lateral leads and aborted SCD (ASCD). The proband displayed accentuation of the ER pattern following calcium injection.[14]

Figure 2A presents a 12-lead ECG of one of the BrS probands (#747), showing a Type 1 ST segment elevation >2 mm in V1 and V2 and a saddleback ST segment elevation in V3. Figure 2B displays the right precordial leads of another BrS proband (#171) showing ST segment elevation in V1 and V2 following an ajmaline challenge (left panel) and inducible polymorphic VT during EP study (right panel). Figure 2C shows a 12-lead ECG of the ERS proband (#485), displaying an ER pattern in the infero-lateral leads with prominent J point elevation in leads I, II, AVF, V4, V5 and V6, associated with development of monomorphic VT, this case with early repolarization and *KCNJ8* mutation reported here is the same patient previously reported by Haissaguerre's group,[14] who was independently genotype by our group.

Identification of S442L mutation in *KCNJ8*

All 204 probands clinically diagnosed with J wave syndromes were screened for *KCNJ8* variations. Molecular genetic screening revealed a missense heterozygous mutation consisting of a C-to-T transition at nucleotide 1265 (c.1265 C>T) in *KCNJ8* predicting a substitution of a leucine for a serine at residue 422 (p.Ser422Leu) of Kir6.1 channel (S422L) (Figure 3A). This mutation was not found in 430 reference alleles from healthy controls, but was found in a family member clinically affected with BrS (Figure 1B). Residue S422 is located at the carboxyl terminal region of Kir6.1 (Figure 3B).

K_{ATP} channels are thought to be hetero-octameric structure (Figure 3C), consisting of four pore-forming subunits (*KCNJ8* encoded Kir6.1 or *KCNJ11* encoded Kir6.2), and four regulatory subunit sulfonylurea receptors (SUR): SUR1 is encoded by *ABCC8* or *SUR2A* encoded by *ABCC9*.[17, 18] Alignment of the amino acid of sequence of Kir6.1 proteins shows that serine at position 422 is highly conserved among species (Figure 3D). This missense mutation predicts substitution of a serine (Ser or S), a polar amino acid with leucine (Leu or L), an uncharged, nonpolar, hydrophobic amino acid.

All patients carrying the *KCNJ8*-S422L mutation were screened for all other known BrS and ERS gene variations as detailed in the Methods section. A D601E polymorphism in *CACNB2b* was identified in 3 cases.

Biophysical characterization of the functional effects of the S442L mutation on K_{ATP} channels

Figure 4 shows current-voltage relations of $I_{K\text{-}ATP}$ recorded using whole-cell patch clamp techniques in TSA201 cells transiently transfected with *KCNJ8*-WT or *KCNJ8*-S422L and

ABCC9-WT. Intracellular ATP was reduced by using a pipette solution containing 100 μM Mg_2ATP. Macroscopic whole cell currents were measured during the voltage protocol shown in the inset. A ramp protocol was introduced from −100 mV to 100 mV over 400 ms. Traces obtained before and after glibenclamide (10 μM) were digitally subtracted to obtain the glibenclamide-sensitive currents shown in Figure 4A. $I_{K\text{-}ATP}$ measured as glibenclamide-sensitive current showed a two-fold gain of function at 0 mV and +40 mV (Figure 4B).

KCNJ8-S422L causes a gain of function in $I_{K\text{-}ATP}$ by reducing sensitivity of K_{ATP} channels to ATP

K_2ATP sensitivity was measured using excised inside-out patches of TSA201 cells expressing *KCNJ8*-WT/*SUR2A*-WT and *KCNJ8*-S422L/*SUR2A*-WT channels (Figure 5). Currents were elicited using a 3 s voltage ramp from −80 to +80 mV every 15 seconds from a holding potential of 0 mV (see Figure 5 inset). Test solutions were applied to the intracellular membrane surface. Currents elicited were nearly linear in the range of potentials tested. Figure 5A shows normalized current traces for *KCNJ8*-WT/*SUR2A*-WT and *KCNJ8*-S422L/*SUR2A*-WT following exposure to increasing concentrations of K_2ATP. K_2ATP decreased current amplitude in a concentration-dependent manner. Concentration-response curves for the inhibitory effects of K_2ATP on currents measured at −80 mV in both channels are shown in Figure 5B. The IC_{50} value of K_2ATP in *KCNJ8-WT*/*SUR2A*-WT channels (38.4±3 μM; n=5) was significantly increased in mutant *KCNJ8*-S422L/*SUR2A*-WT channels (785.5±2 μM; $n = 5$, $p < 0.01$). At physiological levels of intracellular ATP (yellow bar; 2–4 mM) WT channels were totally inhibited, whereas mutant channels remained partially open (Figure 5B).

Modulatory effect of secondary gene variants on QTc interval in probands with the S442L-*KCNJ8* gain of function mutation

The S422L-*KCNJ8* mutation is often associated with a relatively short QTc interval (Table 1). In probands in which QTc was not abbreviated, we uncovered a secondary genetic variant in *CACNCB2b* (D601E) which is known to augment late calcium channel current.[19] The reduced net repolarizing current is likely responsible for diminishing the effect of the $I_{K\text{-}ATP}$ mutation to abbreviate QTc. Using mathematical modeling, we previously demonstrated that a gain of function in late I_{Ca} secondary to the *CACNCB2b* (D601E) variant can prevent abbreviation of the epicardial action potential caused by a mutation in SCN5A.[19] The longest QTc was found in females in whom a D601E-*CACNB2b* polymorphism was detected (Figure 6).

Discussion

Our data show molecular genomic and functional association of Brugada and early repolarization syndromes with a S422L missense mutation in *KCNJ8*, which encodes the pore-forming subunit (Kir6.1) of the cardiac K_{ATP} channel. While a gain of function in $I_{K\text{-}ATP}$ has previously been attributed to this mutation using whole cell patch clamp techniques, that study was done in the presence of high concentration of the K_{ATP} channel opener pinacidil and the mechanism responsible was not probed. The present study uses inside-out patch clamp technology to demonstrate a gain of function in the absence of pinacidil and for the first time demonstrates that the gain of function in $I_{K\text{-}ATP}$ is due to a reduced sensitivity of the mutant channel to intracellular ATP (Figure 5). Our data also point to S422L as a possible hotspot mutation associated with the J wave syndromes.

ATP-sensitive potassium channels, originally discovered in the heart,[20] have since been found in many tissues including pancreatic, skeletal muscle, kidney, and brain cells.[21] K_{ATP}

channels, which remain closed at normal $[ATP]_i$, open as $[ATP]_i$ and the ATP/ADP ratio decline during hypoxia or ischemia, thus abbreviating the action potential. These action of $I_{K\text{-}ATP}$ contribute to regulation of calcium entry and thus to modulation of contraction and oxygen utilization.[22] K_{ATP} channels are thought to be hetero-octameric in structure, consisting of four pore-forming subunits (*KCNJ8* encoded Kir6.1 or *KCNJ11* encoded Kir6.2), and four regulatory subunits SUR: *ABCC8* encoded SUR1 or *ABCC9* encoded *SUR2A* in the heart.[17, 18]

Co-association of *SUR2A* with Kir6.2 was long thought to be the principal subunit combination comprising K_{ATP} channels in the heart and conferring cardioprotection under ischemic conditions. A number of studies have raised serious questions as to the predominant role of Kir6.2. Recent studies suggest that within a single K_{ATP} channel, more than one Kir6.x or SURx subunits can co-exist.[16, 23–27] Several studies point to relatively high expression of Kir6.1 in cardiomyocytes.[28–32]

Morrissey and co-workers used immunolocalization assays to demonstrate that both Kir6.1 and Kir6.2 as well as SUR2 (but not SUR1) are expressed in mouse in a sarcomeric striated pattern, suggesting their presence in T-tubules in ventricular cardiomyocytes, and that Kir6.1 is more strongly expressed in epicardial ventricular myocytes.[33] This transmural distribution is consistent with the observation that K_{ATP} channels are more prominently activated by ischemia in epicardium vs. endocardium[34] and may be related to the finding of Furukawa and co-workers that ATP-regulated K^+ channels are activated by a smaller reduction in intracellular ATP in feline epicardial vs. endocardial cells.[35] If present in humans, this heterogeneous transmural distribution of Kir6.1 likely contributes to the development of ST segment elevation observed in patients with BrS and ERS with mutations in *KCNJ8* or *SUR2A*.

The presence of an additional repolarizing force during the early phases of the epicardial action potential (AP), due to a gain of function of $I_{K\text{-}ATP}$, can generate an early repolarization pattern in the ECG by causing depression of the epicardial AP dome.[3] In the presence of a prominent transient outward current (Ito), commonly seen in right ventricular epicardium,[36] the addition of $I_{K\text{-}ATP}$ can lead to an accentuation of the AP notch, thus amplifying the normal J wave and resulting in an ST segment elevation, characteristic of BrS. A further outward shift in the balance of currents active during the early phases of the epicardial AP, due to augmented vagal influence ($I_{K\text{-}ACh}$ activation), mild ischemia (more $I_{K\text{-}ATP}$) or bradycardia (greater availability of Ito) can then lead to heterogeneous loss of the action potential dome, thus creating a dispersion of repolarization within epicardium and between epicardium and endocardium. This marked electrical heterogeneity may then facilitate the development of phase 2 reentry and polymorphic VT.[3, 37]

The association of S422L-*KCNJ8* with ERS and/or BrS has been previously reported.[14, 16] Haissaguerre and co-workers first identified this variation in a single case of ERS; they failed to find the variant in 764 alleles of healthy controls.[14] Meideros-Domingo et al. uncovered the S422L-*KCNJ8* in both ERS and BrS probands and further established it as a missense mutation, failing to detect it in 1,200 reference control alleles.[16] They also reported that the missense mutation causes a gain of function in *pinacidil-induced* $I_{K\text{-}ATP}$ using whole-cell patch-clamp techniques.[16]

The present study identified this same missense mutation in *KCNJ8* in 4 additional unrelated families with BrS and ERS and has delineated the molecular basis for the $I_{K\text{-}ATP}$ gain of function, demonstrating a reduced sensitivity of the K_{ATP} channel to ATP. These results are consistent with our demonstration over a decade ago of the effect of a $I_{K\text{-}ATP}$ agonist pinacidil to induce ERS and BrS phenotypes, leading to the development of polymorphic

VT.[3, 38, 39] It is noteworthy that transgenic mice with a gain of function in $I_{K\text{-}ATP}$ secondary to mutations in Kir6.2 have been shown to exhibit VT.[40]

In experimental models, $I_{K\text{-}ATP}$ activation with pinacidil, in addition to producing J wave and ST segment elevation, leads to abbreviation of action potential duration and, as a consequence, abbreviation of the QT interval. Consistent with this finding, in the absence of other long QT variants, the S422L-*KCNJ8* mutation is associated with a relatively short QTc interval (see Table 1). In probands carrying a polymorphism in *CACNCB2b* (p.D601E), known to be associated with QT interval prolongation, the QT interval is generally longer.[15]

Limitations

Like other investigators, we used 0.1 mM of Vanadate at room temperature to prevent $I_{K\text{-}ATP}$ channel rundown. Justification for the use of vanadate is provided in the online supplement.

Our patients with a *CACNB2b*-D601E polymorphism displayed longer QT/QTc intervals, likely due to the effect of the polymorphism to induce a gain of function in $I_{Ca\text{-}L}$. Because of the limited number of patients, this modulatory effect of *CACNB2b*-D601E should be viewed with caution until additional confirmatory data are available.

Conclusion

Our results provide direct evidence in support of the hypothesis that *KCNJ8* is a susceptibility gene for both BrS and ERS, both of which can be categorized as J wave syndromes. Our data point to S422L as a possible hotspot mutation causing a reduced sensitivity of the mutant channel to ATP, thus giving rise to a major gain of function of $I_{K\text{-}ATP}$, leading to manifestation of J wave syndrome phenotypes. The particular ERS or BrS phenotype depends on what part of the heart is principally affected. Understanding the molecular genetic and functional basis for the Brugada and early repolarization syndromes may assist with diagnosis and approach to therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AF	atrial fibrillation
AP	action potential
ASCD	aborted sudden cardiac death
BrS	Brugada syndrome
ECG	electrocardiogram

ER	early repolarization
ERS	early repolarization syndrome
IC_{50}	half maximal inhibitory concentration
IVF	idiopathic ventricular tachycardia
K_{ATP}	ATP-sensitive potassium channel
IK_{ATP}	ATP-sensitive potassium channel current
SCD	sudden cardiac death
SUR	subunit sulfonylurea receptors
VT	ventricular tachycardia
VF	ventricular fibrillation
WT	wild type

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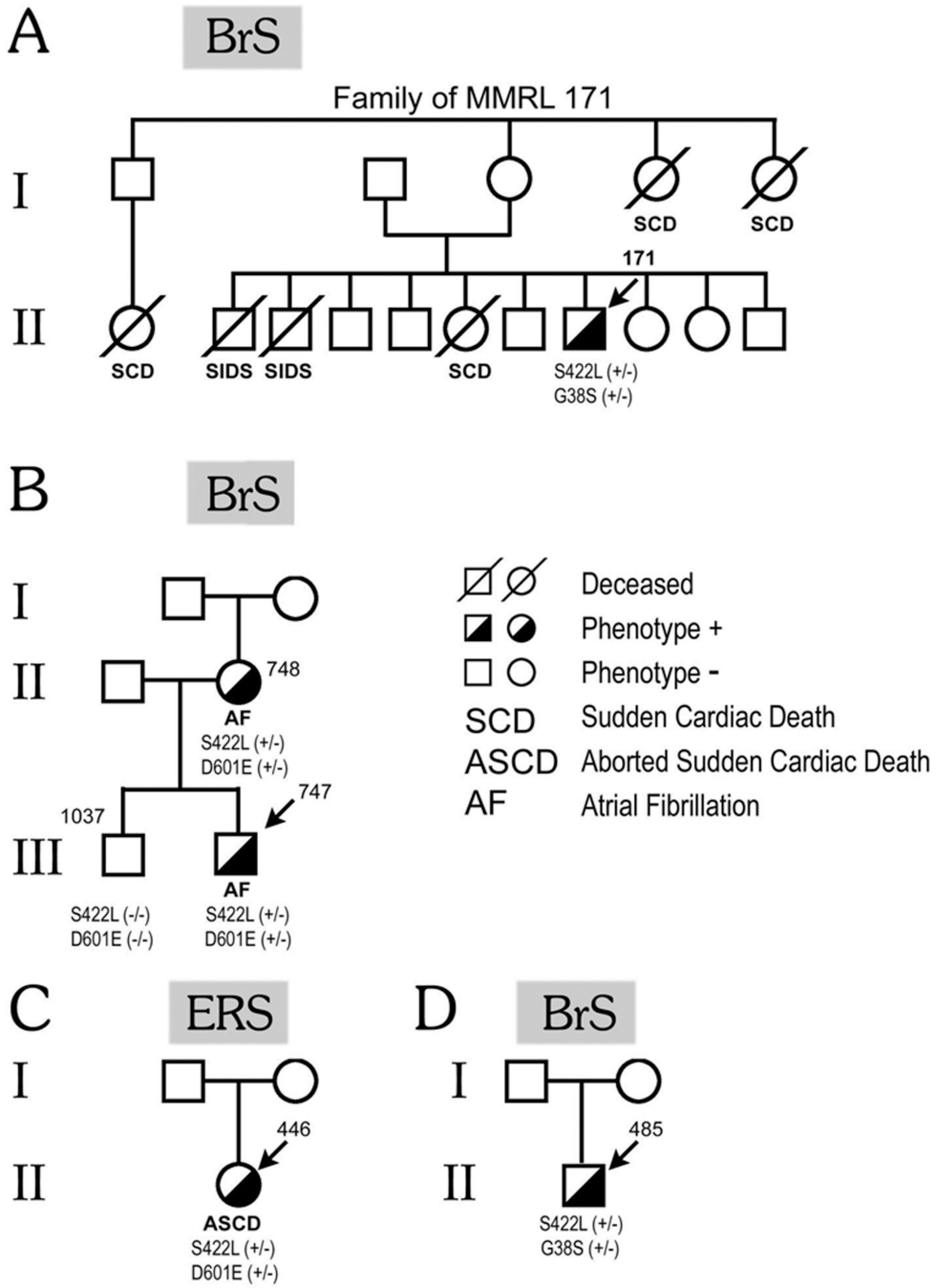

Figure 1. Pedigrees of Brugada syndrome (BrS) and early repolarization syndrome (ERS) families

A: BrS family with atrial fibrillation (AF), sudden cardiac death (SCD) and sudden infant death syndrome (SIDS). **B:** BrS with AF. **C**: ERS with Aborted Sudden Cardiac Death (ASCD). **D**: BrS family. (+/−) indicates heterozygosity for *KCNJ8*-S422L and or *CACNB2b*-D601E. (−/−) indicates a negative genotype. Arrows denote the probands. Numbers represent MMRL ID of probands. Variable penetrance is due in part to age and gender of family members in the case of J wave syndromes.

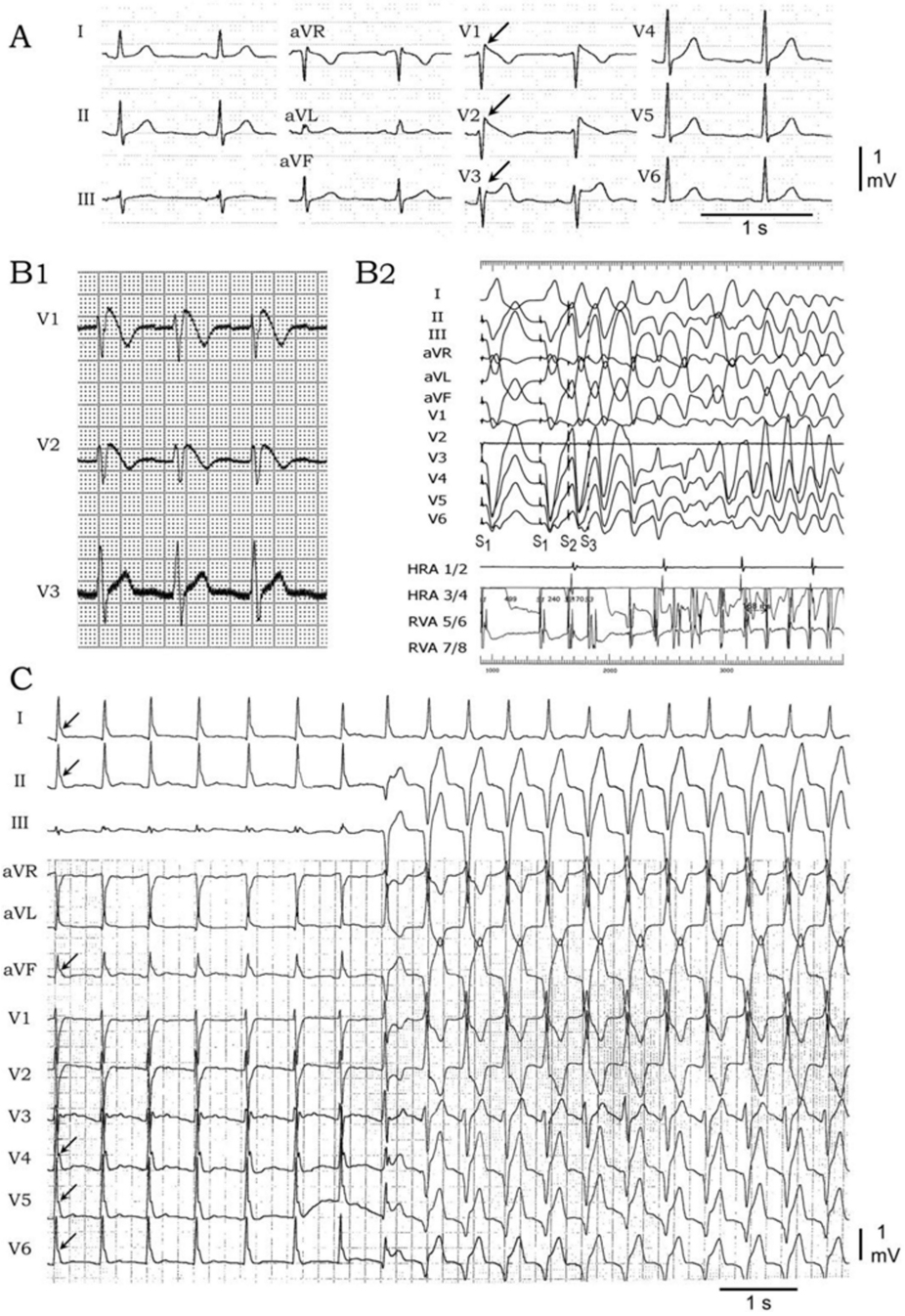

Figure 2. Electrocardiograms (ECGs) of Brugada and early repolarization syndromes probands

A: 12-lead ECG of Brugada syndrome (BrS) proband (#747) recorded after ajmaline displaying ST segment elevation in V1, V2 and V3. **B**: BrS proband (#171) shows ST segment elevation after ajmaline challenge (left panel) and polymorphic ventricular tachycardia (VT) induced using 2 extrastimuli (240 ms and 170 ms) applied to the right ventricular apex (RVA, right panel). **C**: Early repolarization syndrome (ERS) (pedigree shown in Figure 1D) J wave elevation denoted by arrows in leads I, II, AVF, V4, V5 and V6 preceding initiation of monomorphic VT.

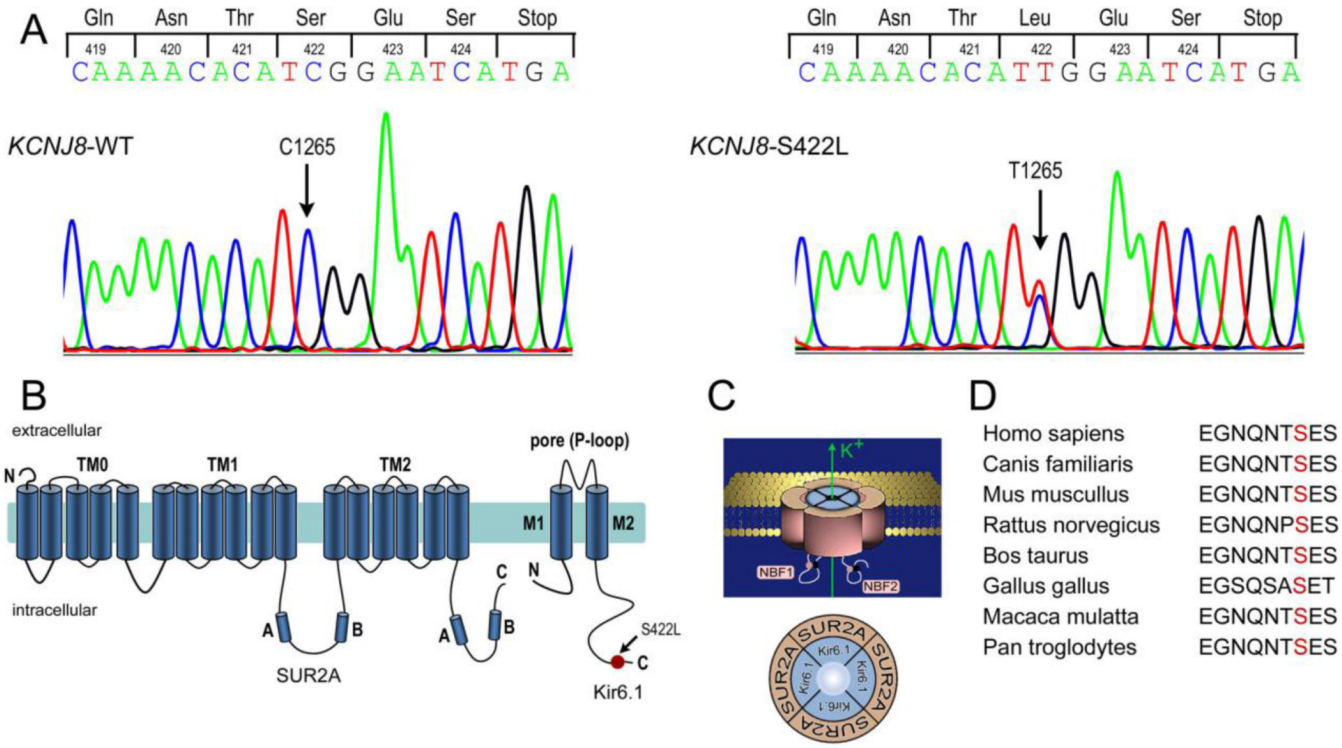

Figure 3. Genetic mutation in *KCNJ8* gene

A: DNA chromatogram showing a heterozygous C-to-T transition at nucleotide 1265, predicating a substitution of a leucine (Leu) for serine (Ser) at residue 422 (S422L) of *KCNJ8*, which encodes the Kir6.1 subunit of the ATP-sensitive K (K_{ATP}) channel current ($I_{K\text{-}ATP}$). **B:** Predicted topology of the Kir6.1 and *SUR2A* subunit showing the location of the S422L mutation (red circle) at the carboxyl terminal region of Kir6.1; **C:** Functional K_{ATP} channels have an octameric subunit structure with four pore-forming subunits (Kir6.1) and four sulfonylurea receptors (*SUR2A*). **D**: Amino acid alignments performed using GenBank accession numbers corresponding to protein sequences shows a serine at position 422 is highly conserved among species.

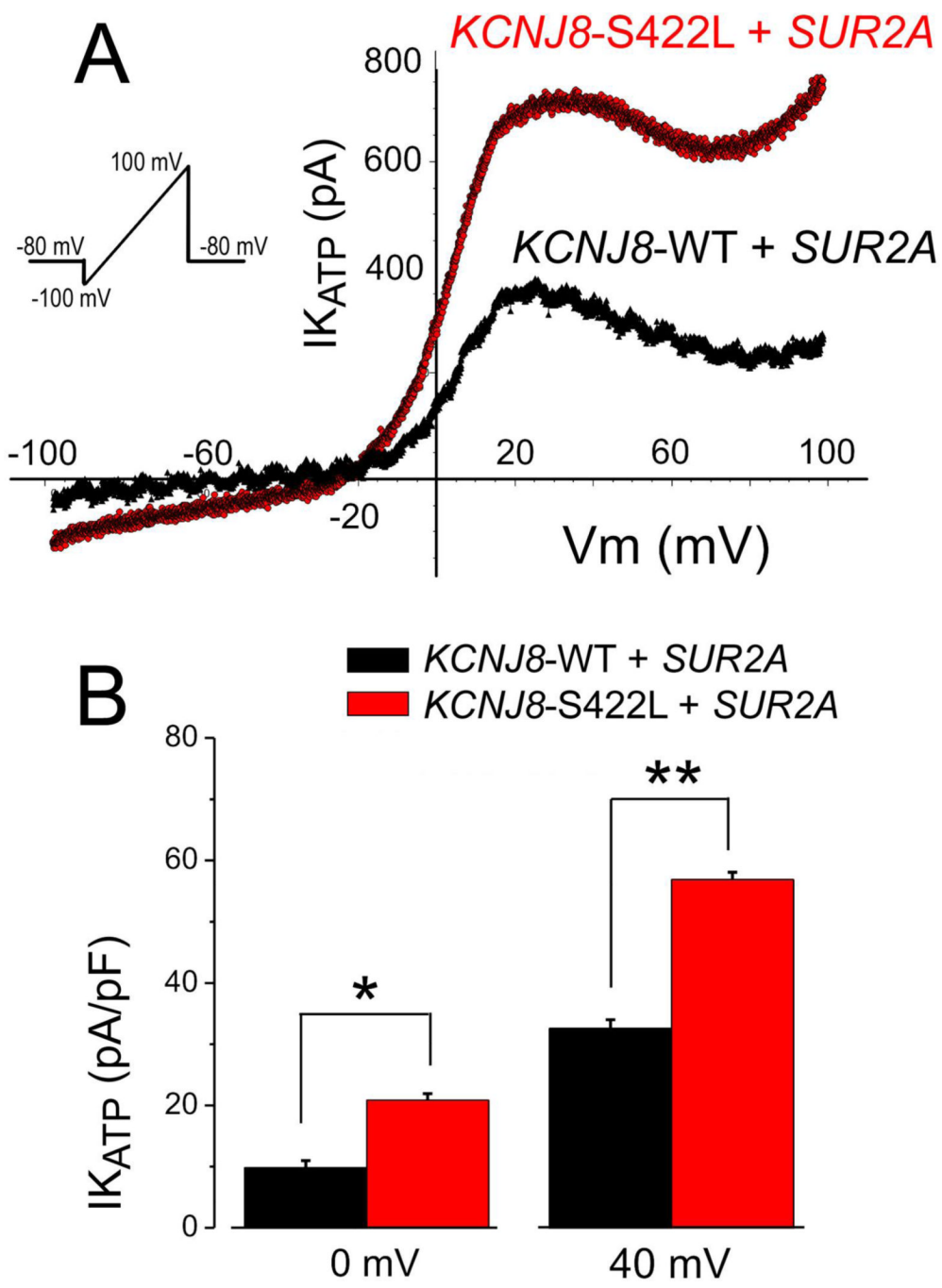

Figure 4. Whole-cell patch clamp studies suggesting that *KCNJ8*-S422L is associated with a gain of function in ATP-sensitive K (K_{ATP}) channel current ($I_{K\text{-}ATP}$)

A: Current-voltage relations of $I_{K\text{-}ATP}$ channel obtained 48–72 hours following transient transfection of human Kir6.1-wild type (WT) (*KCNJ8*-WT) and Kir6.1-S422L (*KCNJ8*-S422L) with human *SUR2A*-WT genes in TSA201 cells. Representative traces recorded before and after gliblenclamide (10 μM) were digitally subtracted to obtain the glibenclamide-sensitive K_{ATP} current. Macroscopic currents were recorded from a holding potential of −80 mV followed by ramp protocol from −100 mV to 100 mV for 400 ms (see insert). **B:** Summary of the difference in $I_{K\text{-}ATP}$ between *KCNJ8*-WT (black bar) and *KCNJ8*-S422L (red bar) channels at 0 mV and +40 mV, respectively. Data points are the

mean±SEM of peak whole-cell currents (n=8 cells for each group). *P<0.05 and **P<0.01 mutant vs WT.

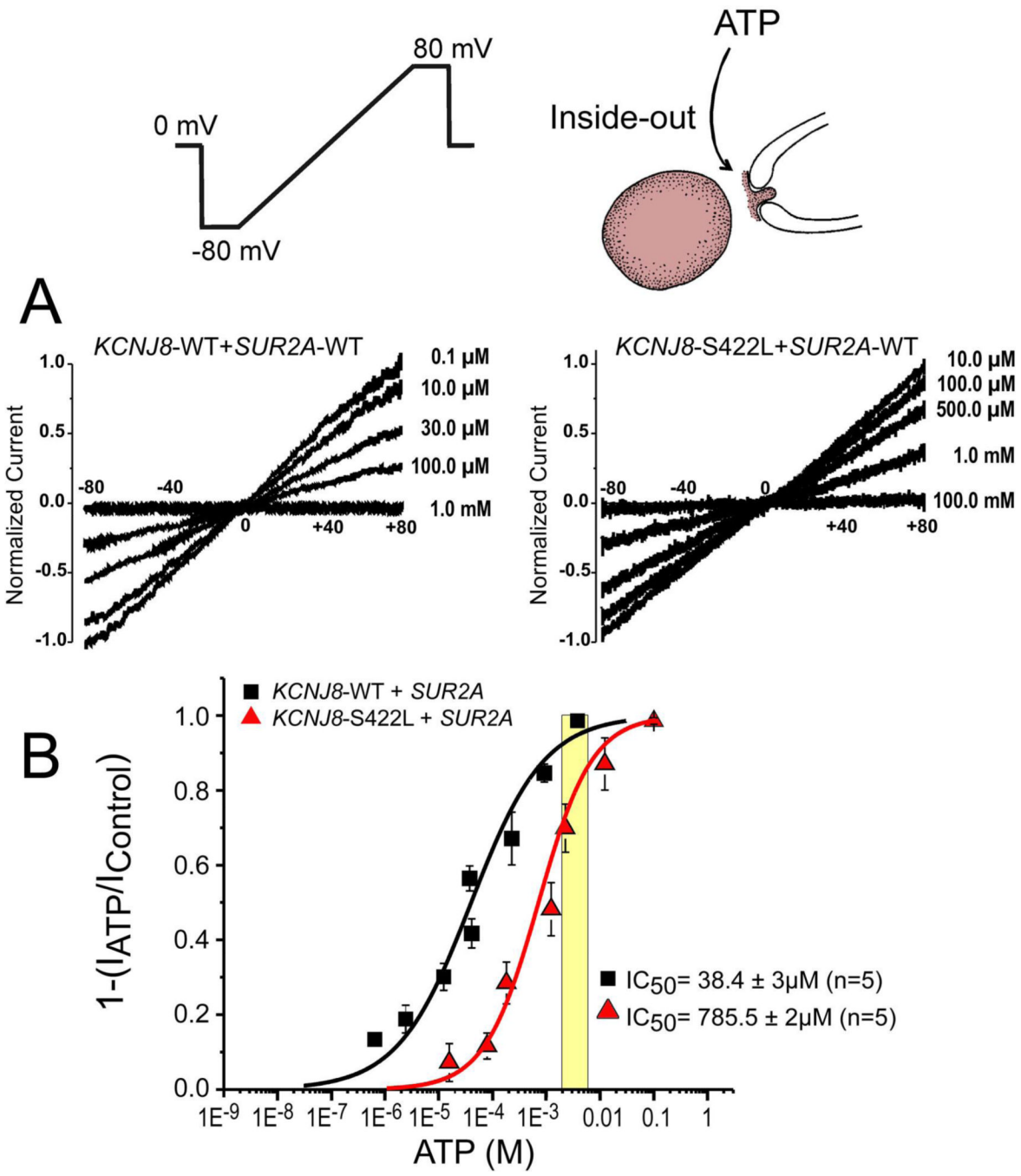

Figure 5. *KCNJ8*-S422L mutation causes a gain of function in ATP-sensitive K (K_{ATP}) channel current ($I_{K\text{-}ATP}$) by reducing sensitivity of K_{ATP} channels to ATP

Sensitivity to K_2ATP of *KCNJ8*-S422L/*SUR2A*-wild type (WT) and *KCNJ8*-WT/*SUR2A*-WT channels measured using excised inside-out patches. **A**: $I_{K\text{-}ATP}$ traces from excised inside-out macropatches, obtained by holding the patch at 0 mV and applying ramps from −80 to +80 mV (3 s), before and after attaining steady-state inhibition with K_2ATP. **B:** Concentration-response relationships. Currents were normalized to the current recorded at −80 mV under control conditions. The IC_{50} value of K_2ATP for *KCNJ8*-WT/*SUR2A*-WT was 38.4±3 μM (n=5), whereas the IC_{50} for the mutant *KCNJ8*-S422L/*SUR2A*-WT was 785.5±2 μM (n=5, p<0.01). Mean ±SEM. **P<0.01 vs. WT.

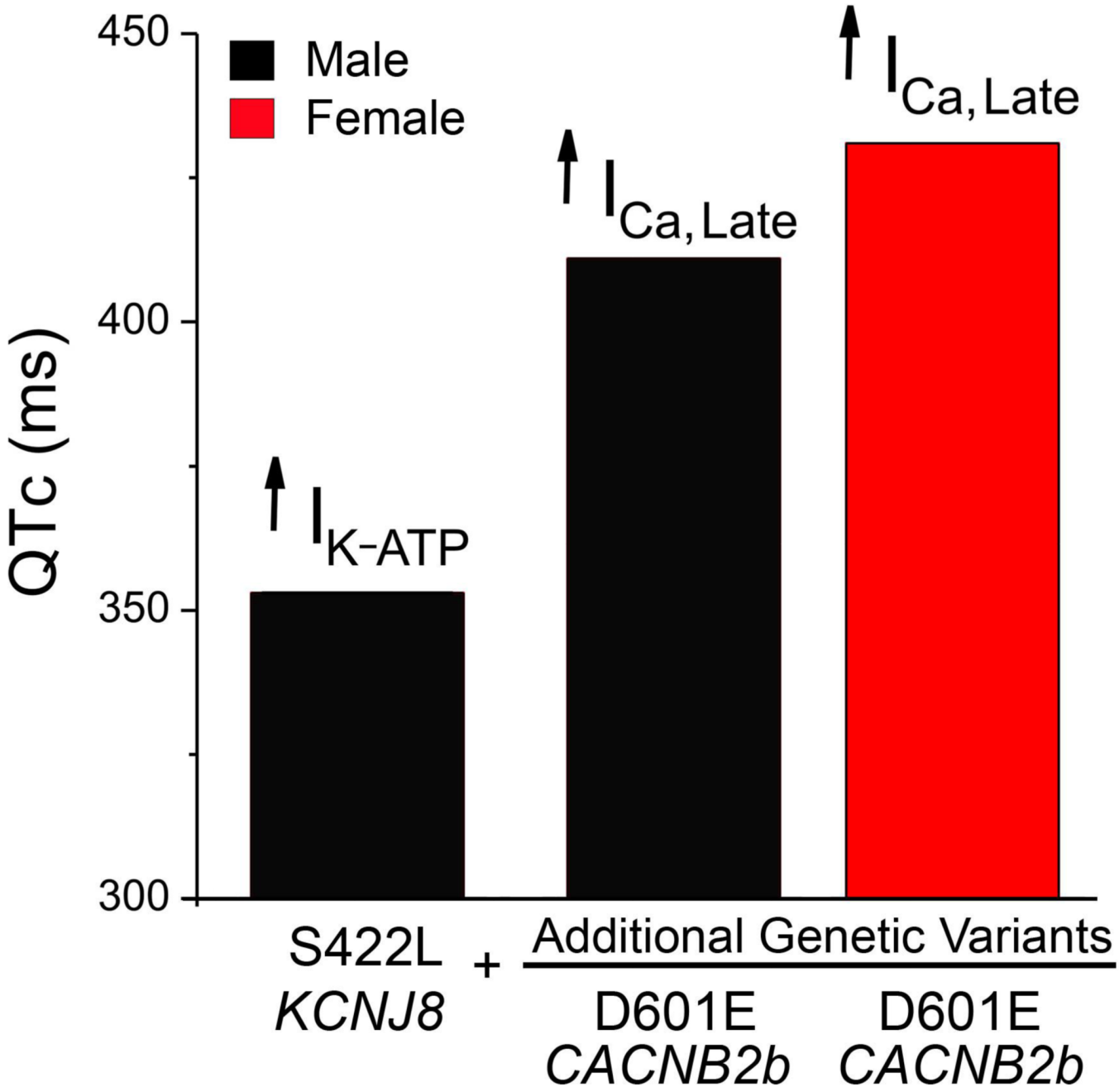

Figure 6. Modulatory effect of a secondary gene variant on QTc interval in probands with the S442L-*KCNJ8* gain of function mutation

The S422L-*KCNJ8* mutation alone is associated with a relatively short QTc interval. Probands with a second genetic variant causing an increase in late calcium channel current display a relatively longer QTc interval. The longest QTc is found in females in whom a gain of function genetic variant in *CACNB2b* is detected leading to an increase in late I_{Ca}.

Table 1

Patients with S422L-*KCNJ8* associated with J-wave syndromes

Patient MMRL #	Proband	Syndrome	Gender/ Age	Symptoms	QT/QTc (ms)	Additional Genetic Variants
1 / 171	Yes	BrS-01	Male / 33	AF/VF	310/352	None
2 / 747	Yes	BrS-02	Male / 45	AF / 1stAVB	384/411	D601E-*CACNB2b*
3 / 748	No	BrS -02	Female / 66	AF / 1stAVB	432/401	D601E-*CACNB2b*
4 / 446	Yes	ERS-01	Female / 23	ASCD	422/431	D601E-*CACNB2b*
5 / 485	Yes	BrS-03	Male / 46	VT	415/353	None

AF=Atrial Fibrillation
VF=Ventricular Fibrillation
ASCD=Aborted Sudden Cardiac Death
VT= Ventricular Tachycardia