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Kcne2 Deletion Creates a Multisystem Syndrome Predisposing to Sudden Cardiac Death

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Background—Sudden cardiac death (SCD) is the leading global cause of mortality, exhibiting increased incidence in patients with diabetes mellitus. Ion channel gene perturbations provide a well-established ventricular arrhythmogenic substrate for SCD. However, most arrhythmia-susceptibility genes, including the KCNE2 K^+ channel β subunit, are expressed in multiple tissues, suggesting potential multiplex SCD substrates.

Methods and Results—Using whole-transcript transcriptomics, we uncovered cardiac angiotensinogen upregulation and remodeling of cardiac angiotensinogen interaction networks in P21 *Kcne2*$^{-/-}$ mouse pups and adrenal remodeling consistent with metabolic syndrome in adult *Kcne2*$^{-/-}$ mice. This led to the discovery that *Kcne2* disruption causes multiple acknowledged SCD substrates of extracardiac origin: diabetes mellitus, hypercholesterolemia, hyperkalemia, anemia, and elevated angiotensin II. *Kcne2* deletion was also a prerequisite for aging-dependent QT prolongation, ventricular fibrillation and SCD immediately after transient ischemia, and fasting-dependent hypoglycemia, myocardial ischemia, and AV block.

Conclusions—Disruption of a single, widely expressed arrhythmia-susceptibility gene can generate a multisystem syndrome comprising manifold electric and systemic substrates and triggers of SCD. This paradigm is expected to apply to other arrhythmia-susceptibility genes, the majority of which encode ubiquitously expressed ion channel subunits or regulatory proteins. (***Circ Cardiovasc Genet*. 2014;7:33-42.**)

Key Words: arrhythmias, cardiac ■ death, sudden, cardiac ■ ion channels ■ ischemia ■ potassium

Sudden cardiac death (SCD) is the term given to sudden, unexpected cessation of cardiac function. It is estimated that >1 in 1000 people in developed nations succumb to SCD annually, and it is the leading cause of death worldwide.[1] Distinct from a heart attack, in which the heart continues to beat but blood flow is blocked, in SCD, the heart ceases to beat because of catastrophic, sustained ventricular fibrillation. Without defibrillation within minutes, this type of event is fatal; individuals who survive require an implantable cardiac defibrillator to protect against future SCD incidence.[2] SCD typically occurs without evidence of a myocardial infarction but is considered frequently to require an ischemic substrate or heart failure. Diabetes mellitus is an established risk factor for both myocardial ischemia[3] and SCD, the latter even after adjustment for other risk factors that accompany diabetes mellitus.[4] Dead-in-bed syndrome, the overnight sudden unexplained death of someone with type I diabetes mellitus, may be linked to nocturnal hypoglycemia precipitating lethal ventricular arrhythmias[5]; hypoglycemia linked to tight glycemic control is also associated with and predictive of increased mortality in people with type II diabetes.[6]

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Younger victims of SCD may lack detectable ischemic substrates, and in these cases in particular, a genetic electric disorder of cardiac origin is often implicated. To date, >25 genes have been identified, which are linked or associated with ventricular arrhythmias predisposing to SCD.[2] These genes all encode cardiac-expressed ion channel subunits or channel-interacting proteins; their disruption is considered sufficient to cause monogenic, lethal ventricular arrhythmias by direct perturbation of ion currents orchestrating ventricular myocyte excitability.[2]

Most arrhythmia-susceptibility genes are not, however, expressed exclusively in the heart, suggesting the possibility that monogenic arrhythmia syndromes are complex, multisystem disorders. Here, we report that deletion of the widely expressed KCNE2 K^+ channel β subunit gene[7,8] creates a multifactorial substrate for SCD that includes diabetes mellitus, dyslipidemia, hyperkalemia, and progressive loss of ventricular repolarization reserve. The findings support the idea of multiplex origins even in monogenic ventricular arrhythmias.

Methods

We generated and genotyped the wild-type, heterozygous, *Kcne2*$^{-/-}$, *Kcne3*$^{-/-}$, and double-knockout *Kcne2*$^{-/-}$*Kcne3*$^{-/-}$ mice as previously described[9,10] and housed and used them according to the US National Institutes of Health Guide for the Care and Use of Laboratory

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Animals. Animal procedures were approved by the Animal Care and Use Committees at Weill Medical College of Cornell University and University of California, Irvine. All adult mice used in this study, aside from breeding males, were female and generated from *Kcne2*$^{+/-}$ ×*Kcne2*$^{+/-}$ crosses. Except where indicated, 2 age groups of adult mice were used, referred to as 4m-old and 7m-old. For functional studies, the 4m-old group were of mean age when studied: 4.3±0.1 months, n=27 (*Kcne2*$^{+/+}$) versus 4.2±0.1 months, n=28 (*Kcne2*$^{-/-}$). The 7m-old group were of mean age when studied: 7.4±0.1 months, n=30 (*Kcne2*$^{+/+}$) versus 7.4±0.4 months, n=42 (*Kcne2*$^{-/-}$). The P21 pups studied for the microarray comparison of maternal versus pup genotype effects were all male and were generated from either *Kcne2*$^{+/-}$ ×*Kcne2*$^{+/-}$ or *Kcne2*$^{-/-}$×*Kcne2*$^{-/-}$ crosses as indicated.

Briefly, statistical analyses were generally conducted as follows: unpaired Student *t* test for comparison of 2 groups; 1-way ANOVA followed by Tukey Honest Significant Difference test or Bonferroni test for comparison of multiple groups; repeated measures 2-way ANOVA followed by post hoc Bonferroni correction for ST heights over time. Raw microarray data were analyzed using Partek Express software (Partek, St Louis, MO). Ingenuity Systems iReport software (Qiagen, Hilden, Germany) was used to compare raw microarray data and for pathway and network analysis independently, using default settings. Unless otherwise described, the Kolmogorov–Smirnov test was used to verify the assumption of normal distribution. Statistical significance was assumed with $P<0.05$. Detailed methods and associated references are described in the Methods in the Data Supplement.

Results

Kcne2 Deletion Causes Maternal Genotype-Dependent and Genotype-Independent Cardiac Remodeling

Kcne2$^{-/-}$ pups of *Kcne2*$^{-/-}$ dams exhibit much more substantial cardiac hypertrophy at P21 than *Kcne2*$^{-/-}$ pups of *Kcne2*$^{+/-}$ dams do (Figure 1A), primarily arising from complications of maternal hypothyroidism during gestation and lactation.[11] Specifically, we previously demonstrated that thyroid KCNQ1-KCNE2 potassium channels are required for optimal thyroid hormone biosynthesis; *Kcne2* deletion causes hypothyroidism during gestation and lactation in mice but not in nonpregnant/lactating adult mice <12 months of age. The most profound consequences of this are observed preweaning in the pups of *Kcne2*$^{-/-}$ dams, which exhibit alopecia, stunted growth, and cardiac hypertrophy. These manifestations are closely associated with maternal hypothyroidism because they can be largely prevented by thyroid hormone treatment of the dam during gestation and lactation or by surrogacy with wild-type dams at P1. Conversely, these symptoms can be initiated in wild-type pups by surrogacy with *Kcne2*$^{-/-}$ dams. One of the main mechanistic explanations for these effects is that maternal hypothyroidism impairs milk ejection.[11]

Here, global whole-transcript microarray analysis revealed differential atrial and ventricular gene network remodeling dependent on pup and maternal *Kcne2* genotype (Figure 1B); for all differentially expressed genes, see the Data Supplement. Surprisingly, gene interaction hierarchy analysis of predicted differentially expressed gene interaction networks uncovered *AGT*, which encodes angiotensinogen, as the most common predicted upstream effector in pup genotype-dependent cardiac remodeling (Figure 1C–1F). Dam genotype-dependent, pup genotype-independent predicted pathways were, in contrast, headed by *PPARG* and *PNPLA2* in atria (Figure 1C and 1D) and *TLR4* in the ventricles (Figure 1E and 1F); the latter finding is consistent with the previously reported role for this gene in ventricular hypertrophy.[12]

Kcne2 Deletion Elevates Serum Angiotensin II and Causes Adrenal Dysfunction and Lipid Accumulation

Angiotensinogen is cleaved by renin (in response to decreased renal perfusion) to generate angiotensin I, which is converted by angiotensin-converting enzyme to angiotensin II. Cardiac myocyte angiotensinogen expression is increased in an autocrine loop by angiotensin II.[13] Accordingly here we found that serum angiotensin II concentration in *Kcne2*$^{-/-}$ mice was twice that of *Kcne2*$^{+/+}$ littermates (n=10–14; $P=0.012$; Figure 2A). However, serum aldosterone, which is typically released by the adrenal gland upon angiotensin II stimulation, was unaltered (n=9; $P=0.95$; Figure 2B). The adrenal unresponsiveness could not be explained by disruption of a direct adrenal role of *Kcne2* because it is not expressed in the adrenal glands (Figure 2C). We detected *Kcne2* in the lung (Figure 2C), the primary source of angiotensin-converting enzyme, but *Kcne2* deletion reduced pulmonary angiotensin-converting enzyme expression (Figure 2D). This is consistent with activation of feedback mechanisms to reduce angiotensin I to II conversion and suggests against the possibility that pulmonary *Kcne2* deficiency increased serum angiotensin II by directly increasing angiotensin-converting enzyme expression in the lung.

Microarray analysis of the adrenal transcriptome (Figure 2E) indicated predominantly downregulation, in adult *Kcne2*$^{-/-}$ mouse adrenals, of expression of genes and gene networks, with *PPARG* as the most common upstream element (Figure 2F), associated with lipid metabolism and uptake (for all differentially expressed transcripts, see Data Supplement), glucose metabolism disorders and insulin resistance (Figure 2G), and metabolic syndrome (Figure 2H). The data suggested a compensatory response to hyperstimulation or aberrant adrenal lipid accumulation, a diabetic/hyperlipidemic environment, and a defect in steroidogenesis, in *Kcne2*$^{-/-}$ adrenals.

Adrenal glands of *Kcne2*$^{-/-}$ mice did not exhibit macroscopic hypertrophy as would be expected in primary hyperaldosteronism, but *Kcne2* deletion caused adrenal cortex vacuolation and lipid accumulation consistent with chronic hyperstimulation as occurs with chronic stimulation by angiotensin II (Figure 2I), also consistent with diabetic dyslipidemia.[14]

Kcne2 Deletion Causes Dyslipidemia, Diabetes Mellitus, and Anemia

Examining potential causes of the adrenal steatosis, we discovered that *Kcne2* deletion causes hypercholesterolemia, raising free low-density lipoprotein cholesterol but not altering free high-density lipoprotein cholesterol or cholesteryl esters (Figure 3A). Strikingly, *Kcne2*$^{-/-}$ mice, but not their *Kcne2*$^{+/+}$ littermates, also develop diabetes mellitus, characterized by fasting hypoglycemia (Figure 3B) and impaired glucose tolerance (Figure 3C and 3D). We detected *Kcne2* expression in mouse pancreas, suggesting that *Kcne2* deletion may pathologically impair a pancreatic K^+ current (Figure 3E).

In addition, after observing that *Kcne2*$^{-/-}$ mice often exhibit paler extremities than their *Kcne2*$^{+/+}$ littermates (Figure 3F), we also found that *Kcne2*$^{-/-}$ mice exhibit splenomegaly (4.2-fold increased mean body mass-corrected spleen mass; Figure 3G and 3H). One likely cause for these

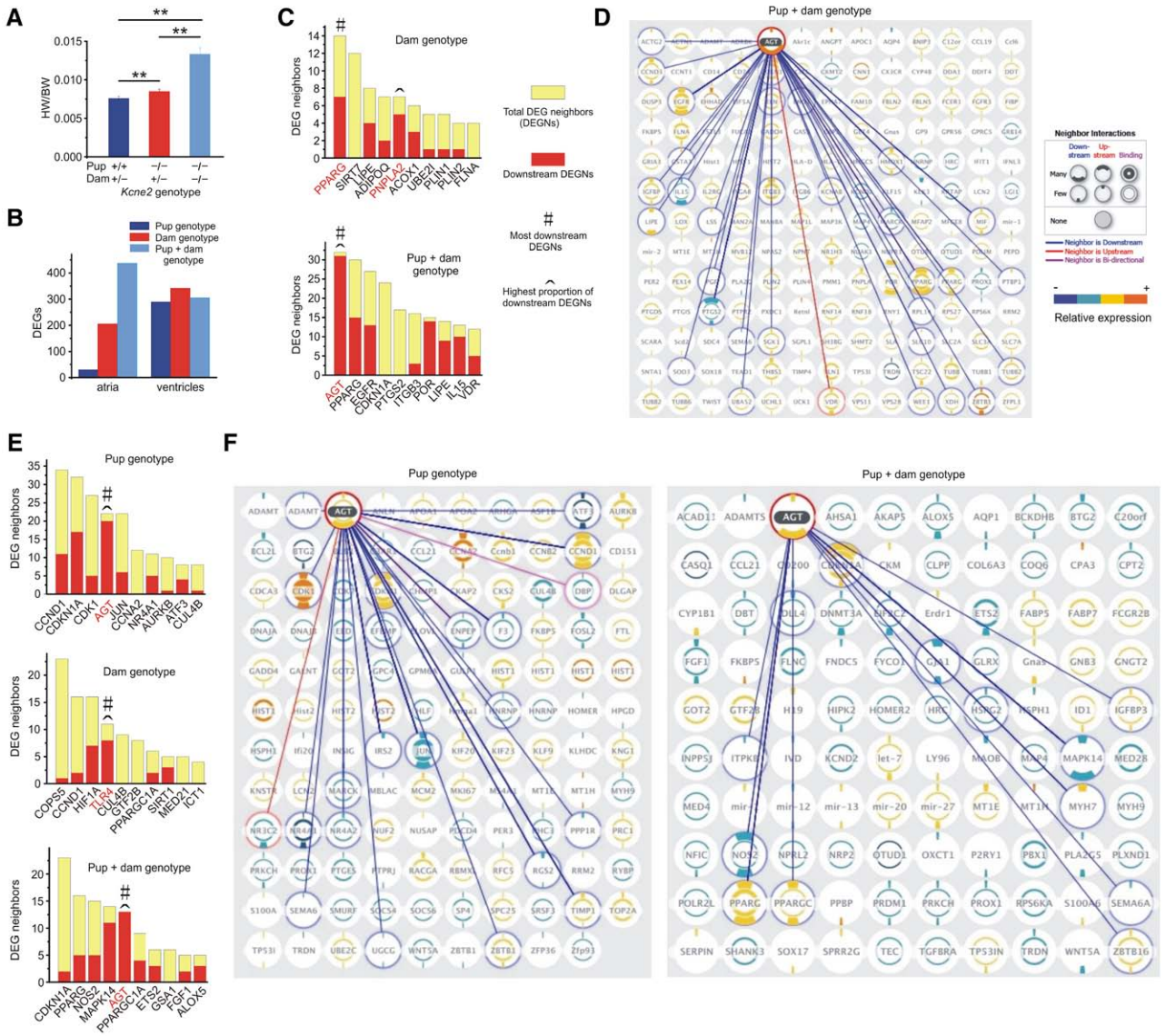

Figure 1. *Kcne2* deletion remodels cardiac angiotensinogen-associated gene interaction networks. **A**, Effects of pup and maternal *Kcne2* genotype on heart weight (HW)/body weight (BW) of P21 pups (n = 8). **P<0.01. **B**, Cardiac differentially expressed genes (DEGs) (quantified by microarray analysis, P<0.05 significance level used for this and all other comparisons, n=7–8 pups per group) in P21 *Kcne2*$^{-/-}$ vs *Kcne2*$^{+/+}$ pups of either *Kcne2*$^{+/-}$ or *Kcne2*$^{-/-}$ dams, grouped as follows: pup genotype—*Kcne2*$^{-/-}$ vs *Kcne2*$^{+/+}$ pups, all from *Kcne2*$^{+/-}$ dams; dam genotype—*Kcne2*$^{+/-}$ vs *Kcne2*$^{-/-}$ dams, all *Kcne2*$^{-/-}$ pups; pup+dam genotype—*Kcne2*$^{-/-}$ pups from *Kcne2*$^{-/-}$ dams vs *Kcne2*$^{+/+}$ pups from *Kcne2*$^{+/-}$ dams. **C**, Top 10 ranked (in terms of number of interacting DEGs) atrial DEGs participating in predicted interaction networks with other DEGs dependent on dam genotype (**left**) or pup+dam genotype (**right**; groups as in **B**). Pup genotype alone yielded no significantly altered interaction networks of >2 DEGs. **D**, **Left**, Atrial DEG interaction network of angiotensinogen (*AGT*), the top ranked upstream effector DEG (atria of *Kcne2*$^{-/-}$ pups from *Kcne2*$^{-/-}$ dams vs *Kcne2*$^{+/+}$ pups from *Kcne2*$^{+/-}$ dams) showing all its predicted interaction DEGs (connected by lines) and all other DEGs predicted to have ≥1 interactions with other DEGs (no connecting lines shown). **Right**, Key for interaction hierarchy, number of interacting DEGs, and relative expression. **E**, Top 10 ranked (in terms of number of interacting neighbors) ventricular DEGs participating in predicted interaction networks with other DEGs dependent on pup genotype (**top**), dam genotype (**middle**) or pup+dam genotype (bottom; groups as in **B**). **F**, Ventricular DEG interaction network of *AGT*, the top ranked upstream effector DEG (**left**, pup genotype dependent; right, pup+dam genotype dependent) showing all predicted interacting DEGs (connected to AGT by lines) and all other DEGs predicted to have ≥1 interactions with other DEGs (no connecting lines shown). Key for interaction hierarchy, number of interacting DEGs, and relative expression as shown in **D**.

2 observations is anemia. *Kcne2* deletion causes achlorhydria because the KCNQ1-KCNE2 K^+ channel is expressed on the apical membrane of parietal cells and is required for gastric acid secretion.[9] One consequence of achlorhydria is anemia because of inadequate iron absorption and vitamin B_{12} deficiency. Here, we quantified blood cell parameters and found that *Kcne2*$^{-/-}$ mice exhibit anisocytosis and microcytic anemia, specific hallmarks of iron-deficiency anemia; the lowered mean corpuscular hemoglobin was also consistent with this (Figure 3I).

Anemia, hypercholesterolemia, and diabetes mellitus all diminish myocardial oxygen supply, predispose to chronic myocardial ischemia,[15] and are associated with increased risk of human SCD.[16–18] In addition, fasting-induced hypoglycemia is considered an important risk factor for diabetic SCD in the form of dead-in-bed syndrome.[19,20]

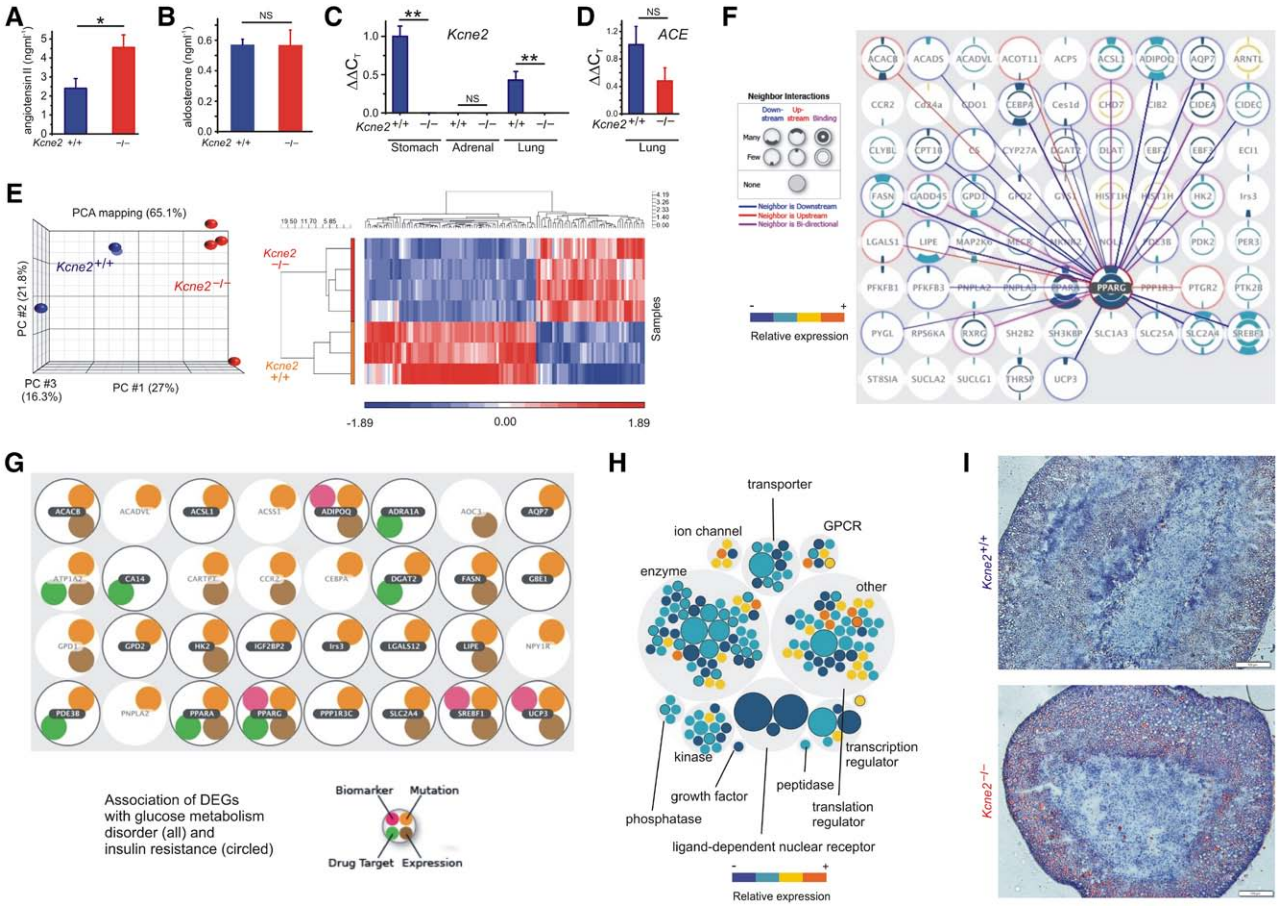

Figure 2. *Kcne2* deletion elevates angiotensin II and causes adrenal remodeling and lipid accumulation. **A**, Mean serum angiotensin II content of 7m-old *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice (n=10–14). *P=0.012. **B**, Mean serum aldosterone content of 7m-old *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice (n=9). **C**, Relative expression of *Kcne2* transcript in *Kcne2*$^{+/+}$ stomach, lung, and adrenal tissue (n=4). **P<0.01. **D**, Relative expression of *ACE* transcript in *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ lung tissue (n=3). **E. Left**, Principle component analysis (PCA); **right**, heat map display of global transcript expression differences, after microarray analysis of the adrenal transcriptomes of *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice (n=3–4). Percentage values indicate the percentage of explained variance. **F**, Adrenal differentially expressed gene (DEG) interaction network (DEGs between *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ adrenals) showing all DEGs with ≥1 predicted DEG interaction neighbors. Predicted interactions are shown between the most common upstream DEG (*PPARG*) and its neighbors. **G**, DEGS within the 2 highest ranking disease processes predicted by analysis of adrenal DEGs in *Kcne2*$^{-/-}$ vs *Kcne2*$^{+/+}$ mice (glucose metabolism disorder, P=8.5×10^{-10}; and insulin resistance, P=2.1×10^{-10}). **H**, Adrenal DEGs grouped by function, sized by number of interacting neighbors, and colored by relative expression (*Kcne2*$^{-/-}$ vs *Kcne2*$^{+/+}$). DEGs associated with the highest ranking category of disorder based on DEGs (metabolic syndrome) are outlined. **I**, Representative (n=4 per genotype) micrographs of Oil Red O stained adrenal gland sections from 6-month-old female *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice (scale bars, 20 μm). GPCR indicates G protein-coupled receptor; and NS, no significant difference between groups.

Kcne2 Deletion Causes Hyperkalemia and QT$_c$ Prolongation

In normal circumstances, hyperkalemia stimulates the renin–angiotensin–aldosterone system, elevating angiotensin II and also aldosterone, increasing sodium reabsorption and potassium excretion by the kidneys, increasing blood pressure. Here, we found that *Kcne2*$^{-/-}$ mice show significant hyperkalemia (8.2±0.44 mmol/L K$^+$; n=23) when compared with *Kcne2*$^{+/+}$ mice (6.4±0.23 mmol/L K$^+$; n=40; P<0.01; Figure 4A). Chronic hyperkalemia would be expected to elevate serum angiotensin II chronically and, in combination with other destructive influences on the adrenal (Figures 2 and 3), lead to impaired aldosterone production (Figure 2B) and inability to restore normokalemia. Interestingly, the high angiotensin II did not elevate blood pressure in *Kcne2*$^{-/-}$ mice, which was slightly lower than that of age-matched *Kcne2*$^{+/+}$ littermates (Figure I in the Data Supplement), possibly reflecting an inability to excrete sufficient K$^+$.

KCNQ1-KCNE2 K$^+$ channels generate K$^+$ recycling currents in gastric parietal cells and other epithelia. KCNE2, in parietal cell apical membrane complexes with KCNQ1, is required to return to the stomach lumen K$^+$ ions brought into the cell by the H$^+$/K$^+$ATPase during gastric acid secretion.[9,21] We previously found that *Kcne2* deletion results in aberrant parietal cell basolateral targeting of KCNQ1 in complexes with pathologically upregulated KCNE3.[10] This could provide a gastric short-circuit current funneling K$^+$ from the stomach through to the blood, robbing the stomach lumen of K$^+$ (consistent with the observed achlorhydria in *Kcne2*$^{-/-}$ mice) and potentially increasing serum K$^+$ (Figure 4B). To test this hypothesis, we first quantified serum K$^+$ in *Kcne3*$^{-/-}$ and double-knockout *Kcne2*$^{-/-}$ *Kcne3*$^{-/-}$ mice. Although *Kcne3*$^{-/-}$ mice were normokalemic (n=18), deletion of both *Kcne2* and *Kcne3* genes caused a statistically nonsignificant trend toward hypokalemia (serum K$^+$ of 5.1±0.46 mmol/L; n=24; P>0.05 versus wild-type). Consistent with this hypothesis, although

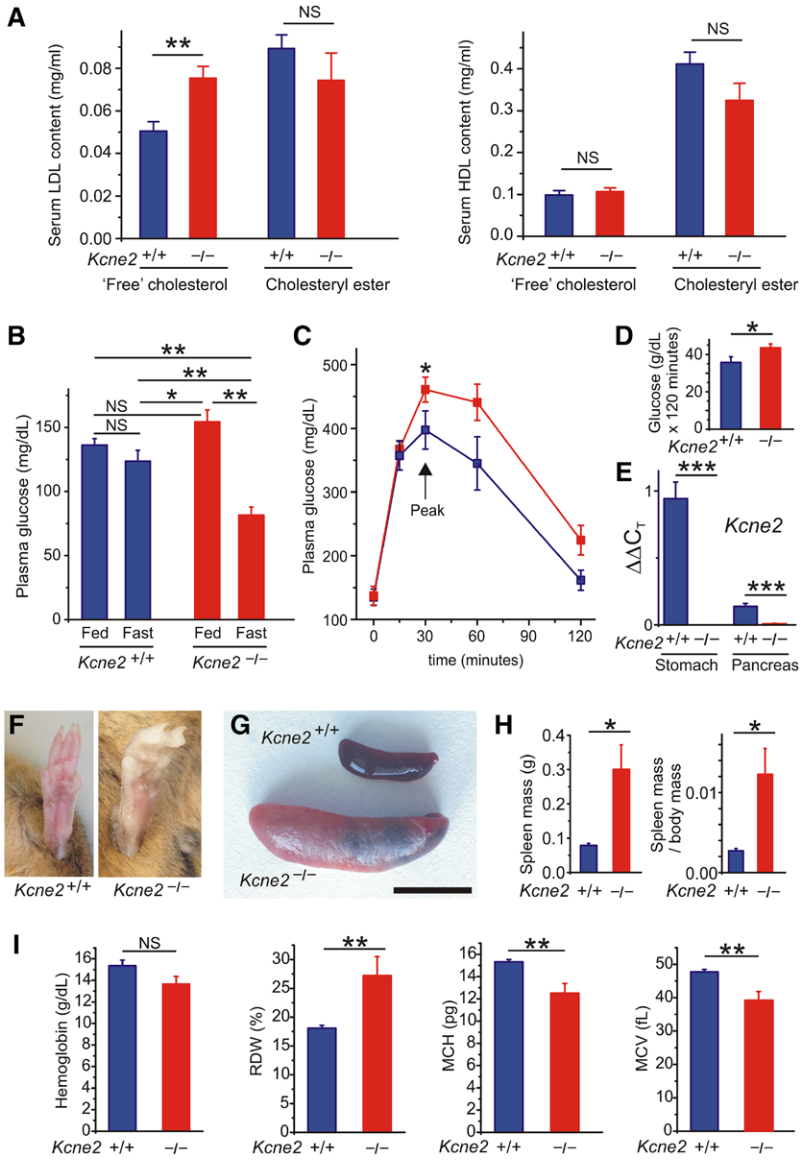

Figure 3. *Kcne2* deletion causes anemia, diabetes mellitus, and dyslipidemia. **A**, Mean serum lipid content of 7m-old female *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice (n=11). **P=0.002. **B**, Mean blood glucose content of 7m-old *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice (n=16–19). *P<0.05; **P<0.01. **C**, Glucose tolerance test results: mean blood glucose after glucose injection in 7m-old *Kcne2*$^{+/+}$ (n=11) and *Kcne2*$^{-/-}$ (n=16) mice; *P<0 0.05 between genotypes at peak glucose level. **D**, Integration of area under curve from data in **C** using trapezoidal rule; *P<0.05. **E**, Real-time quantitative polymerase chain reaction detection of *Kcne2* cDNA in stomach and pancreas of *Kcne2*$^{+/+}$ mice with *Kcne2*$^{-/-}$ mouse tissue as a negative control; ***P<0.001. **F**, Hind feet of adult *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice showing example of pale extremities in the latter. **G**, Exemplar spleens from adult *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice showing splenomegaly in the latter. Scale bar, 1 cm. **H**, Mean spleen mass and spleen mass/body mass for 7m-old *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice (n=7–9). *P<0.05. **I**, Hemoglobin and red blood cell parameters of 4m-old *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice (n=5–7). **P<0.01. MCH indicates mean corpuscular hemoglobin; MVC, mean corpuscular volume; NS, no significant difference between genotypes; and RDW, red blood cell distribution width.

not achieving statistical significance when comparing mean slopes between genotypes, using an ex vivo stomach preparation, we found that the *Kcne2*$^{-/-}$ stomach mucosa exhibits a trend toward an increased luminal-to-serosal K^+ flux when compared with either wild-type or double-knockout *Kcne2*$^{-/-}$ *Kcne3*$^{-/-}$ mucosae, as predicted by our model (Figure 4A–4C).

The findings (Figures 1–4) present a picture of a single gene deletion causing interactive disruption of multiple epithelia and consequent cardiac gene network remodeling, creating a multifactorial systemic substrate comprising a panoply of known risk factors for SCD. We next examined potential electric substrates. Hyperkalemia depolarizes cardiac myocyte membranes, counteracting the effects of repolarizing K^+ currents, and is in itself proarrhythmic.[22] In the mouse ventricular myocardium, *Kcne2* regulates I_{to} and $I_{K,slow}$ by forming complexes with, and augmenting currents through, the Kv4.2 and Kv1.5 Kv α subunits, respectively.[23] We previously demonstrated that *Kcne2* deletion reduces ventricular myocyte I_{to} and $I_{K,slow}$ density in young adult (3–4 months old) mice. Although it does not alter baseline QT or QT_c interval, it compromises ventricular myocyte repolarization sufficiently to predispose mice to drug-induced QT_c prolongation.[23]

Here, modeling in silico the effects on ventricular action potentials of the hyperkalemia in *Kcne2*$^{-/-}$ mice, the hyperkalemia was found to be a significant component in prolonging action potential duration and was predicted to depolarize the resting membrane potential of myocytes in both the ventricular apex and septum by 6.1 mV (from –78.7 to –72.6 mV; Figure 4D and 4E). Next, we recapitulated the previously observed lack of effects on baseline ventricular repolarization in 4m-old mice,[23] but examination of older mice (7m old) revealed an abnormally high-amplitude T wave and a striking delay in returning of the T wave to baseline (Figure 4F) quantified as an 85% increase in heart rate–corrected QT interval (QT_c) in *Kcne2*$^{-/-}$ mice when compared with age-matched wild-type littermates (n=8–15; Figure 4G). The age-dependent QT_c lengthening cannot readily be explained by a pure electric defect caused solely by loss of KCNE2 from ventricular ion channels and instead is consistent with a more complex pathogenesis involving chronic pathological changes such as

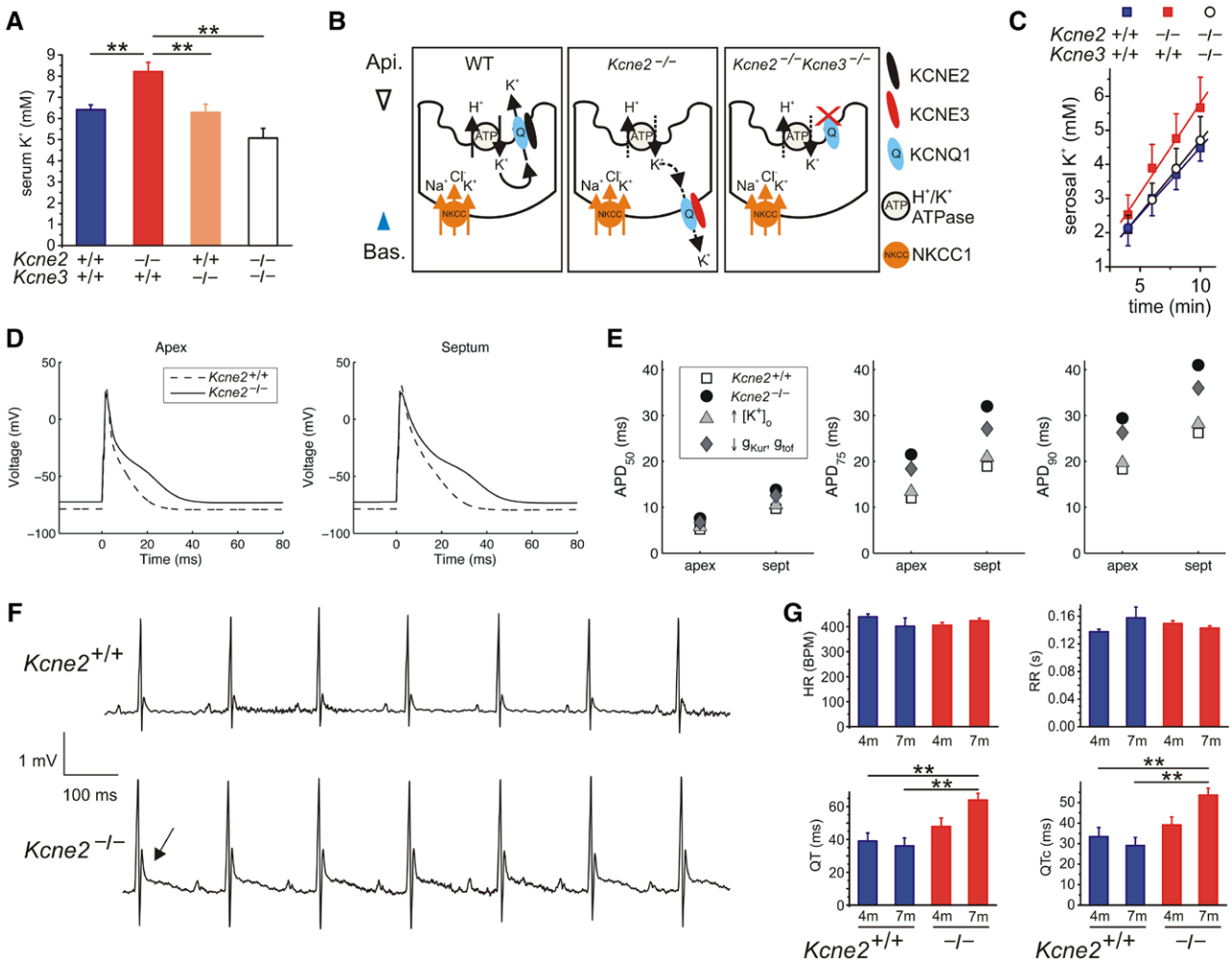

Figure 4. *Kcne2* deletion causes hyperkalemia and age-dependent QT_c prolongation. **A**, Mean serum [K^+] for 4m-old *Kcne2*$^{+/+}$*Kcne3*$^{+/+}$ mice (n=40) or mice lacking *Kcne2* (n=23), *Kcne3* (n=18), or both *Kcne2* and *Kcne3* genes (n=24) as indicated by –/–. ***P*<0.01. All other group comparisons showed *P*>0.05. **B**, Schematic[10] showing hypothesized short circuit K^+ current in the gastric parietal cells of *Kcne2*$^{-/-}$ mice (**middle**) when compared with wild-type mice (**left**), arising from the previously observed switch in KCNQ1 trafficking from apical (**left**, wild-type [WT]) to basolateral because of KCNE3 upregulation (**middle**).[10] In double-knockout *Kcne2*$^{-/-}$ *Kcne3*$^{-/-}$ mice, KCNQ1 is apical but probably nonfunctional because it lacks KCNE2 and KCNE3, which confer constitutive activation (**right**).[10] **C**, Mean K^+ flux across whole ex vivo histamine-stimulated stomach mucosae compared by quantifying the linear phase of serosal K^+ accumulation with a constant supply of K^+ to the luminal side. WT (*Kcne2*$^{+/+}$*Kcne3*$^{+/+}$) stomachs (n=8) were compared with those harvested from *Kcne2*$^{-/-}$ (n=9) and *Kcne2*$^{-/-}$ *Kcne3*$^{-/-}$ (n=8) mice. Slopes were not significantly different between genotypes. **D**, In silico models of *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mouse ventricle apex and septum myocyte action potentials generated using serum K^+ data (**A**) and our previous ventricular myocyte patch clamp recordings.[23] **E**, Time taken for 50%, 75%, and 90% repolarization (APD_{50}, APD_{75}, and APD_{90}, respectively) from models as in **D**, with either *Kcne2* deletion-linked myocyte conductance (reduced g_{Kur} and g_{tof}), or serum K^+ perturbation (increased $[K^+]_o$), or both (*Kcne2*$^{-/-}$), incorporated and compared with the *Kcne2*$^{+/+}$ simulation. **F**, Exemplar surface ECGs from 7m-old female *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice showing abnormal T wave (arrow) in the latter. **G**, Quantification of parameters from ECGs as in **F** showing age-dependent QT and QT_c prolongation in *Kcne2*$^{-/-}$ mice (n=8–15). ***P*<0.01. All other group comparisons *P*>0.05. APD indicates action potential duration; HR, heart rate; and RR, RR interval.

are observed in the cardiac remodeling associated with diabetes mellitus and hypercholesterolemia in human subjects and mouse models,[24,25] the molecular underpinnings of which can be detected as early as P21 in *Kcne2*$^{-/-}$ mice (Figure 1).

Kcne2 Deletion Generates an Electric Substrate for Postischemic SCD

The systemic defects reported above would be predicted to generate myocardial ischemia in aging mice or humans and, in combination with the profound QT_c prolongation (Figure 4G), would be expected to predispose to dangerous ventricular tachycardias (VTs). *Kcne2*$^{-/-}$ mice from heterozygous crosses exhibit hypothyroidism and cardiac hypertrophy at 12 to 15 months,[11] complicating attempts to correlate specific systemic abnormalities with cardiac events at these advanced ages. Therefore, here we instead standardized ischemia using surgery in 7m-old female mice from *Kcne2*$^{+/-}$ crosses that are euthyroid[11] and do not yet exhibit cardiac hypertrophy (Figure II in the Data Supplement) to compare the role of electric and other perturbations caused by *Kcne2* deletion in an ischemic context directly but without the pathological structural remodeling that occurs in these mice after 12 months because of hypothyroidism.[11]

ECG analysis during a 10-minute left anterior descending coronary artery ligation revealed similar ST elevation 7m-old

Kcne2$^{-/-}$ mice when compared with age-matched *Kcne2*$^{+/+}$ littermates (Figure 5A and 5B); myocardial extracellular signal-regulated kinase activation measured in hearts isolated after 20 minutes of reperfusion suggested similar ischemic insult in both genotypes (Figure 5C). Although two thirds of *Kcne2*$^{+/+}$ mice remained in sinus rhythm throughout reperfusion and the remainder exhibited monomorphic VT returning to sinus rhythm within 20 minutes, only 2/13 *Kcne2*$^{-/-}$ mice remained in sinus rhythm, the rest exhibiting VT. Most strikingly, in 4/13 *Kcne2*$^{-/-}$ mice, monomorphic VT degenerated into polymorphic VT, ventricular fibrillation, and SCD; this was not observed in *Kcne2*$^{+/+}$ mice (Figure 5D and 5E). In addition, 2 *Kcne2*$^{-/-}$ mice died during the ligation period, with ECGs consistent with acute heart failure (no fibrillation). These are not included in the analysis in Figure 5 because they did not reach the reperfusion stage.

Kcne2 Deletion Causes Fasting-Induced Ischemia, AV Block, and SCD

Because of the heightened risk of SCD in hypoglycemic diabetics, we repeated the ECG studies in a further cohort of 7m-old female mice that had been fasted overnight. Strikingly, fasted *Kcne2*$^{-/-}$ mice, but not *Kcne2*$^{+/+}$ mice, showed marked ST segment depression (Figure 6A and 6B), a hallmark of myocardial ischemia[26] (an additional *Kcne2*$^{-/-}$ mouse, but no *Kcne2*$^{+/+}$ mice, died suddenly during transfer from its cage before an ECG could be recorded). The acute ischemia was caused by overnight fasting because ST depression was not observed in fed *Kcne2*$^{-/-}$ mice (Figure 5A and 5B). Suggesting against a structural defect, cardiac hypertrophy was not detectable (Figure 6C), contrary to what is observed in *Kcne2*$^{-/-}$ mice at 12 to 15 months.[11] Overnight fasting further increased serum [K^+] in *Kcne2*$^{-/-}$ but not in *Kcne2*$^{+/+}$ mice (Figure 6D); this would be predicted to prolong the QT_c further, but accurate QT_c quantification was not possible because of the ST depression.

Although overnight fasting did not alter the outcome of coronary artery ligation in *Kcne2*$^{+/+}$ mice (Figure 6E and 6F), overnight-fasted *Kcne2*$^{-/-}$ mice showed significantly depressed ST height when compared with that of *Kcne2*$^{+/+}$ mice throughout ligation (Figure 6F), despite exhibiting equivalent cardiac extracellular signal-regulated kinase activation (Figure 6G). *Kcne2*$^{-/-}$ mice also developed severe AV block, which was not observed in the absence of fasting (Figure 6E, 6H, and 6I). This severity of AV block would strongly predispose to syncope and SCD in a human subject.[27] Notably, hyperkalemia, which was markedly exacerbated here in *Kcne2*$^{-/-}$ mice by overnight fasting, is associated with potentially lethal AV block, particularly in human subjects with existing factors causing myocardial ischemia.[28] Interestingly, hypoglycemia more typically results in hypokalemia, itself arrhythmogenic,[29] highlighting the degree of dysregulation occurring in multiple homeostatic systems because of *Kcne2* deletion. Indeed, *Kcne2* deletion seems to disrupt the renin–angiotensin–aldosterone system to the extent that normal homeostatic mechanisms are lost, probably because of chronic hyperkalemia in combination with diabetic dyslipidemia.

Summing all observed mortality in 7m-old mice immediately before or during ischemia/reperfusion experiments (30-minute duration) in this study, we observed a significant predisposition to sudden death in *Kcne2*$^{-/-}$ mice (8/23 *Kcne2*$^{-/-}$ mice versus 0/16 *Kcne2*$^{+/+}$ mice; P=0.008 by 1-tailed Fisher exact test).

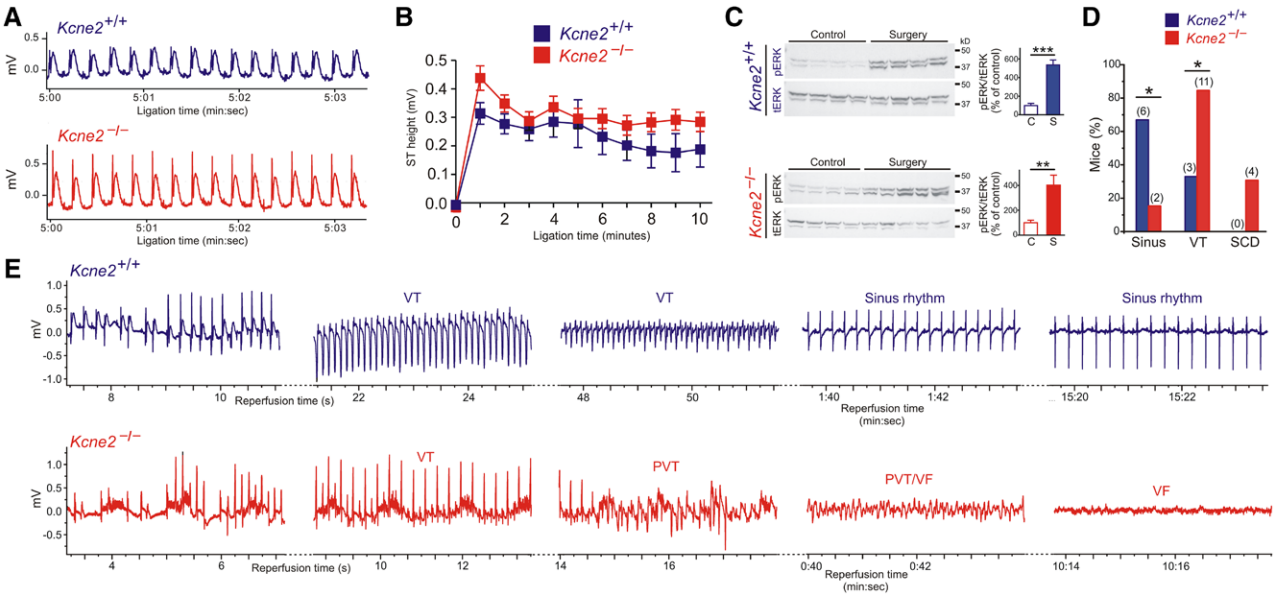

Figure 5. *Kcne2* deletion predisposes to sudden cardiac death (SCD) early in reperfusion. **A**, Representative ECGs recorded during coronary artery ligation of 7m-old female *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice (n=8–12). **B**, Mean ST heights from traces as in **A**, P>0.05 between genotypes at all time points (n=8–12). Groups were not significantly different (by repeated measures ANOVA). **C**, **Left**, Western blots of ventricular phosphorylated extracellular signal-regulated kinase (pERK) and total extracellular signal-regulated kinase (tERK) from mice as in **B**, isolated from control (nonoperated) mice or after ischemia/reperfusion (surgery); **right**, pERK/tERK protein band density measured from blots on left (n=4). **P<0.01, ***P<0.001 for control (C) vs surgery (S). **D**, Cardiac arrhythmia incidence and mortality during postischemia reperfusion in 7m-old female *Kcne2*$^{+/+}$ (n = 9) and *Kcne2*$^{-/-}$ (n=13) mice.. Numbers of animals per category are indicated in parentheses. *P<0.05 between genotypes. **E**, Exemplar ECGs of the most severe arrhythmias by genotype, recorded during postcoronary artery ligation reperfusion of 7m-old female *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice (n=9 to 13). *Kcne2*$^{+/+}$ mice always returned to sinus rhythm, unlike *Kcne2*$^{-/-}$ mice. PVT indicates polymorphic VT; Sinus, remained in sinus rhythm; VF, ventricular fibrillation; VT, ventricular tachycardia.

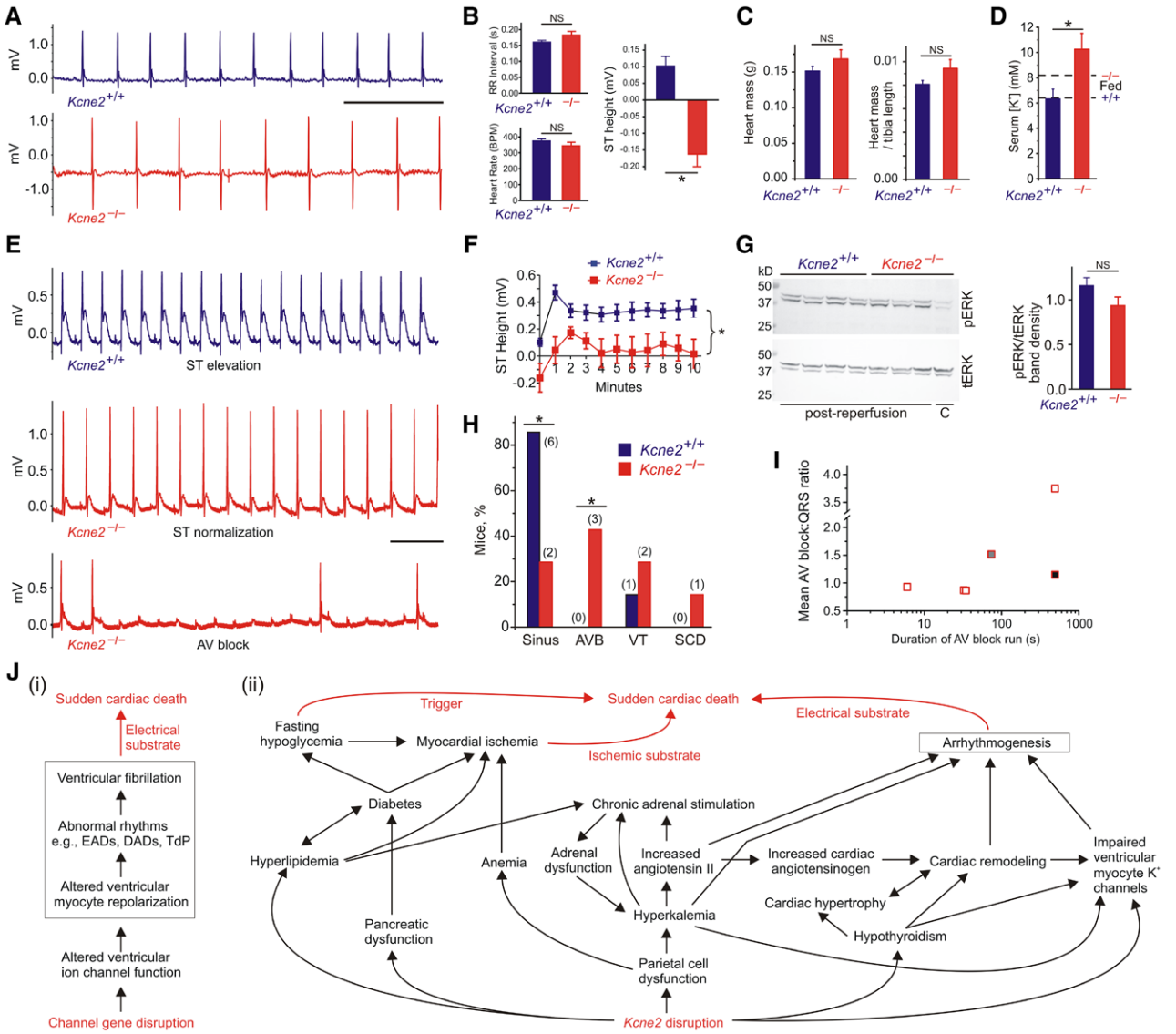

Figure 6. *Kcne2* deletion causes fasting-dependent ischemia and AV block during ischemia. **A**, Representative ECGs recorded from overnight-fasted 7m-old female *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice (n=7). Scale bar, 500 ms. **B**, Mean ECG parameters measured from traces recorded from overnight-fasted mice as in **A** (n=7). *P<0.05. **C**, Mean heart mass (absolute and normalized to tibia length) for mice as in **B**. **D**, Mean postreperfusion serum [K$^+$] for fasted 7m-old female *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice (n=7). *P<0.05. Values for fed mice (dashed lines) from Figure 4A are shown for comparison (n=23–40). **E**, Representative ECGs recorded during coronary artery ligation of overnight-fasted 7m-old female *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice (n=7). Scale bar, 500 ms. **F**, Mean ST heights from traces as in **E**, *P<0.05 between genotypes by repeated measures ANOVA (n=7). **G. Left**, Western blots of ventricular phosphorylated extracellular signal-regulated kinase (pERK) and total extracellular signal-regulated kinase (tERK) from mice as in **B**, isolated from control (C, nonoperated) mice or after ischemia/reperfusion (postreperfusion); **right**, pERK/tERK protein band density in postreperfusion mouse ventricles measured from blots on left (n=3–4). **H**, Cardiac arrhythmia incidence and mortality during ischemia and postischemia reperfusion in 7m-old female *Kcne2*$^{+/+}$ and *Kcne2*$^{-/-}$ mice as in **F** (n=7). Numbers of animals per category are indicated in parentheses. *P<0.05. **I**, Mean AV block:QRS ratio plotted vs duration of runs of AV block for the 3 *Kcne2*$^{-/-}$ mice summarized in **H** that exhibited AV block. Each shade indicates a different mouse; 1 mouse (□) exhibited 4 distinct runs of AV block. **J**, (i) Existing model for the mechanism of monogenic predisposition to sudden cardiac death (SCD). (ii) Multifactorial pathogenesis of *Kcne2*-associated arrhythmogenesis and SCD supported by the findings herein. DADs indicates delayed after-depolarizations; EADs, early after-depolarizations; NS, no significant difference; Sinus, remained in sinus rhythm; TdP, torsades de pointe; and VT, ventricular tachycardia then recovery to sinus.

Discussion

The current model for monogenic arrhythmogenesis (Figure 6J, i) relies heavily on a myocentric mechanism, but given the new findings for KCNE2, this may be too simplistic a model, which ignores the extracardiac effects of disrupting arrhythmia-susceptibility genes, the large majority of which are also expressed outside the heart. Although extrapolation of mouse models to human disease must be conducted with extreme caution, 35% of *Kcne2*$^{-/-}$ mice in the present study succumbed to sudden death immediately before, during, or after surgery to induce transient ischemia, supporting the hypothesis that more subtle single-allele effects of human *KCNE2* mutations could be significant when considering large populations. We suggest a new model for KCNE2-linked arrhythmogenesis in mice, elements of which may be applicable to some human arrhythmias (Figure 6J, ii), and aspects of

which are likely to apply to other broadly expressed arrhythmia-susceptibility genes, especially KCNQ1, which partners KCNE1, 2, and 3 in a variety of epithelial cell types.[7]

KCNQ1 gene variants are strongly linked to type II diabetes mellitus,[30] yet the mechanism has not been delineated. Nor has a dissection of the potential arrhythmogenic contribution of KCNQ1-linked diabetes mellitus in humans, or systemic effects of *Kcnq1* deletion in mice, been reported, to our knowledge (although *Kcne1* disruption has been shown to affect K^+ homeostasis in mice, with the suggestion that this could contribute to arrhythmogenesis).[31] However, a recent study showed that hypergastrinemia, a marker of parietal cell dysfunction, correlated well with QT_c prolongation in *KCNQ1*-linked Jervell and Lange–Nielsen Syndrome.[32] Interestingly, we previously found that even single-allele disruption of *Kcne2* causes hypochlorhydria in mice, intermediate between wild-type mice and the achlorhydria we found in *Kcne2*$^{-/-}$ mice.[9] Furthermore, we previously found that the gastric effects of *Kcne2* deletion worsened with age,[10] providing indirect support for the hypothesis that extracardiac effects of *Kcne2* disruption could contribute to age-dependent worsening of the cardiac phenotype. KCNQ1 and KCNE1 are also reportedly expressed in neuronal networks and the brain stem, and KCNQ1-linked seizures suggested as being arrhythmogenic, potentially explaining some cases of sudden unexplained death in epilepsy[33]; an analogous situation was recently reported for Rett syndrome.[34]

Diabetes mellitus, hypercholesterolemia, and anemia all ultimately reduce myocardial oxygen supply, leading to myocardial ischemia, and are recognized risk factors in SCD, especially in combination. In addition, nocturnal hypoglycemia in diabetics predisposes to SCD; cardiac events in overnight-fasted *Kcne2*$^{-/-}$ mice reported here provide insights into the type of catastrophic cardiac events that might lead to the dead-in-bed syndrome form of SCD in a hypoglycemic diabetic. Particularly striking is that a single gene disruption provides the systemic substrate (diabetes mellitus, hypercholesterolemia, elevated angiotensin II), the electric substrate (impaired ventricular repolarization caused by direct ventricular channel subunit loss, hyperkalemia, and probably remodeling in response to the elements of the ischemic substrate), and also a trigger (hypoglycemia, causing in this case acute ischemia, acute exacerbation of hyperkalemia, and probably AV block).

Although results obtained from knockout mice cannot be directly extrapolated to explain human disease states, the AV block observed in fasted *Kcne2*$^{-/-}$ mice suggests parallels to arrhythmogenesis mechanisms linked to increased mortality in large-scale studies of diabetes mellitus[35] and thought to underlie nocturnal SCD in nondiabetics.[36] ST depression (as we observed in fasted *Kcne2*$^{-/-}$ mice) was also observed in type 2 diabetics in whom hypoglycemia was induced by insulin infusion.[37] The general consensus is that there must be an underlying genetic component involving a ventricular ion channel in dead-in-bed syndrome to provide an electric substrate in addition to systemic substrates and the trigger (eg, hypoglycemia).[20] We now show that a single gene disruption can underlie all of these factors, at least in a mouse model.

One of the mysteries surrounding monogenic arrhythmia syndromes has been why some individuals die young from SCD, whereas others live without incident into advanced age. Our findings suggest that lifestyle can play a more important role than could be rationalized solely by considering the myocentric arrhythmogenesis mechanism. Individuals who maintain a healthy diet and exercise regimes, for example, would be expected to delay the systemic effects of a pathological *KCNE2* gene variant and thus minimize the ischemic substrate while also diminishing aspects of the electric substrate and potentially eliminating the hypoglycemic trigger. It would require long-term study of a large cohort of individuals to determine whether any aspects of the *KCNE2*-linked multisystem arrhythmogenesis model apply in human arrhythmias. Rare *KCNE2* mutations were recently reported in 2 independent postmortem studies of human sudden unexplained death, but underlying mechanisms were not elucidated. Indeed, rigorous investigation of the mechanisms involved in such human sudden unexplained death cases would be incredibly challenging. In these studies, 4 mutations were found: in 3 cases, the sentinel event was sudden unexplained nocturnal death, and in the other, the sentinel event was syncope during exertion.[38,39] Interestingly, the predicted KCNE2 protein mutation, T10M, of this latter case was similar to that in a patient we previously reported in whom the sentinel event was also syncope and who subsequently experienced ventricular fibrillation after exertion.[40] Unfortunately, many studies of this nature preclude the patients who could be affected by a multisystem syndrome such as we demonstrate in *Kcne2*$^{-/-}$ mice. This is because individuals exhibiting structural heart disease, diabetes mellitus, and hyperlipidemia are often excluded from arrhythmia genetic association studies because these conditions are, understandably, considered as confounding variables, rather than potential manifestations of the channel gene sequence variant.

In summary, new findings from the *Kcne2*$^{-/-}$ mouse suggest that a more holistic view of the pathogenesis of monogenic cardiac arrhythmias could provide a clearer picture of the mechanisms underlying SCD if results from this mouse model are found to recapitulate aspects of human cardiac arrhythmogenesis. This could include enhancing the ability to recognize individuals with an inherited predisposition to SCD, particularly in the context of diabetes mellitus, and to design more effective avoidance, management, and therapy strategies that lessen the overall SCD substrate and reduce the probability of a triggering event. Strategies might involve routine genetic screening of diabetics for pathological single-nucleotide polymorphisms in known monogenic arrhythmia genes (particularly KCNQ1 and KCNE2), especially before embarking on aggressive glycemic control regimens.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Sudden cardiac death (SCD) is a leading cause of mortality, thought to require both an ischemic and electric substrate and perhaps an additional trigger. Recognizing genetic and environmental risk factors that predispose to SCD will reduce mortality by facilitating early prevention and therapeutic strategies. Genetic variants within genes encoding ion channel subunits (including *KCNE2*) are known to underlie ventricular arrhythmias that can provide the electric substrate for SCD. The established paradigm is that these gene mutations directly impair the function of ventricular myocyte ion channels, and that other, unrelated substrates or triggers superimposed on this electric substrate can lead to SCD. We now show in a mouse model that genetic disruption in the *KCNE2* gene causes a multisystem syndrome that can provide both the electric and ischemic substrates, and the trigger, for SCD. In human populations, polymorphisms in *KCNE2* and other ion channel genes predispose to inherited and drug-induced long-QT syndrome, but extracardiac pathologies arising from the same sequence variants, and their potential role in cardiac and extracardiac diseases, have been largely overlooked. Indeed, polymorphisms in the human *KCNQ1* locus, which encodes a potassium channel regulated by *KCNE2*, are strongly linked to both diabetes mellitus and long-QT syndrome, but whether superimposition of multiple *KCNQ1*-linked disorders predisposes to SCD is not known. Our findings suggest that consideration of the extracardiac pathologies potentially caused by mutations in known arrhythmia genes will lead to a fuller understanding of inherited predisposition to SCD and ultimately to improved early detection and prevention strategies.