SUPPLEMENT MATERIAL

Methods:

Creation of the N629D mice.

mERG genomic DNA spanning intron 5 to exon 15 [1] was used to construct a targeting vector containing a pol II-driven neomycin resistance cassette flanked by loxP sites which was cloned into intron 7 and the N629 residue in exon 7 was mutated to D (Online Figure I A). The targeting construct was assembled in lambda2TK [2] . The arms of the phage-targeting vector were removed via NotI digestion. The targeting vector was introduced into 5 -7 x 10^6 mouse R1 embryonic stem (ES) cells by electroporation (Bio-Rad Gene Pulser). Positive and negative selections were carried out using 300 U of Geneticin (G418)/ml and $2X^{10-7}$ M 1-(2-deoxy-2-fluoro-D-arabinofuranosyl)-5-iodouracil, respectively. ES cell clones containing the neomycin cassette were identified by Southern blot analysis with a 3' probe external to the ERG genomic DNA present in the vector and was confirmed by a 5' probe. The karyotype of targeted ES cell clones was determined to verify diploid status. Three targeted ES cell clones were injected into 3.5-day-postcoitum (dpc) C57BL/6 blastocysts. One male chimera with 60% agouti pigmentation was bred to C57BL/6 females, and agouti pups were genotyped to confirm germ line transmission.
The genotypes from the F1 and F2 generation were determined by Southern blotting, while genotypes of all subsequent mating were determined by PCR.

For PCR analysis, oligonucleotides for common forward (5'-CATCGGCTGGCTGCACAAC-3'), wild type reverse (5'-GCCAACCTTCACCATTTCCT-3') and mutant (neo cassette) reverse (5'-GGAATGGGTTGGTAGAGCGA-3') were used in a 3 primer assay. PCR conditions were as follows: denaturation at 95°C for 1 min followed by 30 cycles of 1 min at 94°C, 1 min at 60°C, and

1 min at 72°C. Analysis thus far has been carried out on the hybrid C57BL/6–129-SV/J background. To remove the neomycin cassette, N629D mice were mated to Mox2-Cre mice.

To identify the neomycin cassette -removed mice, we synthesized a reverse primer (5'-ACGCCAACCTTCACCATCGA-3') that incorporated an intron7 Cla1 site created for neo cassette insertion. This was used for PCR in conjunction with the above common forward primer. The product was sequenced to confirm the existence of the N629D mutation and removal of the neo-cassette.

Intracellular calcium measurement

Fluo-4 AM (Molecular Probes, Invitrogen Inc.) was dissolved at a concentration of 1 mmol/L in 20% Pluronic F127 in dimethylsulfoxide (Molecular Probes, Invitrogen Inc.). 1-1.5 µL of this solution was added to 1 mL of cell culture solution containing a glass coverslip with attached cells. Myocytes were loaded for 30 min at 37ºC then the coverslip was placed on the chamber of an inverted microscope (Axiovert 200, Carl Zeiss Inc.) and perfused with Tyrode's solution at 37ºC. At least 10 min were allowed for de-esterification of the dye. Light from a 100 W mercury arc lamp (HBO 100, Carl Zeiss Inc.) was filtered to give excitation at 488 nm (10 nm bandwidth). Emission light was passed through a 500 nm long-pass beamsplitter and a 525 nm bandpass filter (50 nm bandwidth) before being collected by a photomultiplier tube (PTI Inc.). The voltage output from the PMT was digitized and recorded using pCLAMP 9 software (Axon Instruments, Molecular Devices) to allow simultaneous measurement of membrane potential and calcium signals.

For each cell under study, an adjustable rectangular diaphragm in the emission lightpath was used to mask off light from any other cells or debris in the optical field. Background fluorescence (F_B) was measured by moving the cell of interest outside this aperture. Cellular autofluoresence (F_{Auto}) was estimated to be $1.04 \times F_B$ in separate experiments using unloaded cells. Minimum fluorescence (F_0) was obtained for each cell by perfusing with Ca-free Tyrode's solution containing 1 mM EGTA. Fluorescent signals were expressed as $(F-F_{Auto})/(F_0-F_{Auto})$. If it is assumed that the intracellular Ca concentration in the presence of 1 mM EGTA is the same for WT and N629D cells, then valid comparisons can be made between these cell types in terms of their Ca transient characteristics, without further conversion into absolute $[Ca^{2+}]_i$ (which is difficult for non-ratiometric dyes such as Fluo-4). F_0-F_{Auto} was no different between the two cell types (WT 0.84±0.04 V *vs* N629D 0.89±0.06 V, p=0.5).

Rapid application of 10 mM caffeine was achieved using a custom-built heated solution switcher whose tip was placed 2-3 mm from the cell.

Results:

Online Figure I - Creation of mERG- N629D Mice. Panel A. The targeting vector and the strategy to create the recombinant mouse is shown. A pol II-driven neomycin cassette flanked by loxP sites was cloned into intron 7 and the N629 residue in exon 7 was mutated to D. Subsequent mating to Mox2-Cre mice deleted the neo-cassette insert. **Panel B**. The genotypes at different stage of development are shown. The frequency of the genotypes at E8.5 to 11 versus post natal day 1 (P1) embryos were compared. Note that no N629D/N629D mice (-/-) are observed at P1.

Online Figure II - Mean action potential characteristics are related to the characteristics of the paired recordings of I_{Kr} in each cell. Resting membrane potential (RMP) data, on the left, and action potential duration (APD_{90}) data on the right are related to genotype and to the I_{Kr} phenotype. * indicates a statistically significant difference $p<0.01$ compared to +/+.

Online Figure III. Representative examples of the cellular distribution of the mERG proteins were evaluated in isolated cardiac myocytes show at lower power magnification. The left hand panel shows +/+ protein which is dominantly expressed in the plasma-lemma. The right hand panel shows that the N629D/N629D protein is dominantly expressed intracellularly. The middle panel shows the mERG proteins in heterozygous cells manifesting a spectrum of patterns of protein expression.

Online Videos

1) Shows *in vitro* microscopy of the +/+ embryo with normal heart beating.

2) Shows *in vitro* microscopy of the N629D/N629D embryo showing abrupt asystolic episodes, irregular beating patterns and no propulsive flow into or out of the N629D/N629D embryos.

3) Echocardiographic assessment of *in situ* embryos at E9.5 in +/+ embryos showing normal and regular heart beat without pauses.

4) Echocardiographic assessment of *in situ* embryos at E9.5 in N629D/N629D embryos showing abrupt pauses.

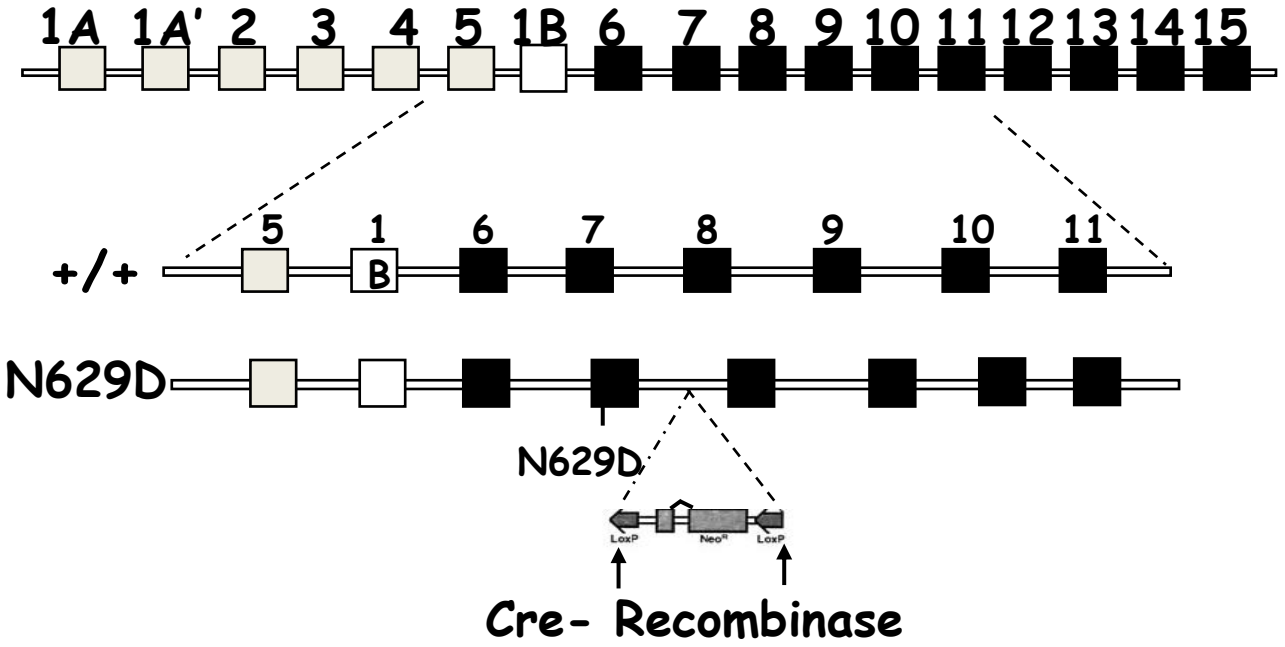
References

1. London B, Trudeau MC, Newton KP, Beyer AK, Copeland NG, Gilbert DJ, Jenkins NA, Satler CA, Robertson GA. Two isoforms of the mouse ether-a-go-go-related gene coassemble to form channels with properties similar to the rapidly activating component of the cardiac delayed rectifier K+ current. *Circ Res*. 1997;81:870-8.

2. Lees-Miller JP, Guo J, Somers JR, Roach DE, Sheldon RS, Rancourt DE, Duff HJ. Selective knockout of mouse ERG1 B potassium channel eliminates I(Kr) in adult ventricular myocytes and elicits episodes of abrupt sinus bradycardia. *Mol Cell Biol*. 2003;23:1856-62.

Online Figure I

A



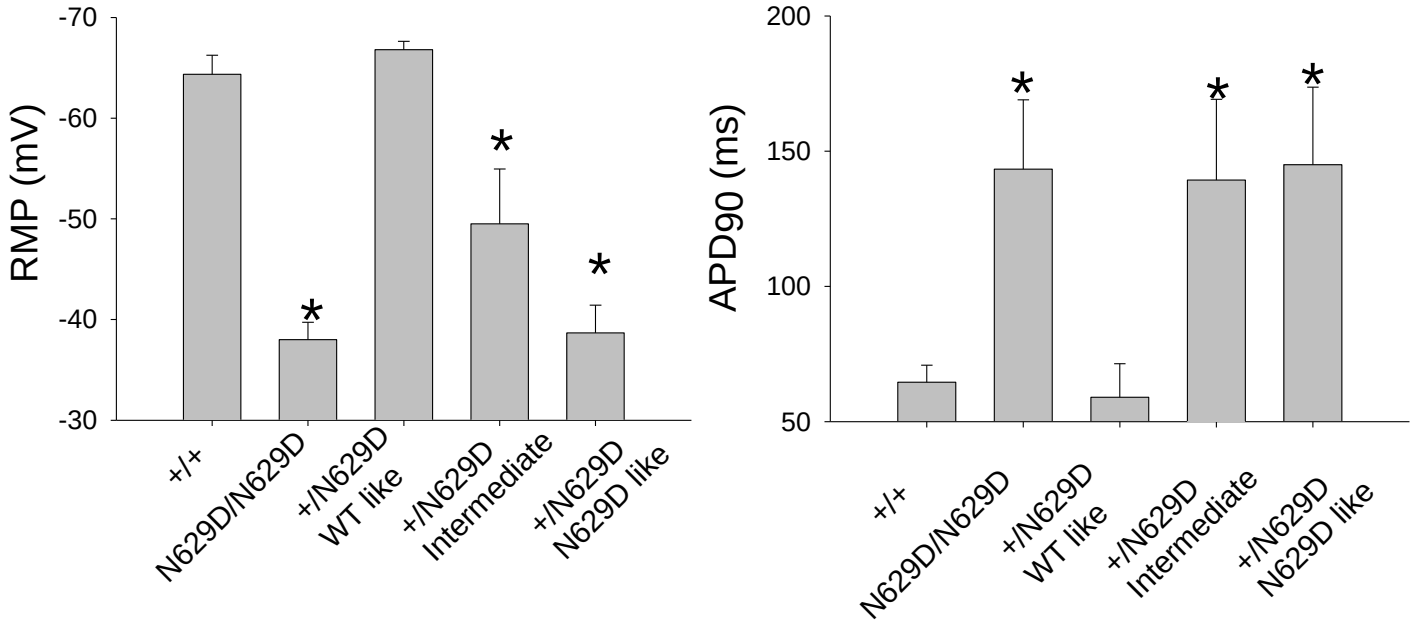
B

Genotype of frequency of embryos from N629D heterozygote intercrosses

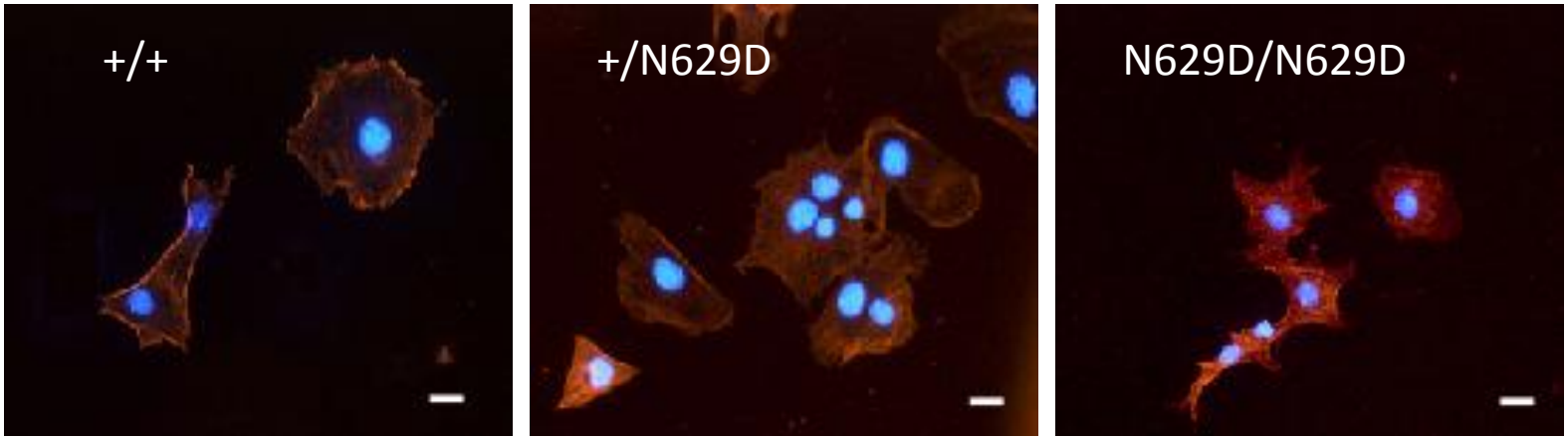
Stage	Litters	Total	+/+	+/-	-/-
P1	20	106	39	67	0
E8.5-11.5	8	48	11	26	11

Online Figure II

Fig .5



Online Figure III



Bar indicates 20uM