ONLINE SUPPLEMENTARY APPENDIX

for BRIEF REPORT

Atrial Natriuretic Peptide Frameshift Mutation In Familial Atrial Fibrillation

Denice M. Hodgson-Zingman, M.D., Margaret L. Karst, B.A, Leonid V. Zingman, M.D., Denise M. Heublein, C.L.T., Dawood Darbar, M.D., Kathleen J. Herron, B.A., Jeffrey D. Ballew, M.S., Mariza de Andrade, Ph.D., John C. Burnett, Jr., M.D., and Timothy M. Olson, M.D.

SUPPLEMENTARY METHODS

Primer pairs for PCR amplification of the 3 translated exons of *NPPA*

NPPA exon 1F: 5'-GGAGACAGGGACAGACGTAG-3'

NPPA exon 1R: 5'-CCCAGACTGCACCCGCTTTC-3'

NPPA exon 2F: 5'-GCCAGGAAAGCGGGTGCAG-3'

NPPA exon 2R: 5'-GGGCACTCTGGGTGTTGGG-3'

NPPA exon 3F: 5'-GTGGGAAGCAGGTGGTCAGTA-3'

NPPA exon 3R: 5'-AGCTTAGATGGGATGATCACA-3'

Radioimmunoassay (technical details)

C-18 Bond Elution cartridges were pre-washed with 4 ml 100% methanol and 4 ml water, after which 1 ml of plasma was applied. Cartridges with adsorbed peptides were then washed with 2 ml saline, 6 ml water, and 1 ml 100% methanol. ANP and mANP were eluted with 2 ml 75% methanol and 1% TFA. Eluates were dried and concentrated overnight on a Savant speed vacuum and re-suspended in 300 _l assay buffer. 100 _l of samples and standards were incubated with 100 _l diluted (1:150,000) anti-human ANP or anti-mANP at 4 °C. After 18 hours, 100 _l (10,000 counts) I^{125}-labelled ANP or mANP was added and incubated at 4 °C for 18 hours. A secondary antibody was then added to each sample, followed by centrifugation to separate free and bound fractions. The free fraction was aspirated and the bound fraction was counted on a gamma counter.

For BNP assays, standard solutions or patients' plasma samples (100 _l) were incubated with a monoclonal antibody that was specific for the carboxyl-terminal region of BNP and bound to a solid bead. A second I^{125}-labeled monoclonal antibody (200_l) specific for the ring structure of BNP was added to form a sandwich complex. After 14-18 hours incubation at 4 °C, the bead was washed to remove the unbound radio-iodinated antibody and the bound fraction was counted on a gamma counter.

Isolated heart model (technical details)

Rapid cardiectomy was performed in male rats weighing 300-350 g, under general anesthesia with pentobarbital. The hearts were retrogradely perfused via the aorta at constant pressure and temperature (100 mmHg and 37 ºC) with Kreb's–Henseleit buffer filtered at 0.22 μm and bubbled with 95% O_2/5% CO_2 at 37 °C and pH 7.4. Coronary perfusion flow was monitored constantly (T402, Transonic Systems) and remained >10 ml/min.

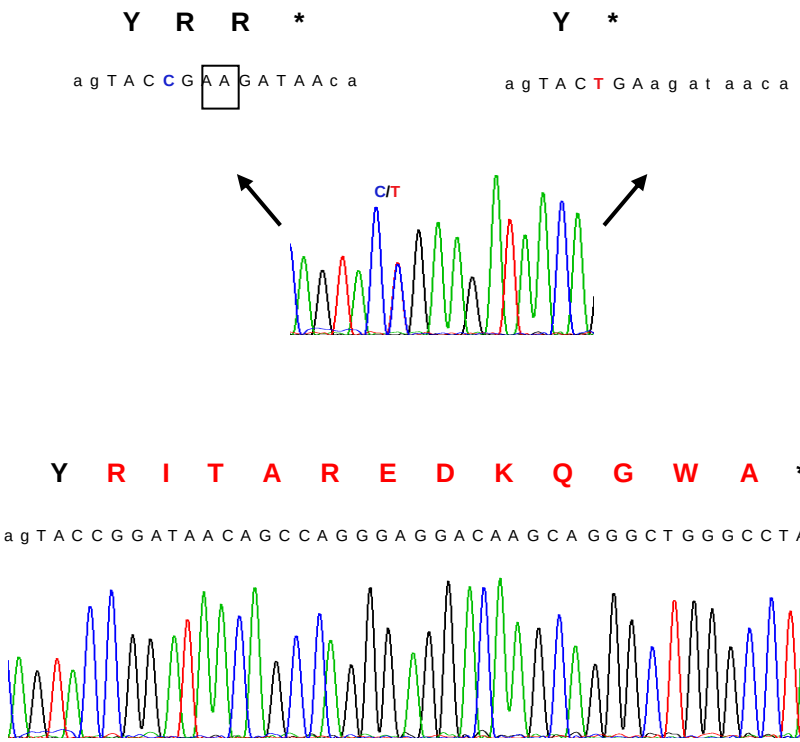
SUPPLEMENTARY FIGURE

Figure S. *NPPA* mutation generates a chimeric ANP protein.

In Panel A, a common 454 C>T polymorphism in exon 3 of wild-type *NPPA* (individual III.4) results in ANP with or without a carboxyl-terminal pair of arginine residues, which are removed in post-translational processing. The mutant allele (individual III.2), on the background of the 454C variant, harbors a 2-bp

frameshift deletion (456-457delAA; cDNA clone BC005893), disrupting the stop codon and generating a 40-amino acid chimeric protein with an anomalous 12-residue carboxyl-terminal tail. In Panel B, alignment of mature human ANP with ANP orthologs demonstrates significant sequence conservation among eukaryotic species. The human paralogs BNP and CNP are divergent, yet retain the “CFGXXXDRIXXXSXLGC” motif (blue font) common to natriuretic peptides.[22] Atrial fibrillation-associated mutant ANP has an extended amino acid carboxyl-terminus (red font) reminiscent of DNP (green font), a natriuretic peptide with distinct biological properties found in the green mamba snake.

A



B

H. sapiens ANP	SLRRSSCFGGRMDRIGAQSGLGCNSFRY
Mutant ANP	SLRRSSCFGGRMDRIGAQSGLGCNSFRYRITAREDKQGWA
P. troglodytes ANP	••••••••••••••••••••••••••••
S. scrofa ANP	••••••••••••••••••••••••••••
C. familiaris ANP	••••••••••••••••••••••••••••
B. taurus ANP	••••••••••••••••••••••••••••
O. cuniculus ANP	••••••••••••I•••••••••••••••
R. norvegicus ANP	••••••••••••I•••••••••••••••
M. musculus ANP	••••••••••••I•••••••••••••••
D. rerio ANP	•KSL•G•••••L••••SS•T•••••KKG
H. sapiens BNP	SPKMVQG•G•••RK••••SSS•••••KVL•RH
H. sapiens CNP	GLSKG•••LKL••••SM•••••
D. angusticeps DN	EVKYDP•••HKI•••NHV•N•••P•L•DPRPNAPSTSA