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Rapid communication

Kcne2 deletion promotes atherosclerosis and diet-dependent sudden death

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ABSTRACT

Coronary artery disease (CAD) is the leading cause of death worldwide. An estimated half of cases involve genetic predisposition. Sequence variants in human *KCNE2*, which encodes a cardiac and epithelial K^+ channel β subunit, cause inherited cardiac arrhythmias. Unexpectedly, human *KCNE2* polymorphisms also associate with predisposition to atherosclerosis, with unestablished causality or mechanisms. Here, we report that germline *Kcne2* deletion promotes atherosclerosis in mice, overcoming the relative resistance of this species to plaque deposition. In female western diet-fed mice, *Kcne2* deletion increased plaque deposition >6-fold and also caused premature ventricular complexes and sudden death. The data establish causality for the first example of ion channel-linked atherosclerosis, and demonstrate that the severity of *Kcne2*-linked cardiac arrhythmias is strongly diet-dependent.

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1. Introduction

CAD results in higher mortality in the United States and globally each year than any other single cause of death. An estimated half of CAD cases involve genetic predisposition, yet it is predicted that reduction of other risk factors could reduce CAD mortality and morbidity by >30%. It is therefore crucial to develop more comprehensive prevention and treatment strategies for both genetic and environmental risk factors for CAD, necessitating a fuller mechanistic understanding of CAD [1].

Another form of fatal heart disorder, Sudden Cardiac Death (SCD), accounts for ~1000 deaths per day in the United States. SCD is proposed to require an electric substrate, an ischemic substrate, and perhaps a trigger [2]. Despite significant advances in our mechanistic understanding of SCD, there is still much to learn. Because most of the 25 genes linked to SCD also serve roles outside the heart, the possibility arises that even monogenic forms of SCD may involve complex, multisystem disease pathogenesis not confined to direct dysfunction of cardiac myocyte electrical activity.

Many SCD-linked genes encode ion channel pore-forming (α) subunits, but the rest encode proteins that regulate them [2]. KCNE2 (which we originally named MiRP1) is a relatively promiscuous, single-transmembrane span ion channel β subunit best known for its ability to co-assemble with and alter the trafficking and functional properties of voltage-gated potassium (Kv) channels [3]. Human KCNE2 mutations that result in impaired function of ventricular myocyte Kv channels are linked to cardiac arrhythmias including Long QT syndrome (LQTS) [3], which predisposes to ventricular fibrillation and SCD.

In addition to the heart, KCNE2 is expressed in a variety of secretory epithelia [4–6], raising the possibility of broader effects of human *KCNE2* disruption. Indeed, a SNP near the human *KCNE2* locus was found to be associated with early onset [7], prevalence, and subsequent mortality [8] of myocardial infarction (MI); a different SNP within the human *KCNE2* gene itself was associated with predisposition to CAD [9]. These findings suggested the possibility of an unanticipated causal link between *KCNE2* disruption and CAD/MI, which we investigated here using germline *Kcne2* deletion in mice.

2. Methods

All mice were housed in pathogen-free facilities and the study was approved by the Animal Care and Use Committee at University of California, Irvine, in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The $Kcne2^{-/-}$ mouse line was generated as we previously described [5], and mice used in this study were bred by crossing $Kcne2^{+/-}$ mice which had been backcrossed >10 times into the C57BL/6 strain. After being genotyped and weaned at 3 weeks of age, pups were assigned to, and maintained on, either a control diet (2020X, Harlan, 16% kcal from fat; 19.1% protein, 2.7% crude fiber, 12.3% neutral detergent fiber and 0% cholesterol) or western diet (TD.88137, Harlan, 42% kcal from fat, >60% of which is saturated; 34% sucrose; 0.2% cholesterol). Detailed methods appear in the online supplement.

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3. Results

3.1. Kcne2 deletion promotes atherosclerosis

We first quantified the effect of *Kcne2* deletion on plaque deposition as a percentage of aortic surface area in mice fed regular mouse chow (normal diet), or a high-fat, high-cholesterol, high sugar (western) diet, comparing $Kcne2^{+/+}$ and $Kcne2^{-/-}$ mice matched for age and sex. Plaques were visible in the aorta of $Kcne2^{-/-}$ mice fed a western diet, suggesting a predisposition to atherosclerosis (Fig. 1A, B). We confirmed this by *en face* quantification of Sudan IV-stained plaques in the aorta of 5–7 month-old mice. Plaque deposition was relatively low in all mice fed a control diet. Nevertheless, control diet-fed female and male $Kcne2^{-/-}$ mice exhibited 10.2-fold ($p = 0.035$) and 2.5-fold ($p = 0.1$) increases in mean plaque surface area, respectively, at 5–7 months of age, compared to $Kcne2^{+/+}$ mice. In mice fed a western diet, *Kcne2* deletion strikingly increased plaque deposition, 6.2-fold ($p = 8 \times 10^{-6}$) in females and 3.2-fold ($p = 0.049$) in males, compared to $Kcne2^{+/+}$ mice (Fig. 1C, D).

3.2. A western diet potentiates the effects of Kcne2 deletion on arrhythmogenesis and mortality

Human *KCNE2* gene variants cause LQTS [3], and *Kcne2* deletion lengthens the QTc interval in aging mice fed a normal diet [4]. In adult mice, *Kcne2* deletion prolongs the QTc because of loss of function of Kv4.2 and Kv1.5 channels, which Kcne2 normally regulates in mouse ventricles [10]. We recently found that *Kcne2* deletion raises serum LDL cholesterol [4], a likely contributory factor in the *Kcne2*-dependent atherosclerosis we describe here. Interestingly, ventricular Kv1.5 current is downregulated by elevated cholesterol [11], suggesting the potential for synergism between *Kcne2* deletion and a western diet in arrhythmogenesis. Accordingly, here we discovered particularly prominent effects of *Kcne2* deletion on arrhythmogenesis and sudden death in female mice fed a western diet. In $Kcne2^{+/+}$ mice, and in male $Kcne2^{-/-}$ mice, the western diet had negligible effects on mortality during the study. In contrast, 4/7 western diet-fed female $Kcne2^{-/-}$ mice died suddenly by the age of 15 weeks. In normal diet-fed $Kcne2^{-/-}$ mice, the first mortality was observed at 18 weeks (Fig. 2A).

Electrocardiographic analysis of surviving mice revealed that *Kcne2* deletion increased the QTc interval in male mice on a western diet, and in female mice on a control diet, the latter recapitulating our previous findings [4,10] (Fig. 2B, C). The western diet did not substantially extend the already-long QTc in female $Kcne2^{-/-}$ mice, but it induced frequent premature ventricular complexes (PVCs) in all 3 female mice still alive for testing beyond 15 weeks, at a mean frequency of 4 PVCs per 100 beats (Fig. 2B–E). The PVCs were typically immediately followed by full compensatory pauses that were double the length of the preceding R–R interval (Fig. 2E, blue arrows), highly characteristic of PVCs observed on human ECGs. In contrast, PVCs were not observed in any of the other groups (Fig. 2B, D), nor in our previous studies of normal diet-fed $Kcne2^{-/-}$ mice [4,10].

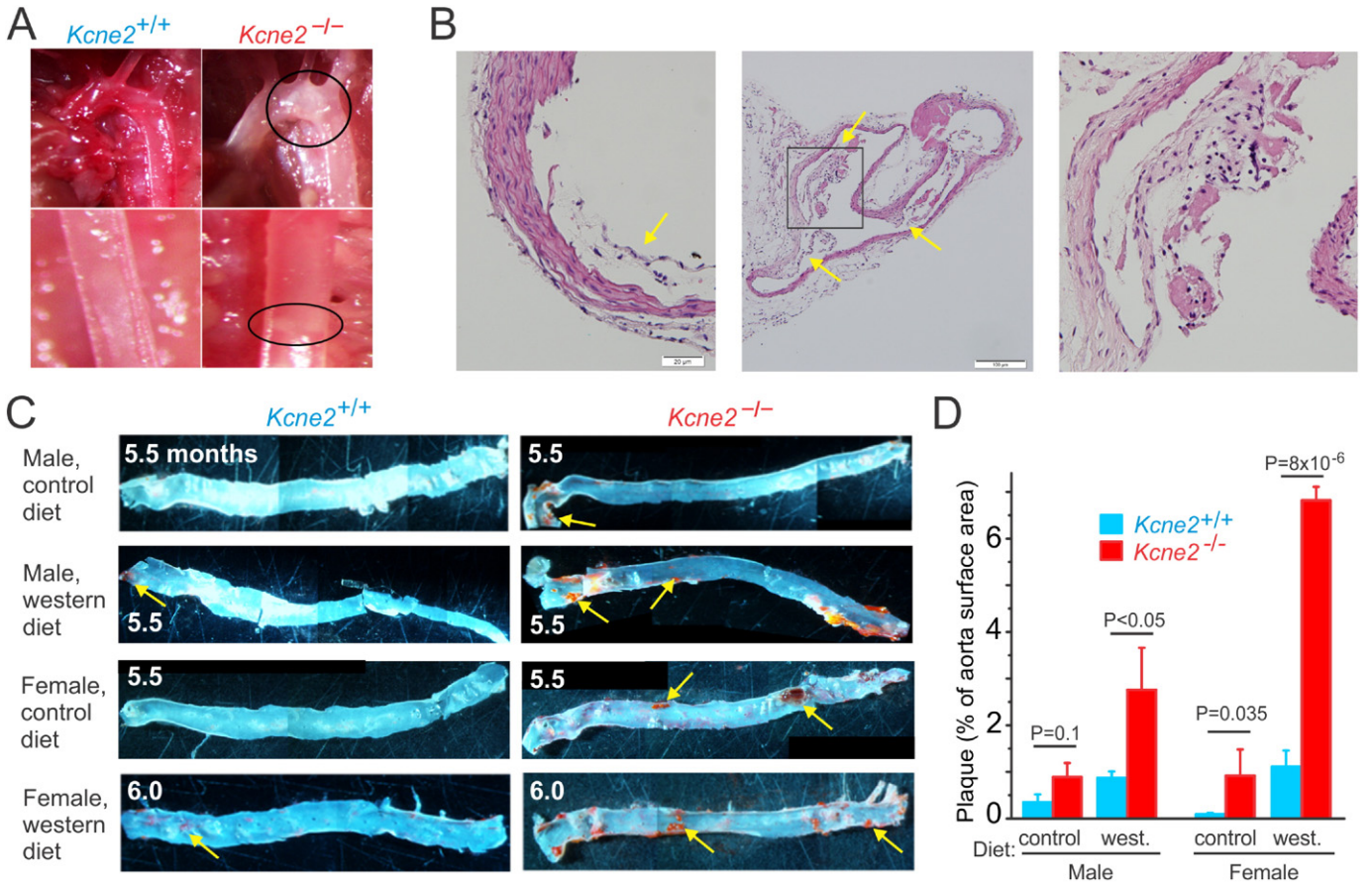

Fig. 1. *Kcne2* deletion promotes atherosclerosis. A. External images of 9–10 month-old, western diet-fed $Kcne2^{+/+}$ (*left*) and $Kcne2^{-/-}$ (*right*) aortic arch (*upper*) and descending aorta (*lower*). Plaque deposition is indicated by black rings. B. H & E-stained aorta (left, scale bar 20 μm) and aortic branch (center, scale bar 100 μm; close-up of boxed region on right) showing plaques (*yellow arrows*) in a 6-month-old female western diet-fed $Kcne2^{-/-}$ mouse. C. Representative images of aortic plaques (*yellow arrows*), visualized with Sudan IV solution, from mice between 5.5 and 6 months of age; genotype, diet, age and sex as indicated. Each aorta image was prepared by digital splicing of panoramic series captured with a dissection microscope-mounted digital camera. D. Quantification of the mean ± SEM percentage of 5–7-month-old mouse aorta surface area covered by plaque as assessed by Sudan IV staining. $n = 5–7$, male control diet; 6–8, male western diet; 4–7, female control diet; 3–6, female western diet; p values are for 1-tailed, unpaired t-tests for inter-genotype comparisons within equivalent sex and diet groups.

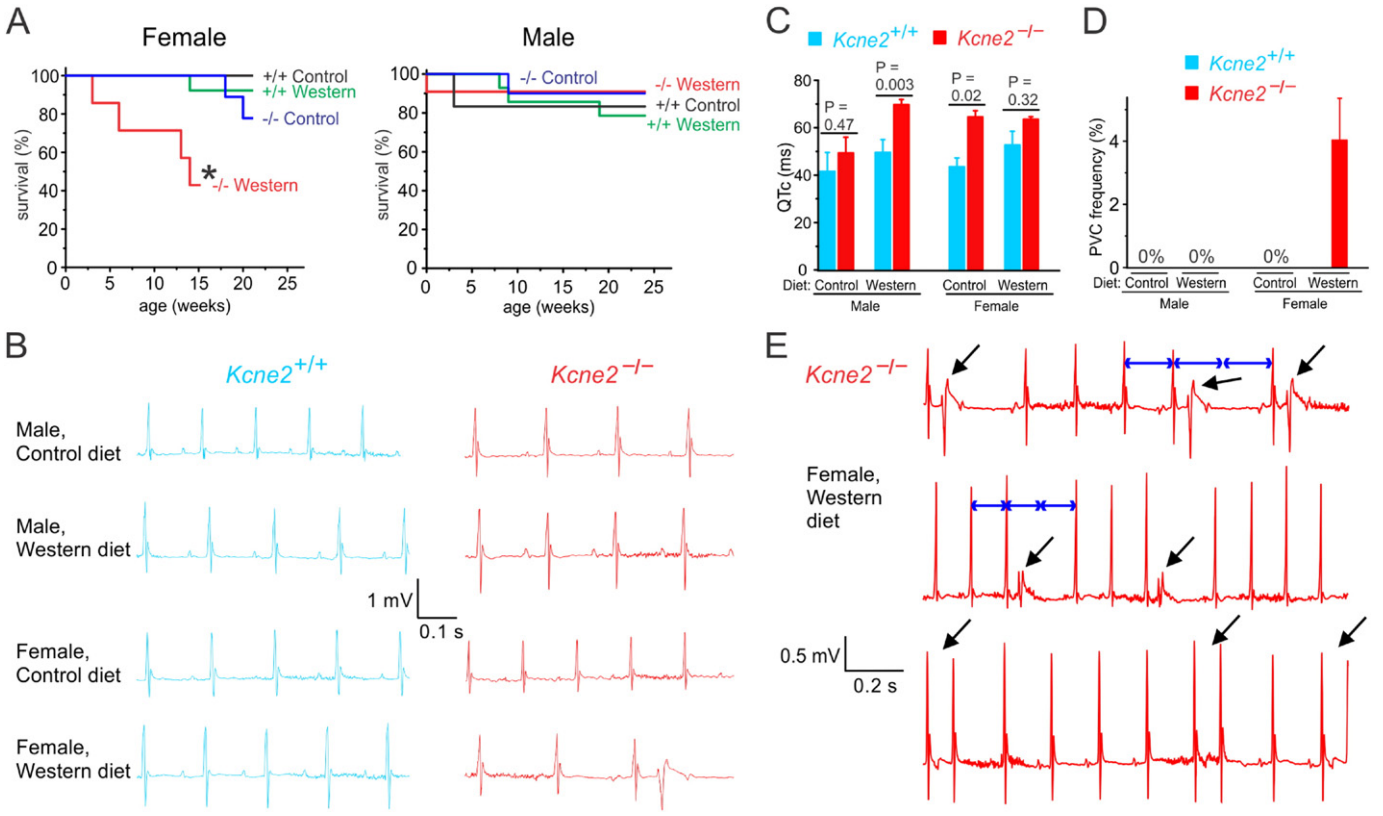

Fig. 2. *Kcne2* deletion causes diet- and sex-dependent premature ventricular complexes and sudden death. A. Effects of *Kcne2* deletion and a western diet on % survival of female (n = 7–13) and male (n = 6–14) mice. Log rank test of survival rates: * female $Kcne2^{-/-}$ western diet versus female $Kcne2^{+/+}$ control diet survival (p = 0.0028), versus female $Kcne2^{+/+}$ western diet survival (p = 0.018), and versus male $Kcne2^{-/-}$ western diet survival (p = 0.034); all other comparisons p > 0.05. B. Representative ECGs recorded from control- or western-diet-fed $Kcne2^{+/+}$ and $Kcne2^{-/-}$ mice. C. Mean (±SEM) QTc values quantified from ECGs as in panel B (n = 3–8 mice per group). D. Mean premature ventricular complex (PVC) incidence quantified for all groups, showing 4% (4 PVCs per 100 beats) for female western diet-fed $Kcne2^{-/-}$ mice and 0% incidence for all other groups. E. Representative ECG segments for all three western-diet-fed $Kcne2^{-/-}$ female mice that survived sufficiently long for ECG recordings. Black arrows: PVCs; blue arrows, spacers demonstrating a full compensatory pause following PVCs.

4. Discussion

Our findings demonstrate for the first time that genetic disruption of an ion channel subunit can cause atherosclerosis, and support recent human genetic studies in which polymorphisms in or near the *KCNE2* locus were associated with increased predisposition to atherosclerosis and early-onset myocardial infarction [7–9]. Mice are inherently resistant to development of atherosclerosis. To our knowledge, only two other single-gene knockout mouse lines ($Apoe^{-/-}$ and $Ldlr^{-/-}$) develop spontaneous atherosclerotic lesions, the Apolipoprotein E gene (*Apoe*) knockout being the most robust example [12].

Future studies will be targeted toward delineating the mechanistic basis for KCNE2-linked atherosclerosis, now that we have established causality. We recently found that *Kcne2* deletion raises serum LDL and impairs glucose tolerance, by unknown mechanisms [4]. *Kcne2* deletion also causes hypothyroidism, which can predispose to hyperlipidemia [6], and elevates serum Angiotensin II [4], which can also cause vascular injury. We suspect that *Kcne2* deletion results in a spectrum of factors, each either impairing lipid or carbohydrate metabolism, or favoring plaque development through other mechanisms.

Premature mortality and PVCs occurred here only in female $Kcne2^{-/-}$ mice fed a western diet. The increased susceptibility for *Kcne2*-linked atherosclerosis, PVCs and premature mortality in female versus male mice we observed may be linked to the positive estrogen dependence of KCNE2 expression, which is presumed to make females more reliant than males upon KCNE2 [13]. When K^+ channel loss-of-function delays ventricular repolarization to the extent that an action potential fires during phase 2 or 3 of the previous action potential, early afterdepolarizations (EADs) and, in turn, PVCs can arise. While human PVCs are in general not particularly uncommon and often benign, when occurring in combination with LQTS they are highly problematic as they promote potentially lethal polymorphic ventricular tachycardias including *torsades de pointes*. It is possible that PVCs contributed to premature mortality in western diet-fed female $Kcne2^{-/-}$ mice; alternatively, the PVCs could represent solely an electrocardiographic marker of their CAD. Human PVCs are associated with increased risk of coronary heart disease [14] and incidence of SCD [15].

In conclusion, we demonstrate for the first time that disruption of an ion channel gene can cause atherosclerosis. Our preliminary findings suggest that, in addition to avoidance of medications known to prolong the QT interval, diet management may be of paramount importance in individuals harboring potentially pathogenic *KCNE2* sequence variants.

Disclosure statement

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yjmcc.2015.08.013.

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