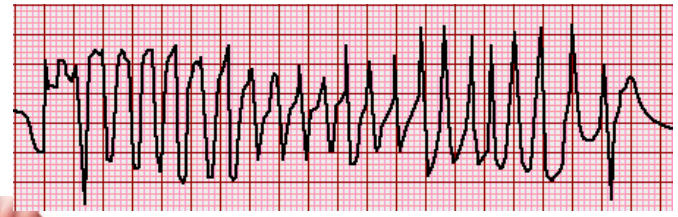
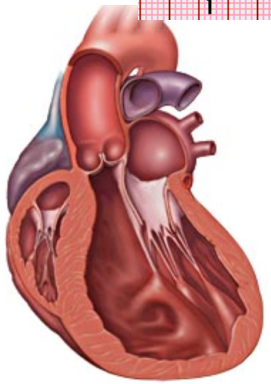
# Why clinical medicine and research?

# Why do both basic and clinical research?






Christopher Newton-Cheh, MD, MPH

Heart Failure & Transplant Section
Cardiovascular Research Center
Center for Human Genetic Research
Massachusetts General Hospital, Harvard Medical School

Program in Medical and Population Genetics
Broad Institute of Harvard and MIT

# In memoriam

- Kenneth Bloch, MD
- Mentor
- Scientist
- AHA

# Potential conflicts of interest

- patents related to use of copeptin and adrenomedullin in cardiovascular risk prediction
- patent related to use of miR-425 antagonism in treatment of hypertension, heart failure
- consultant, Novartis Institute for Biomedical Research
- grants from NIH, Burroughs Wellcome Fund, Doris Duke Charitable Foundation

# Newton-Cheh laboratory

- population genetic studies
- genotype-directed studies in humans
- identification of underlying molecular mechanisms

- blood pressure / hypertension
- QT interval / myocardial repolarization / arrhythmias
- left ventricular hypertrophy

# Lab-related meetings

- Post-doc meetings (4 hrs/wk)
- Research coordinator (30 min/wk)
- Joint lab meeting (1hr/wk)
- Lab meeting (1hr/wk)
- Center for Human Genetic Research seminar (1hr/wk)
- Broad Institute Medical/Population Genetics seminar (1hr/wk)

# Other consortia research meetings

- BP (1-2hr /wk)
- QT interval / SCD (2hrs /mo)
- QRS voltage (1hr /wk)

# Clinical work: pts & mtgs

- Heart Failure, Transplant, MCS, Coronary Care Unit
- 3 half-months inpatient service / year
- 2 half-day clinics / month
- Monday AM intake grp mtg (30 min/wk)
- Thursday noon HF didactic lectures (1 hr/wk)
- Friday AM pre-TXP, PM post-TXP (2hrs/wk)
- Cardiology grand rounds (1hr/wk)

# Administrative/teaching/other

- Run ECG lab (1 hr/mo)
- Read ECGs (4 hrs/mo)
- Direct 2 courses (1 hr/wk)
- Grant reviews (NIH, AHA)
- AHA FGTB, advocacy
- Conferences
- Presentations
- Write grants, edit papers

# human genetics

- novel therapeutic targets
- novel mediators of drug toxicity

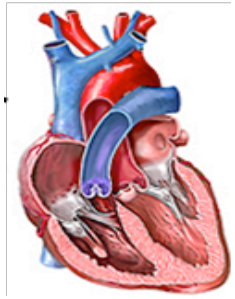
- Atrial natriuretic peptide & blood pressure
- cGMP regulating pathways emerge from GWAS
- QT interval variants & cardiotoxic drug response

- Atrial natriuretic peptide & blood pressure
- cGMP regulating pathways emerge from BP GWAS
- QT interval variants & cardiotoxic drug response

# candidate gene study: ANP & BNP

- ANP + BNP highly correlated (r = 0.60-0.70)
- hormones produced by heart in response to salt-overload, hypertension, age, myocardial dysfunction
- activate particulate guanylate cyclase
- increase intracellular cyclic guanosine monophosphate (cGMP)
- enhance renal sodium excretion
- vasodilatory effects

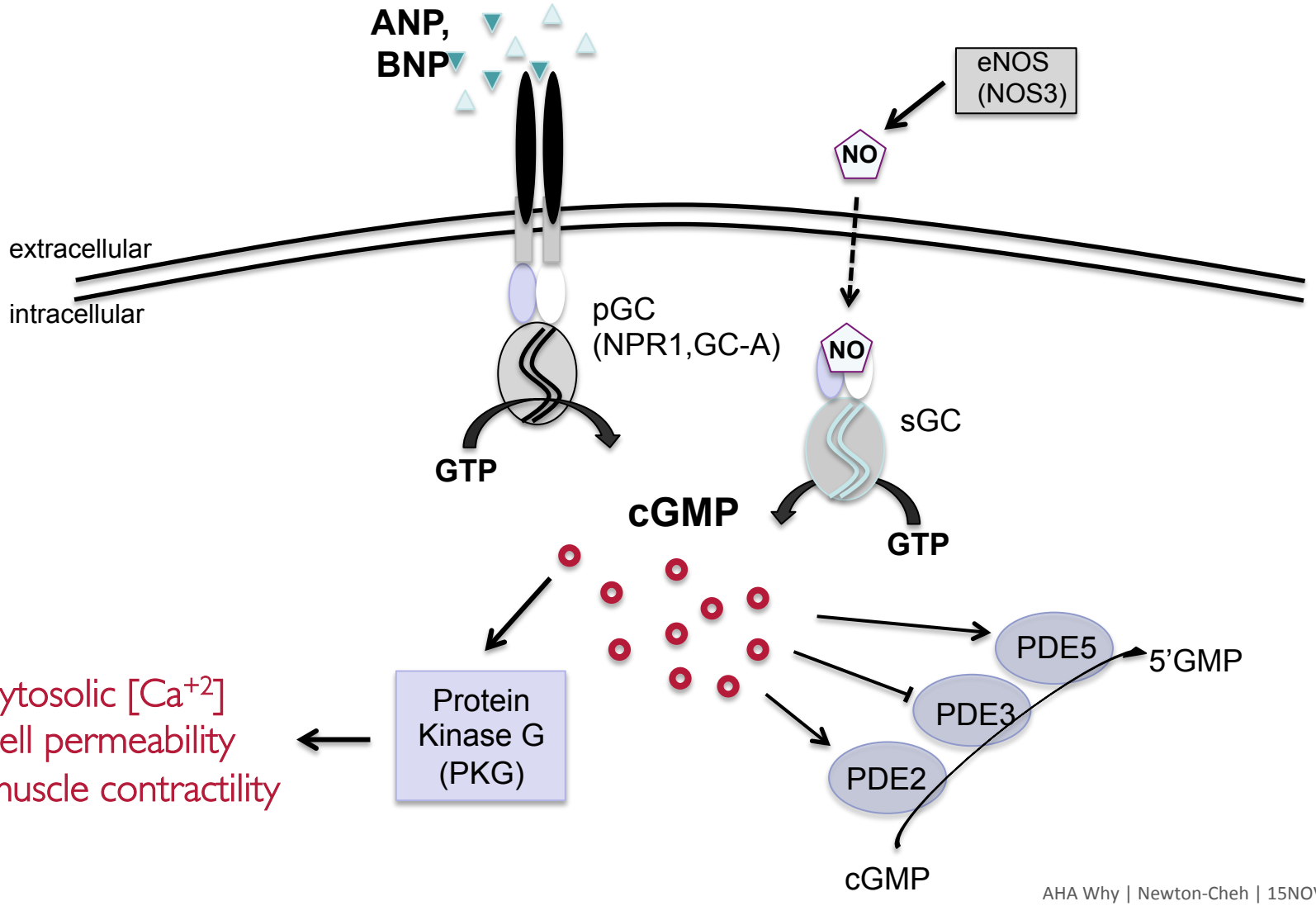
# activation, inactivation of ANP+ BNP



pro-ANP —*corin*→ Nt-ProANP + **ANP** —*neprilysin*→ degradation by proteolysis

pro-BNP —*corin / furin*→ Nt-ProBNP + **BNP** —*neprilysin*→ degradation by proteolysis

# cGMP signaling cascade




- cytosolic [$Ca^{+2}$]
- cell permeability
- muscle contractility

nature genetics

# Association of common variants in *NPPA* and *NPPB* with circulating natriuretic peptides and blood pressure

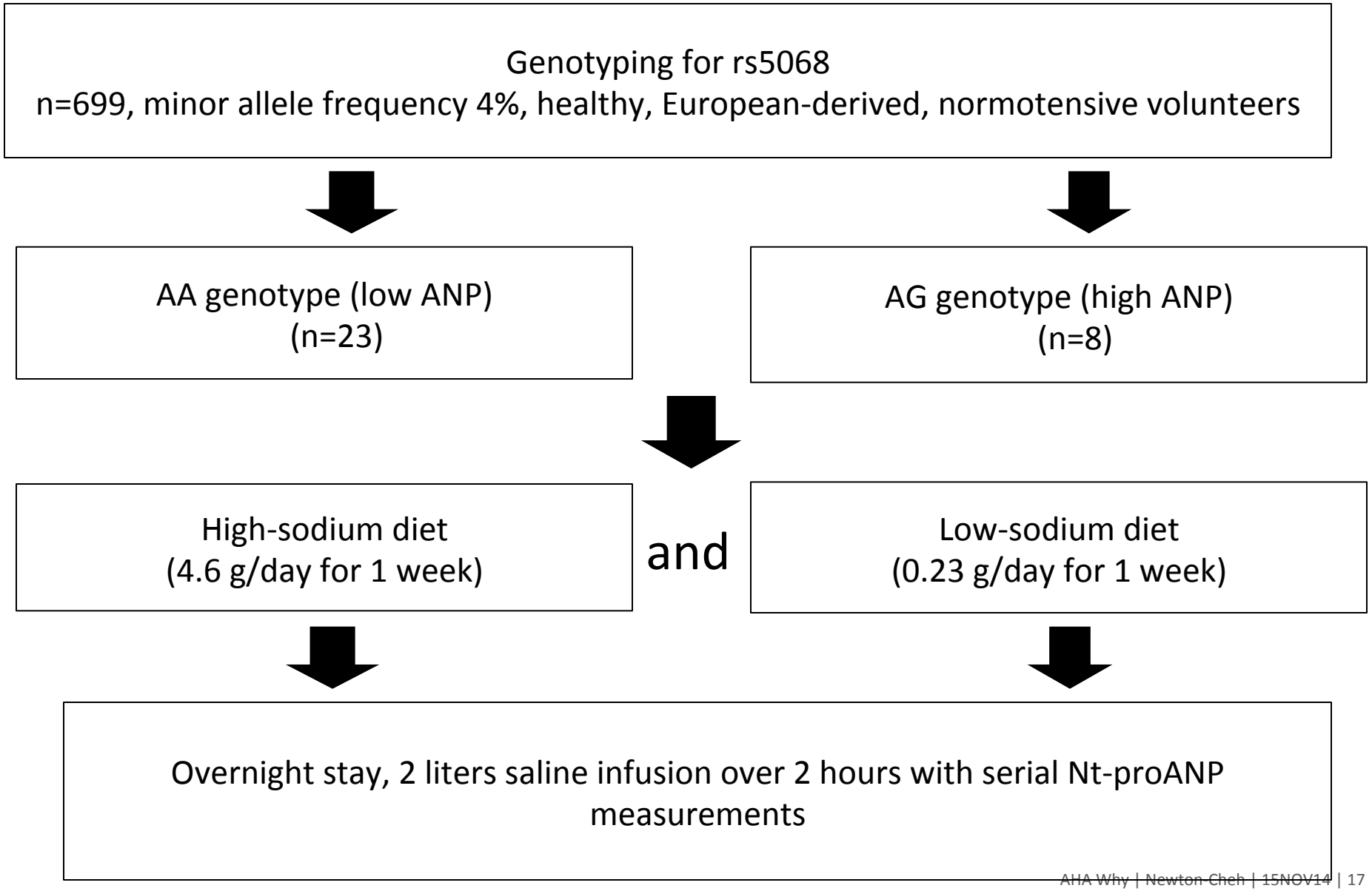
Christopher Newton-Cheh[1–4], Martin G Larson[4,5], Ramachandran S Vasan[4,6,18], Daniel Levy[4,7,18], Kenneth D Bloch[2,8], Aarti Surti[3], Candace Guiducci[3], Sekar Kathiresan[1–4], Emelia J Benjamin[4,6], Joachim Struck[9], Nils G Morgenthaler[9], Andreas Bergmann[9], Stefan Blankenberg[10], Frank Kee[11], Peter Nilsson[12], Xiaoyan Yin[4], Leena Peltonen[13–15], Erkki Vartiainen[13], Veikko Salomaa[13], Joel N Hirschhorn[3,16,17], Olle Melander[12,19] & Thomas J Wang[2,4,19]

# rs5068, plasma ANP levels, blood pressure in humans

- rs5068 minor G allele in 3'UTR of NPPA
- frequency ~4-6% in 15k individuals of European ancestry
- Increasing copy of the rs5068 minor G allele is associated with:
  - increased plasma ANP levels ($P = 8x10^{-70}$)
  - decreased systolic blood pressure ($P = 2x10^{-6}$)
  - decreased diastolic blood pressure ($P = 1x10^{-6}$)
  - reduced odds of hypertension (OR = 0.85)

Newton-Cheh et al, Nature Genetics 2009

# Genotype-directed physiologic study

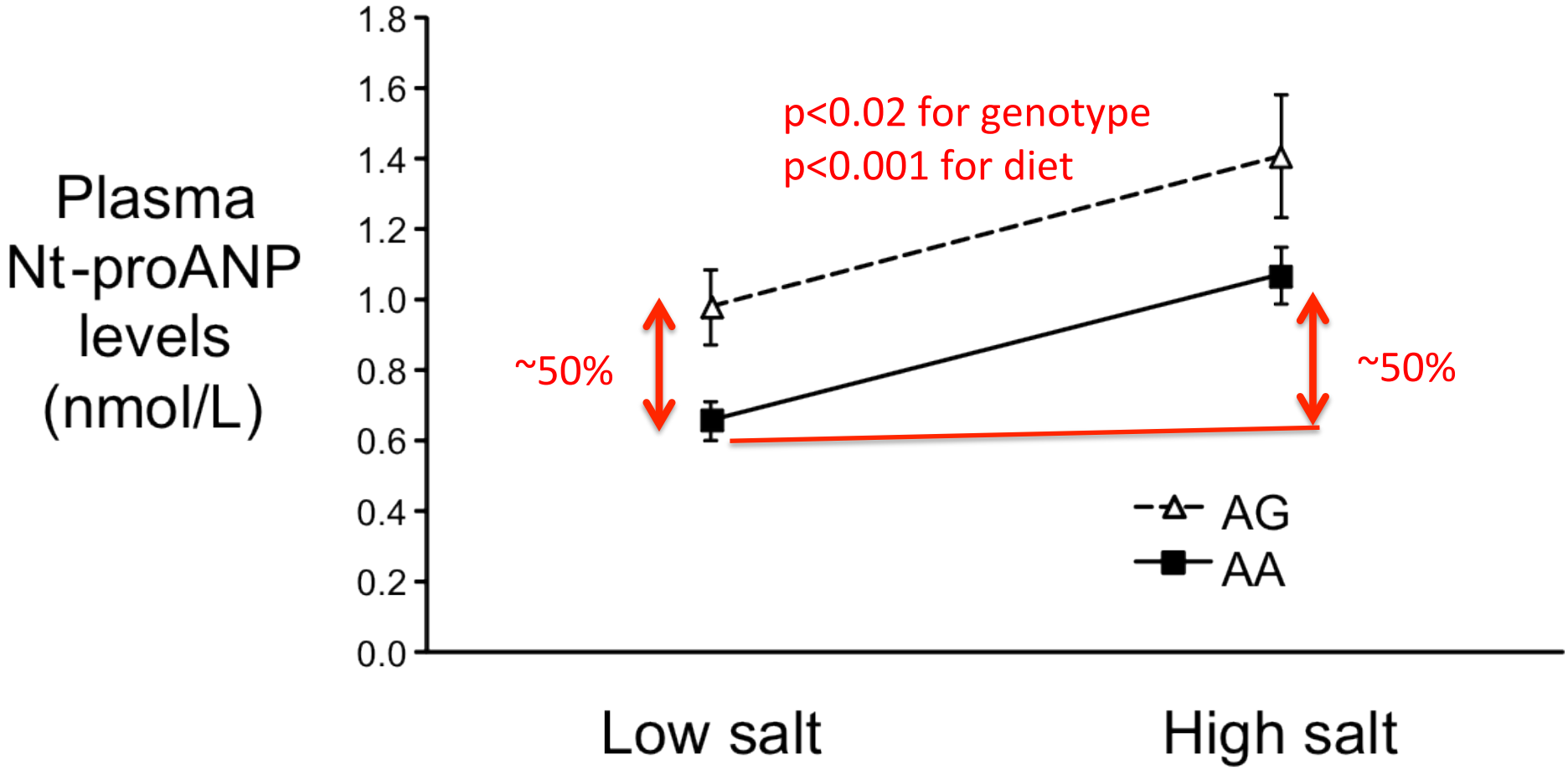


# Genotype-directed study results

- Screened 700 healthy volunteers for rs5068 genotype
- 31 completed both diets based upon genotype
  - 23 Low ANP (AA) genotype
  - 8 High ANP (AG) genotype
- 3 minor homozygotes
  - Only 1 completed both diets

Arora JCI, 2013

# Effect of genotype and dietary sodium on baseline plasma Nt-proANP levels



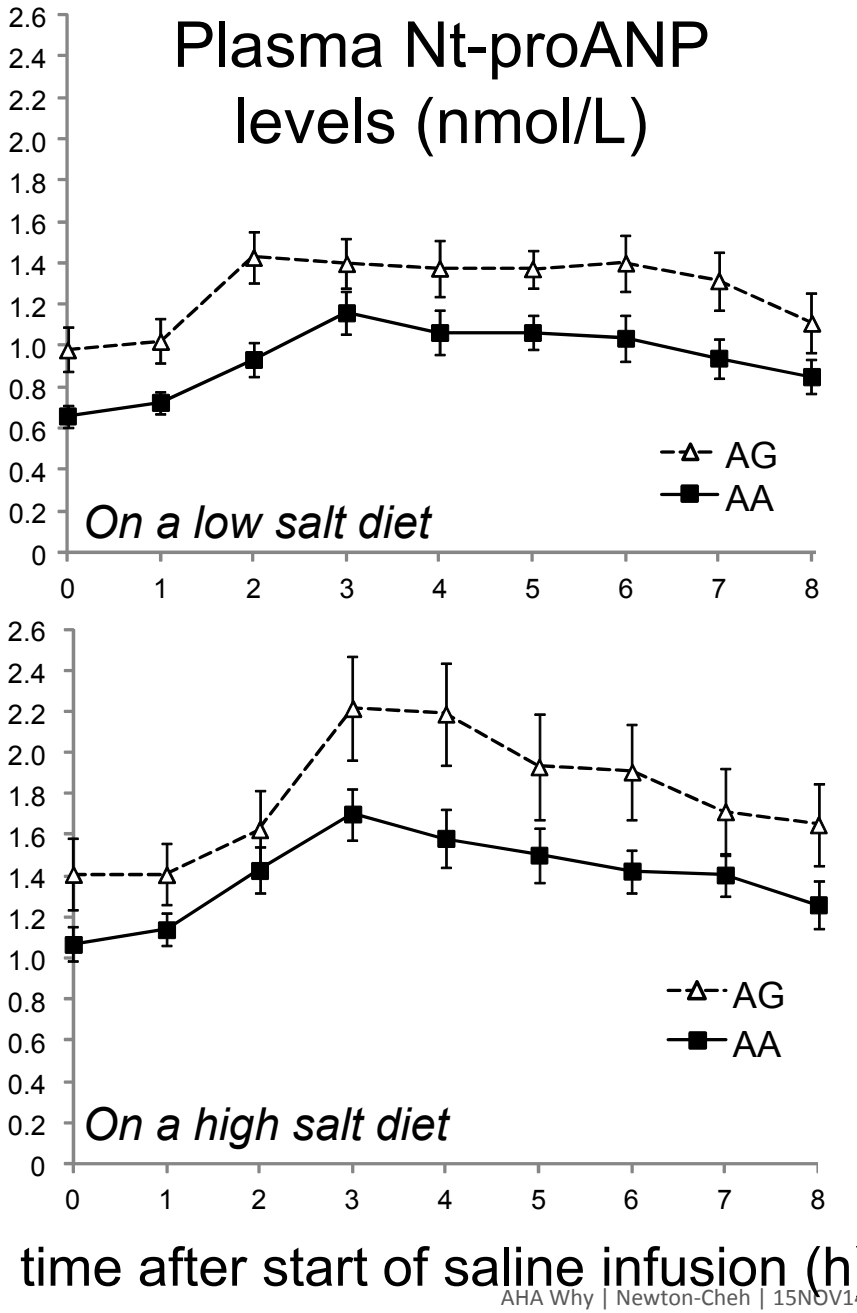

Arora, JCI 2013

# Effect of genotype on plasma Nt-proANP levels during saline challenge

P (saline) < 0.001
P (diet) < 0.001
P (genotype) < 0.02

AG individuals have an increased ANP "set point" without altering the responsiveness to salt loading.

Arora, JCI 2013



# Conclusion

- In a genotype-directed physiologic study, standardization of salt background refined the association between genotype and plasma NT-proANP levels
- The effect of genotype on baseline Nt-proANP levels was similar to that of a marked change in salt intake
- Genotype did not alter ANP response to dietary or intravenous sodium challenge

Brief report

# Atrial natriuretic peptide is negatively regulated by microRNA-425

Pankaj Arora,[1,2,3,4] Connie Wu,[5] Abigail May Khan,[6] Donald B. Bloch,[7,8] Brandi N. Davis-Dusenbury,[9] Anahita Ghorbani,[1,2] Ester Spagnolli,[5] Andrew Martinez,[1,3] Allicia Ryan,[1,3] Laurel T. Tainsh,[5] Samuel Kim,[3] Jian Rong,[10,11] Tianxiao Huan,[10,11] Jane E. Freedman,[12] Daniel Levy,[10,11] Karen K. Miller,[13] Akiko Hata,[14] Federica del Monte,[15] Sara Vandenwijngaert,[16] Melissa Swinnen,[16] Stefan Janssens,[16] Tara M. Holmes,[17] Emmanuel S. Buys,[5] Kenneth D. Bloch,[1,2,5] Christopher Newton-Cheh,[1,2,3,4] and Thomas J. Wang[1,2,18]

Pankaj Arora

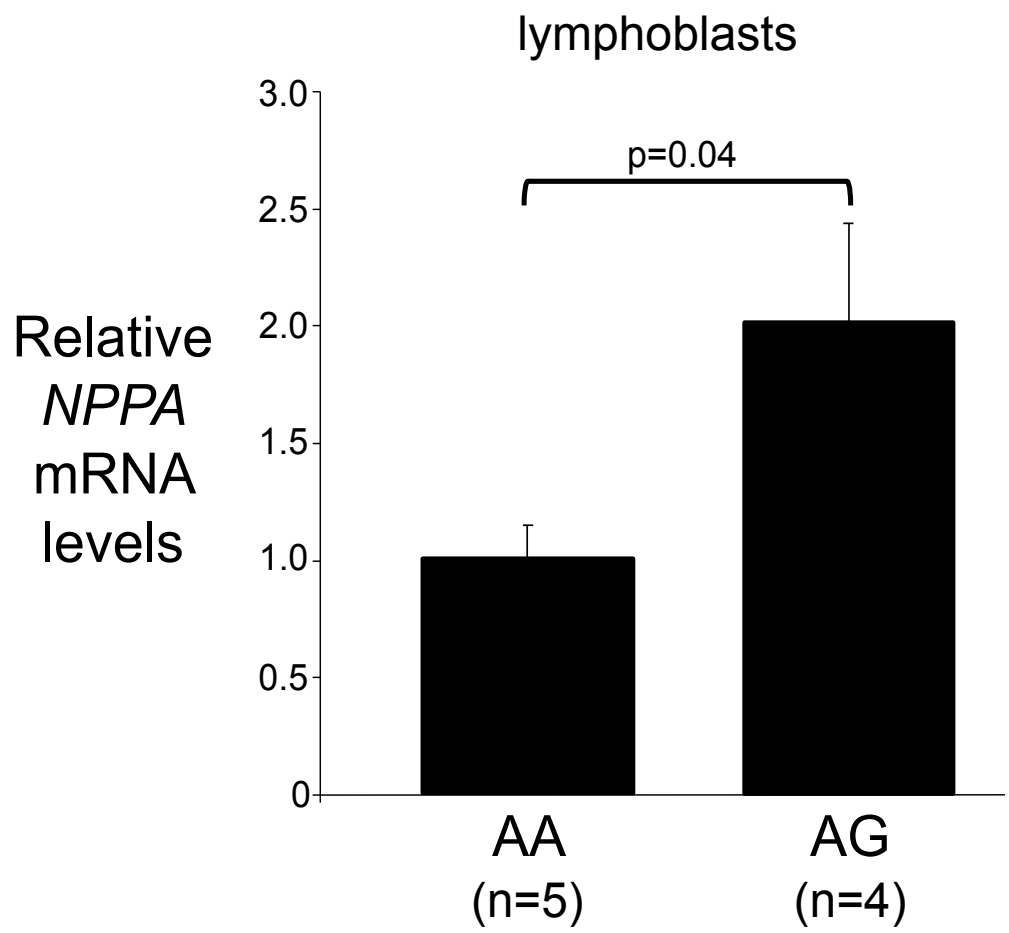
Ken Bloch

Tommy Wang

Chris Newton-Cheh

Arora JCI 2013

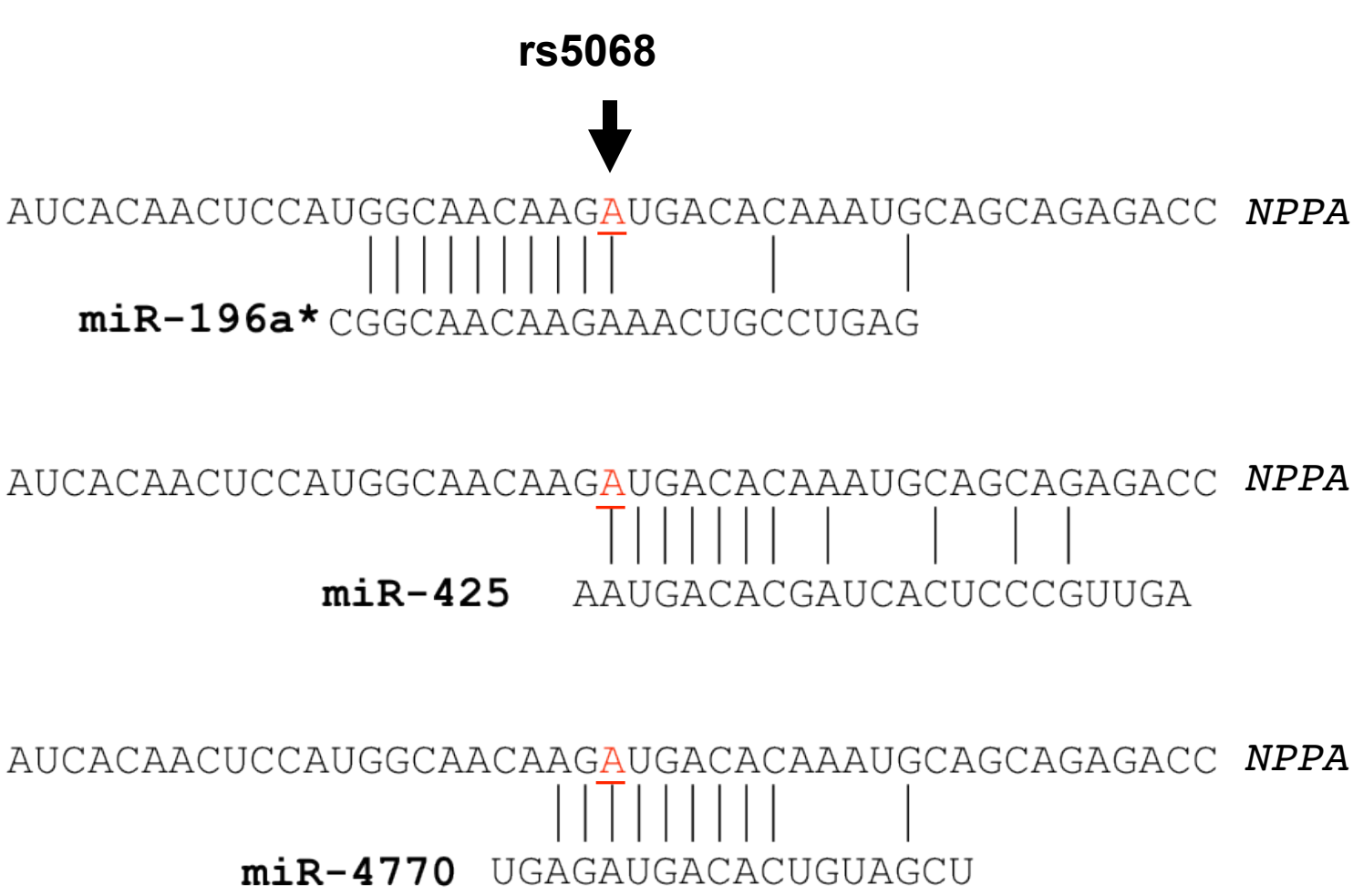
# rs5068 regulates NPPA mRNA levels




n=2,246 Framingham Heart Study participants
*NPPA* gene expression higher in individuals with at least one copy of the G allele (n=203) compared with AA (n=2,043) individuals ($P = 2 \times 10^{-18}$).

Arora, JCI 2013

# miRNAs predicted to target the major A but not minor G allele of rs5068



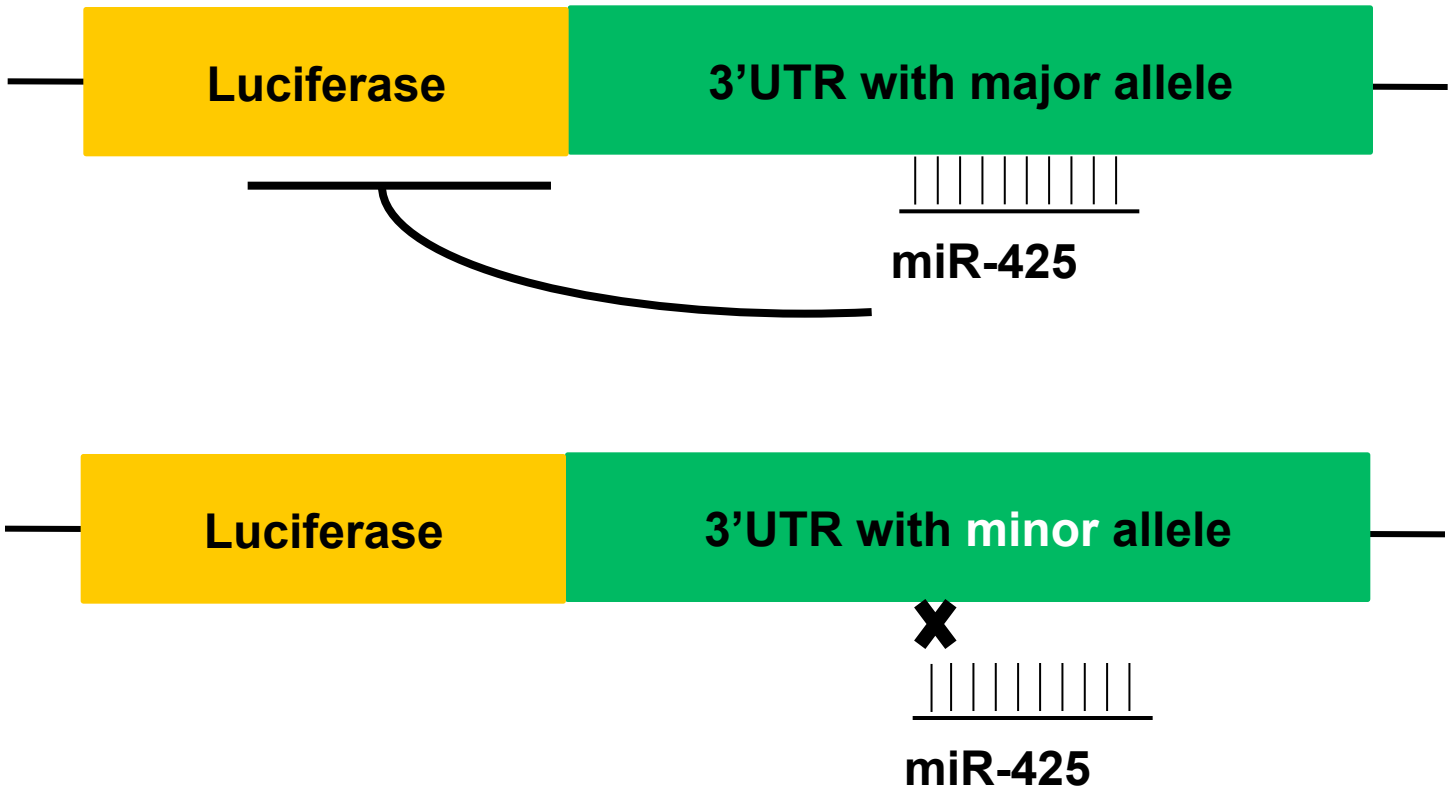

Expressed in cardiac tissues:

no

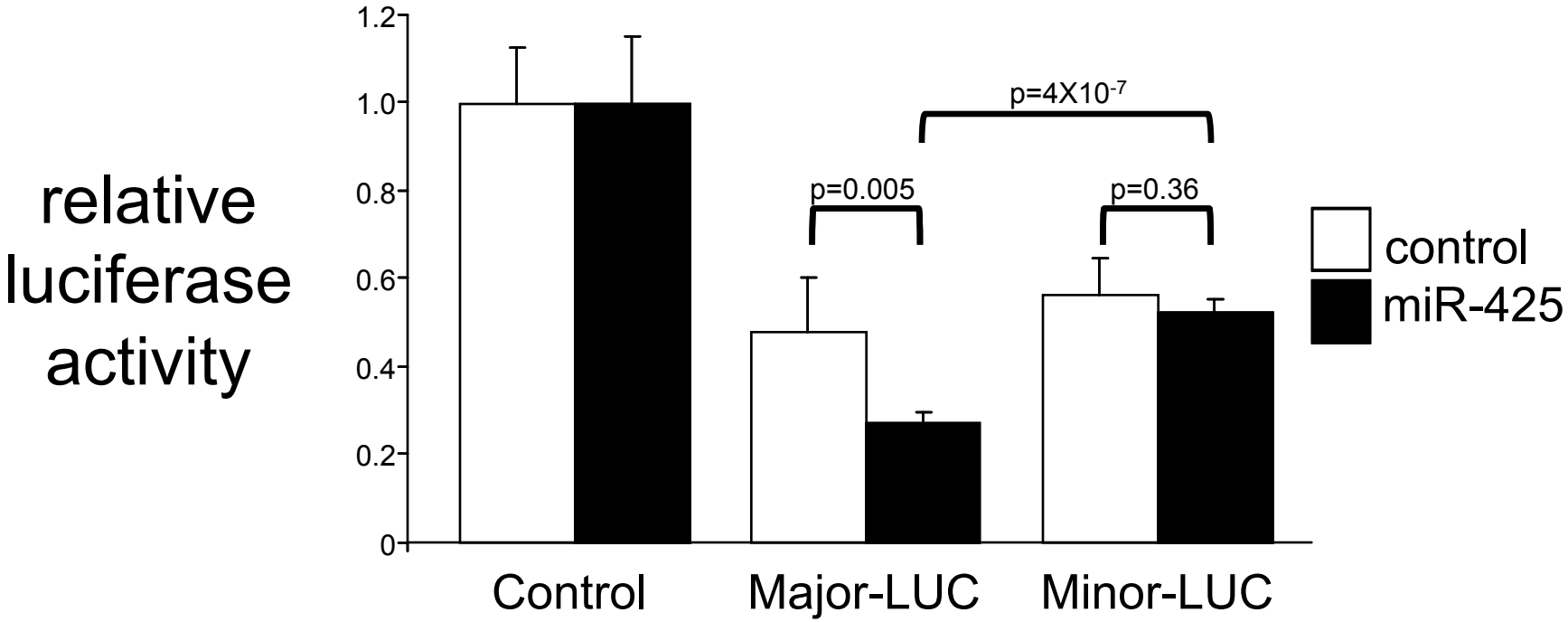
yes

yes ✗

# Validation of the predicted binding of miR-425 with the *NPPA* 3'UTR

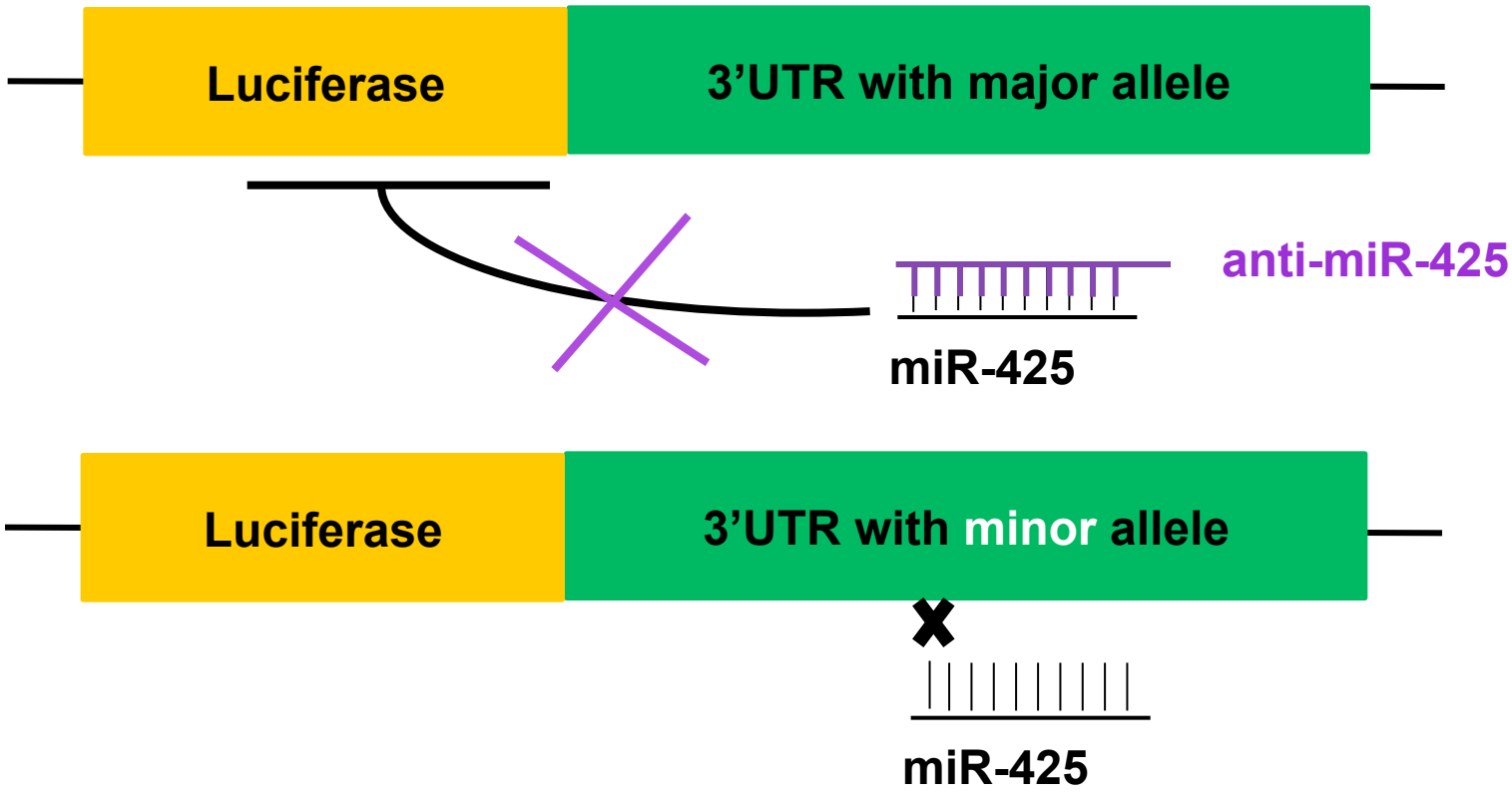


# miR-425 (major allele vs. minor allele)



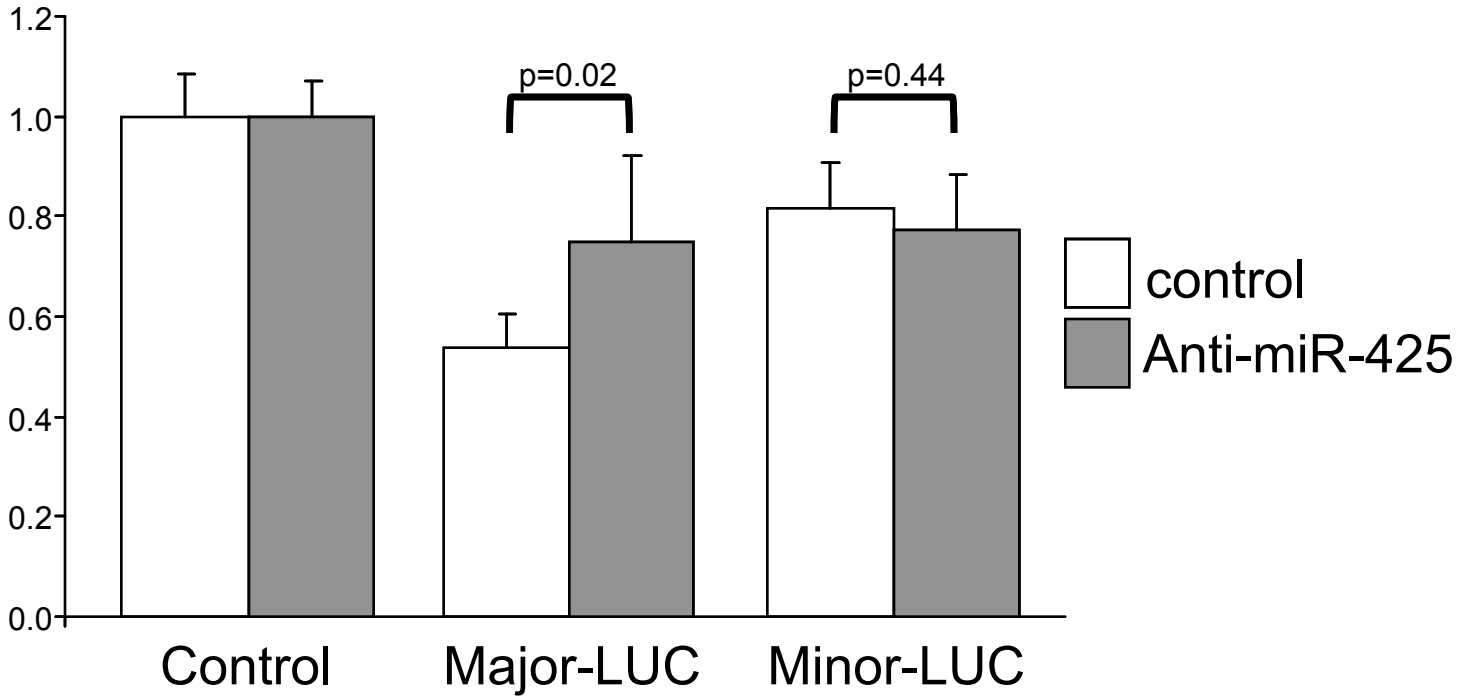

Arora, JCI 2013

# AntimiR-425 reverses *NPPA* 3'UTR inhibition



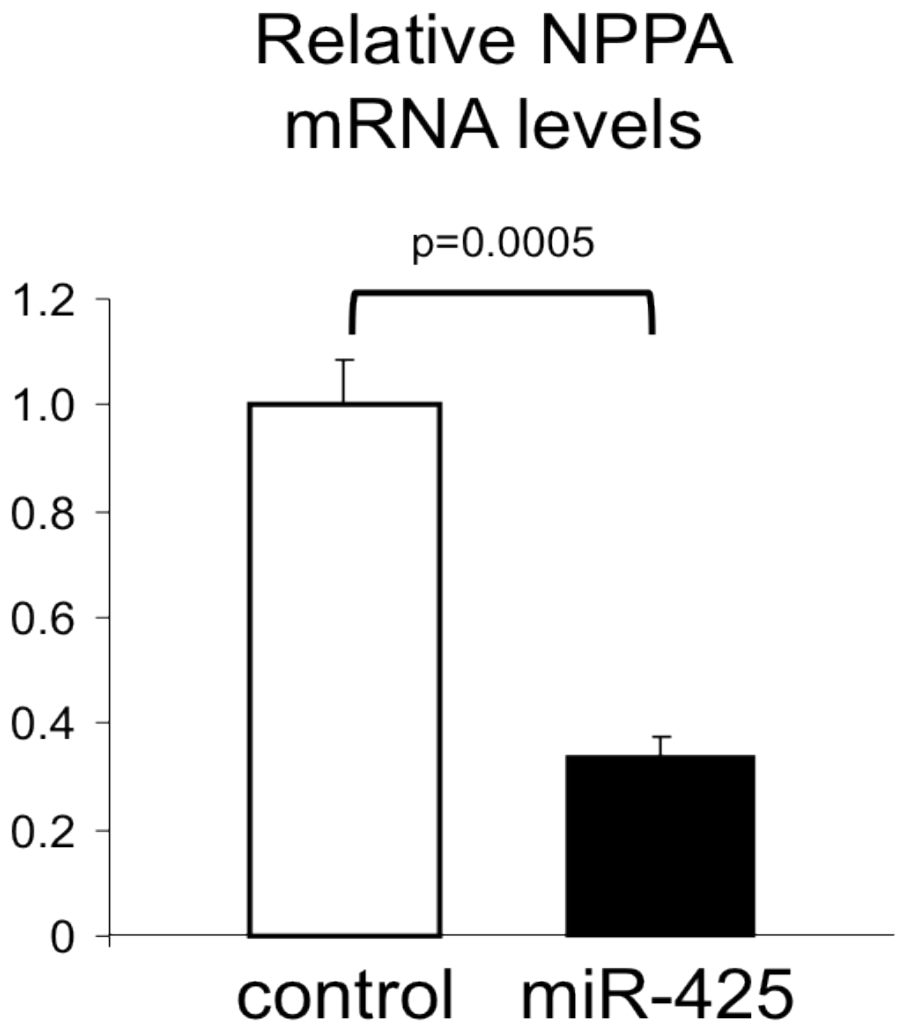
# Anti-miR-425
# (major allele vs. minor allele)

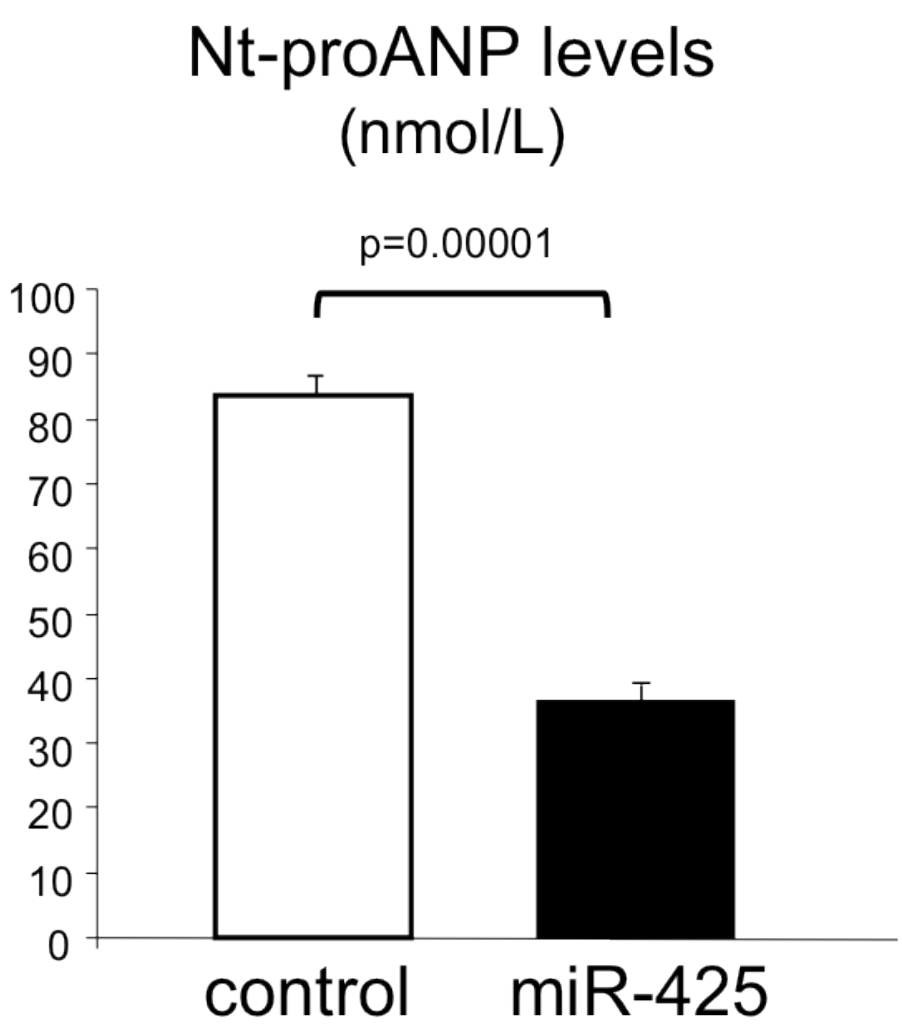
relative luciferase activity




Arora, JCI 2013

# Induced pluripotent stem cell-derived human cardiomyocytes (AA genotype)




Arora, JCI 2013

# Conclusions

- A common genetic variant in the *NPPA* 3' UTR, rs5068, impacts the ability of a miR to target NPPA mRNA
- Inhibiting miR-425 could enhance atrial ANP release in response to salt excess in hypertension and heart failure
- miR-425 target absent from murine Nppa
- Human transgene, crossing to KO

- Atrial natriuretic peptide & blood pressure
- cGMP regulating pathways emerge from BP GWAS
- QT interval variants & cardiotoxic drug response

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LETTER

doi:10.1038/nature10405

# Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk

The International Consortium for Blood Pressure Genome-Wide Association Studies

Ehret et al *Nature* 2011

# Discovery of Genetic Variants Associated with Blood Pressure

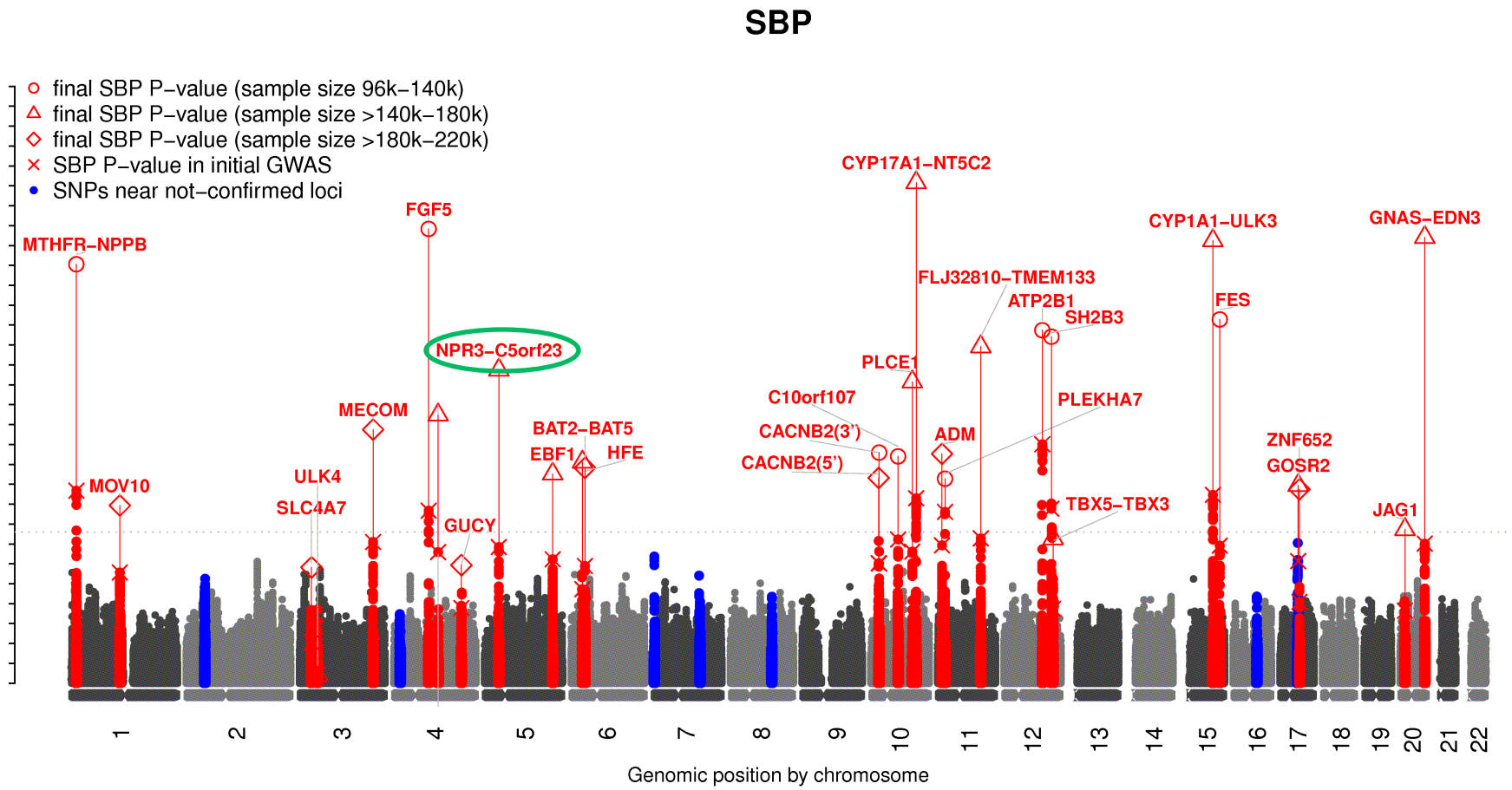
- GWAS of systolic and diastolic blood pressure (BP)

- GWAS discovery (n=70,000)
- Targeted replication (n=130,000)
- Total sample size up to 200,000 individuals of European descent

- 28 genome-wide significant ($P$ < 5E-08) SNPs

Ehret et al *Nature* 2011

# Systolic BP



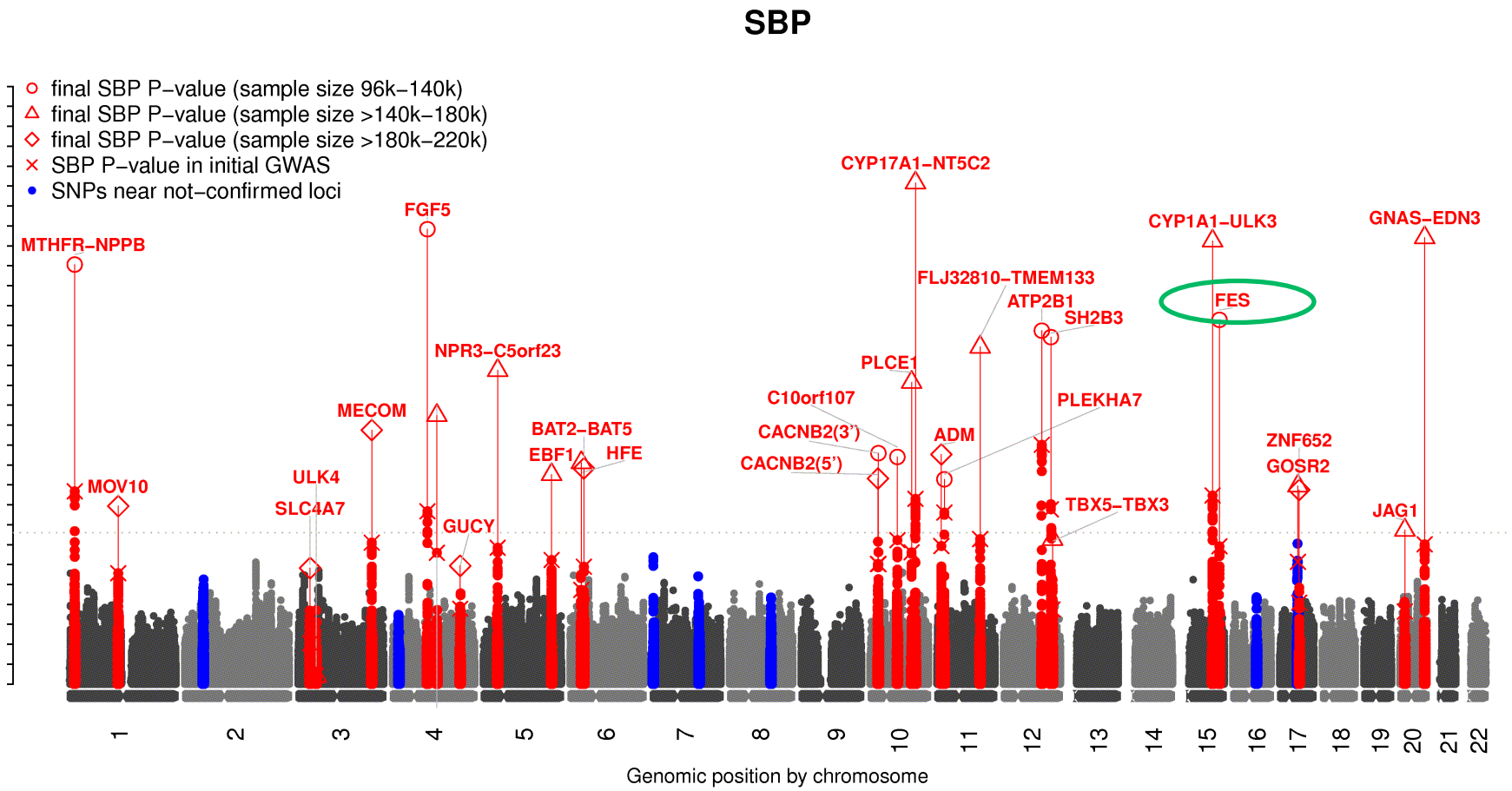

Ehret, *Nature*

# *NPR3* noncoding SNP

- encodes natriuretic peptide receptor C
- no guanylate cyclase activity
- KO results in lower BP and skeletal overgrowth (Matsukawa 1999)
- proxy associated with height (higher BP allele = shorter stature)
- no assoc ANP, BNP, NTproBNP in 7,000 indiv
- NPR3 agonists have a vasodilatory action (Moyes 2014)

Ehret Nature 2011, Lango Nature 2010

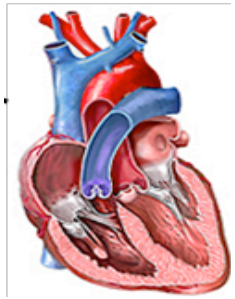
# Systolic BP

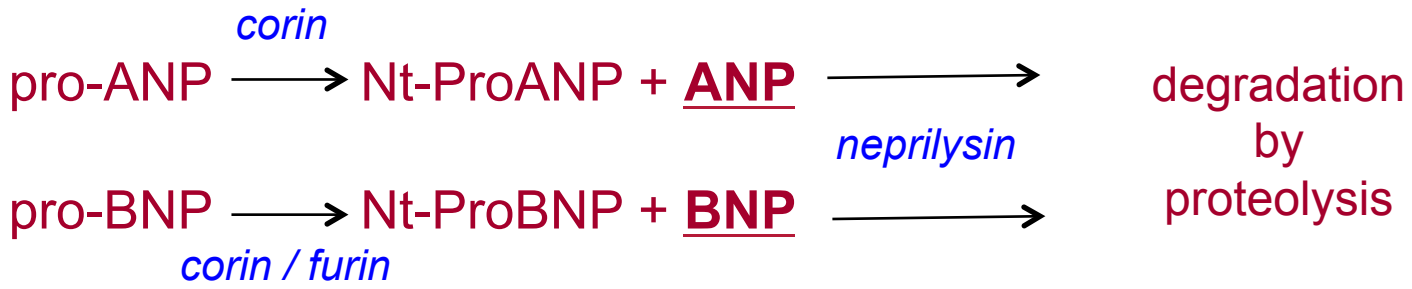



Ehret, *Nature*

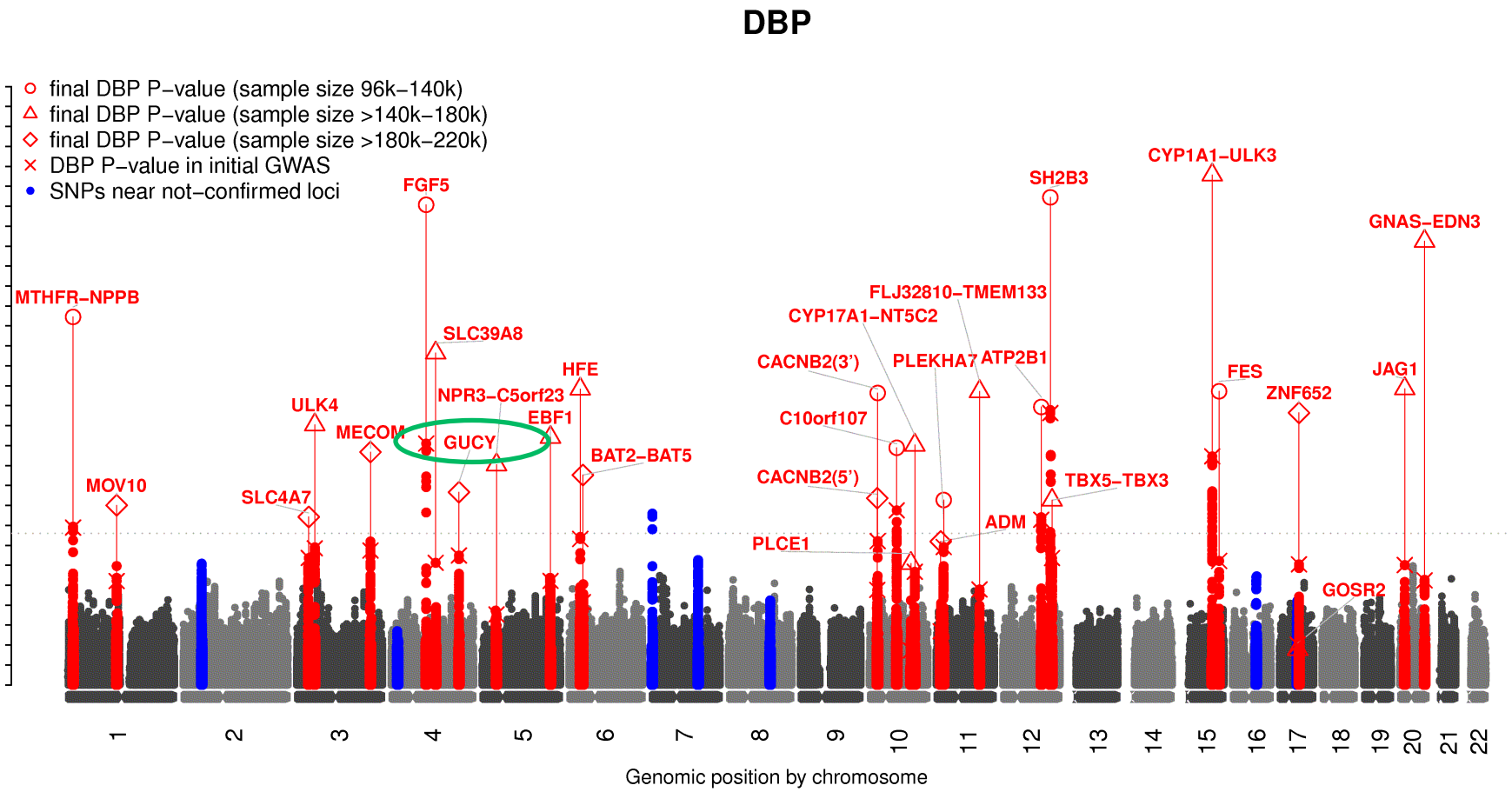
# FES-FURIN

- noncoding SNP
- furin cleaves proBNP to form active BNP



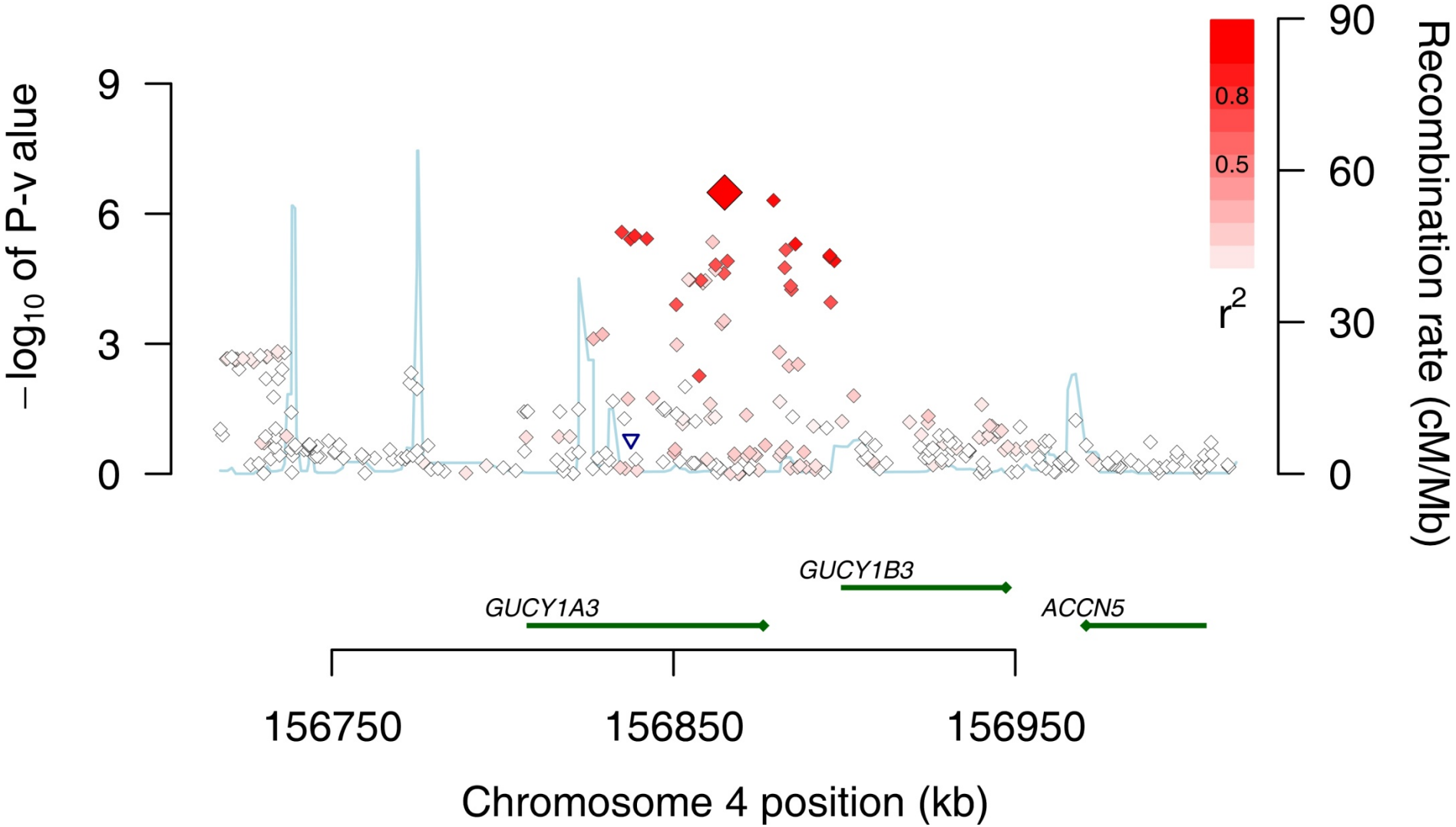


# Diastolic BP




Ehret et al, *Nature*

# noncoding *GUCY1A3, GUCY1B3* SNP




Ehret et al, *Nature*

# SGC and hypertension

- soluble guanylate cyclase is the protein that transduces the effect of nitric oxide (NO)
- SGC α1 mouse knockout -> HTN (Mergia 2006)
- SGC α1 mouse knockout males -> hypertension (Buys 2008)
  - ICBP: stronger effect in male>female (p=0.04)
- SGC β1 mouse knockout -> fatal gastrointestinal obstruction, HTN (Friebe 2007)

ORIGINAL ARTICLE

# Riociguat for the Treatment of Pulmonary Arterial Hypertension

Hossein-Ardeschir Ghofrani, M.D., Nazzareno Galiè, M.D., Friedrich Grimminger, M.D., Ekkehard Grünig, M.D., Marc Humbert, M.D., Zhi-Cheng Jing, M.D., Anne M. Keogh, M.D., David Langleben, M.D., Michael Ochan Kilama, M.D., Arno Fritsch, Ph.D., Dieter Neuser, M.D., and Lewis J. Rubin, M.D., for the PATENT-1 Study Group*

ORIGINAL ARTICLE

# Riociguat for the Treatment of Chronic Thromboembolic Pulmonary Hypertension

Hossein-Ardeschir Ghofrani, M.D., Andrea M. D'Armini, M.D., Friedrich Grimminger, M.D., Marius M. Hoeper, M.D., Pavel Jansa, M.D., Nick H. Kim, M.D., Eckhard Mayer, M.D., Gerald Simonneau, M.D., Martin R. Wilkins, M.D., Arno Fritsch, Ph.D., Dieter Neuser, M.D., Gerrit Weimann, M.D., and Chen Wang, M.D., for the CHEST-1 Study Group*

NEJM 2013

# *NOS3* noncoding SNP

- eNOS (endothelial nitric oxide synthase
- IBC 50,000 SNPs in 2,000 genes
- 25,000 individuals of European ancestry, +replication in 25,000
- common variant associated with DBP ($2x10^{-9}$)
- mouse KO → HTN (Huang, *Nature* 95)

T Johnson, AJHG, 2011

# Targeted genotyping array identifies 28 novel loci

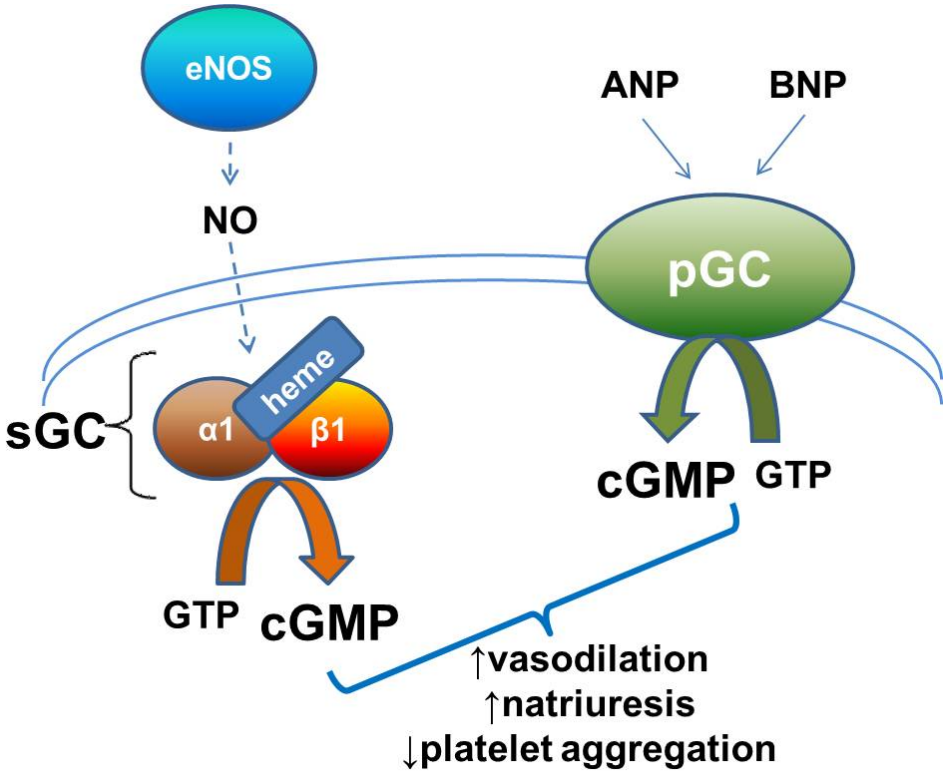
- Metabochip array (200,000 SNPs)
- 200k individuals
- 28 novel loci ($p<5E-8$)
  - one locus includes *PDE3A*
- 2nd independent signal identified at several loci
  - *NPPA-NPPB*, *GUCY1A3-GUCY1B3*, *PDE3A*
- 66 total common variant BP loci

unpublished results

# 8/66 loci involved in cGMP signaling

- *NPPA-NPPB* → ANP/?BNP production
- *NPR3* → ? ANP/BNP clearance vs signalling
- furin -> BNP activation
- eNOS → NO production
- *GUCY1A3-GUCY1B3*

  →SGC α1, β1 prodn
- *PDE3A* (cGMP/cAMP)



# Soluble guanylate cyclase

- Allele-specific enhancer assays luciferase constructs
- Recruiting healthy volunteers by sGC genotype
- Administering iNO, measuring plasmsa cGMP
- Platelet aggregation by ADP, NO donor
- Expression α1, β1 subunit

- Atrial natriuretic peptide & blood pressure
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# QT prolongation & sudden cardiac death

<u>general population</u>

Schouten 91

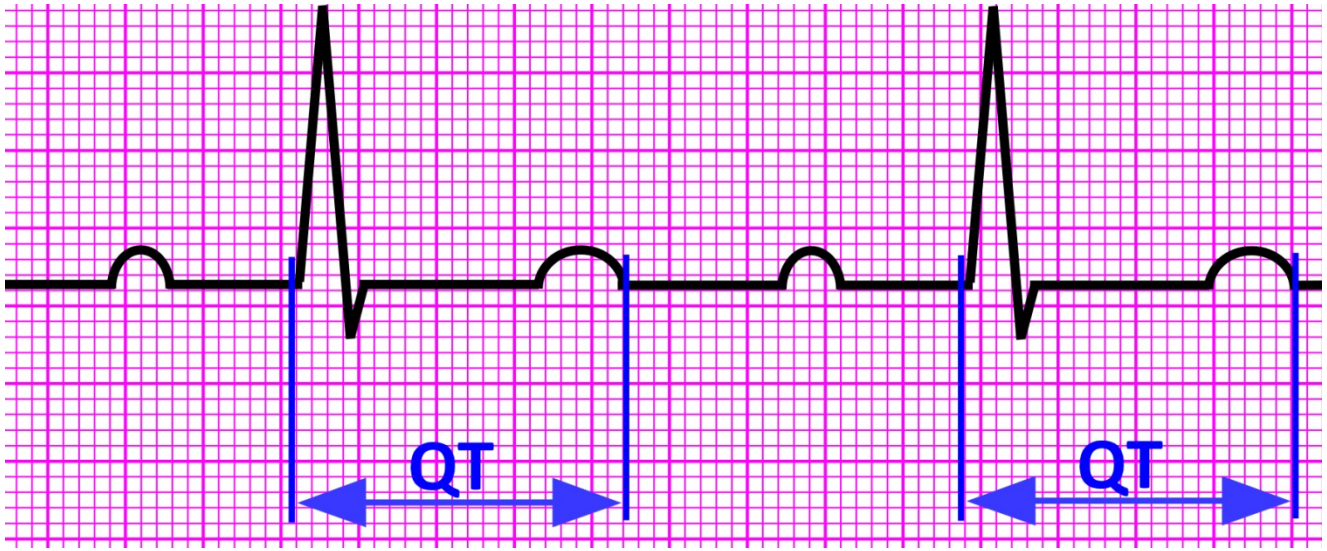
Algra 91

Siscovick 96

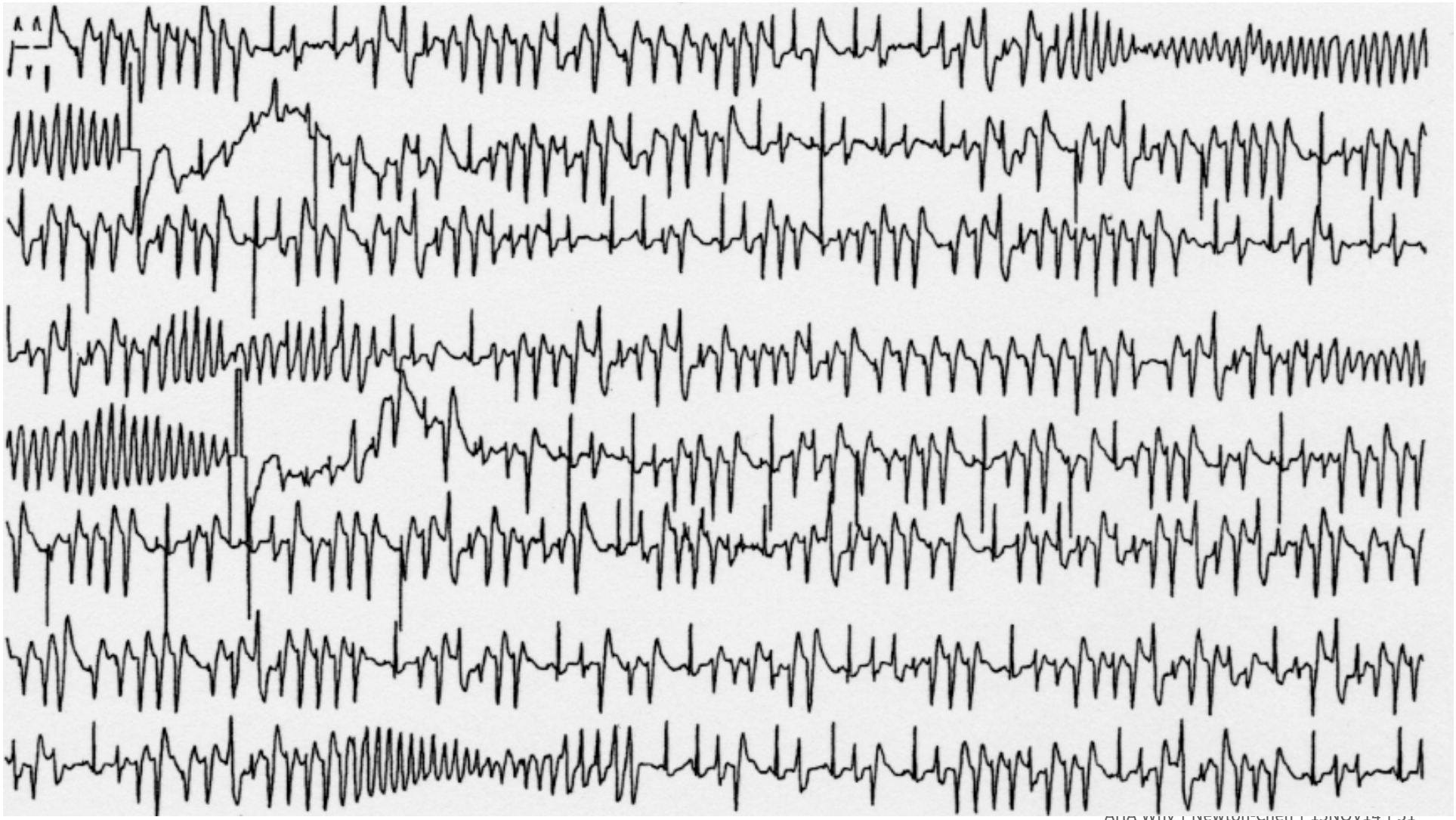
Straus 06

<u>CAD</u>

Chugh 09



# Torsade de pointes

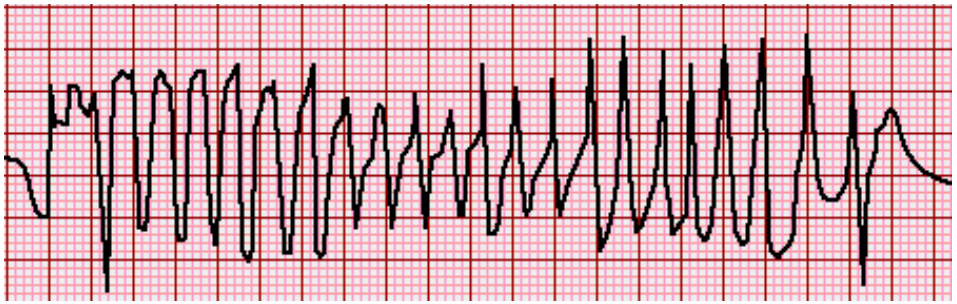


courtesy D Milan

# QT, SCD, pharmacotherapy

- Non-cardiac meds
  - terfenadine
  - cisapride
  - erythromycin
  - Haloperidol
- Anti-arrhythmics
  - sotalol
  - dofetilide
  - procainamide
- QTc prolongation by ≥8-10 msec associated with arrhythmia

www.qtdrugs.org



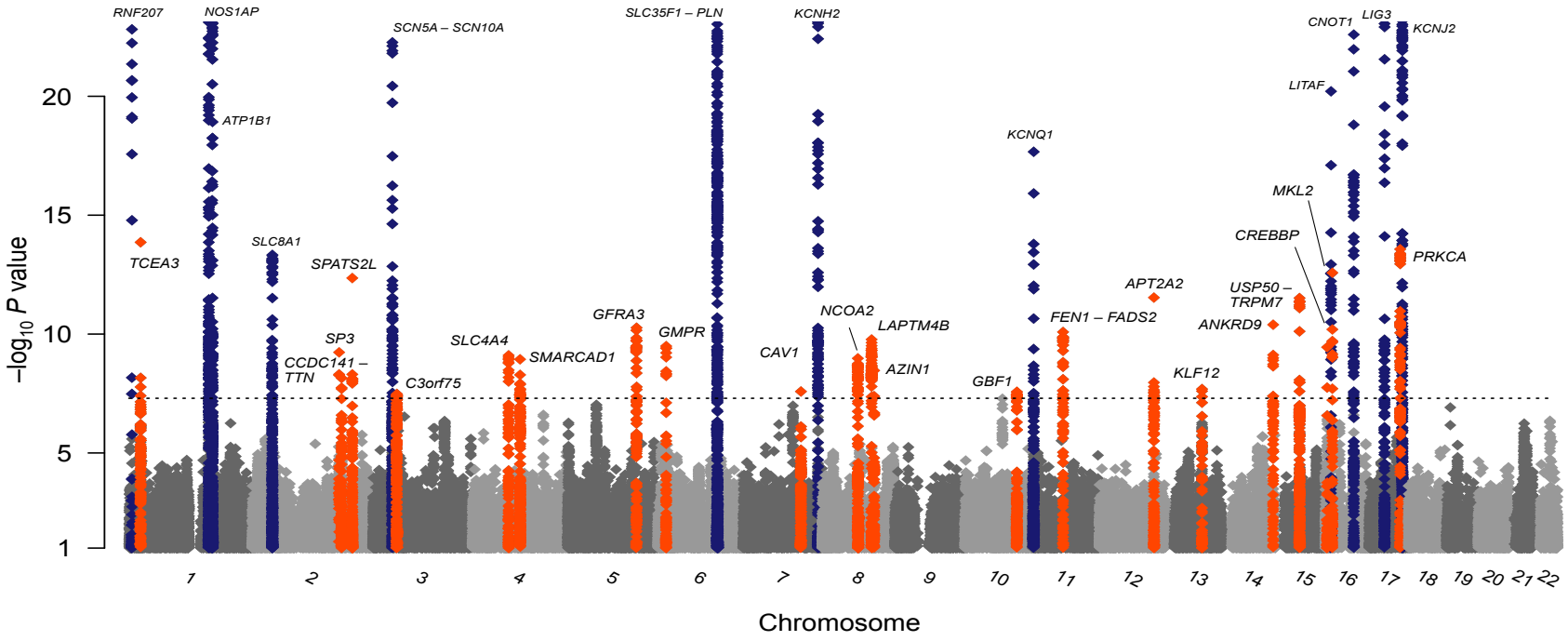
# QT-IGC consortium

- 76,061 European-derived individuals
- replication in 31,962 individuals
- QT adjusted for age, sex, RR interval
- excluding QRS > 120 ms, bundle branch block, AF, pacer
- HapMap2 imputation
- fixed effects meta-analysis



Arking...Newton-Cheh *Nature Genetics* 2014

# 35 loci (22 novel)
# 68 independent
# 10% of QT variation
# 25% of QT heritability




Arking...Newton-Cheh *Nature Genetics* 2014

# left ventricular expression QTLs

| QT SNP | chr | position | transcript | best eSNP for transcript | $r^2$ between QT SNP & eSNP | direction of eSNP effect for QT increasing allele | Transcript association of QT SNP (*P*) | Transcript association of QT SNP with adjustment for best eSNP (*P*) | attenuated signif | QT association of QT SNP (*P*) | QT association of best eSNP (*P*) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **rs17457880** | 1 | 160,434,778 | ***FCGR2B*** | rs17457880 | same | ↑ | $1x10^{-5}$ | 0.99 | YES | $3x10^{-10}$ | same |
| rs17457880 | 1 | 160,434,778 | *FCGR3A* | rs9727076 | 0.00 | ↑ | $1x10^{-7}$ | $9x10^{-9}$ | NO | $3x10^{-10}$ | NA |
| rs295140 | 2 | 200,868,944 | *SPATS2L* | rs295113 | 0.53 | ↓ | $8x10^{-7}$ | 0.74 | YES | $4x10^{-13}$ | 0.76 |
| **rs174583** | 11 | 61,366,326 | ***FADS2*** | rs174548 | 0.80 | ↓ | $6x10^{-8}$ | 0.94 | YES | $1x10^{-10}$ | $8x10^{-8}$ |
| **rs3026445** | 12 | 109,207,586 | ***VPS29*** | rs6606686 | 0.86 | ↑ | $1x10^{-6}$ | 0.84 | YES | $1x10^{-8}$ | $2x10^{-7}$ |
| **rs728926** | 13 | 73,411,123 | ***KLF12*** | rs1886512 | 0.93 | ↑ | $4x10^{-5}$ | 0.25 | YES | $2x10^{-8}$ | $4x10^{-8}$ |
| **rs735951** | 16 | 11,601,037 | ***LITAF*** | rs7187498 | 0.93 | ↑ | $4x10^{-13}$ | 0.62 | YES | $2x10^{-28}$ | NA |
| rs246196 | 16 | 57,131,754 | *SETD6* | rs42945 | 0.30 | ↑ | $4x10^{-7}$ | 0.20 | YES | $2x10^{-57}$ | $9x10^{-22}$ |
| **rs9892651** | 17 | 61,734,255 | ***PRKCA*** | rs11658550 | 0.97 | ↑ | $2x10^{-41}$ | 0.22 | YES | $3x10^{-14}$ | NA |

Arking...Newton-Cheh *Nature Genetics* 2014

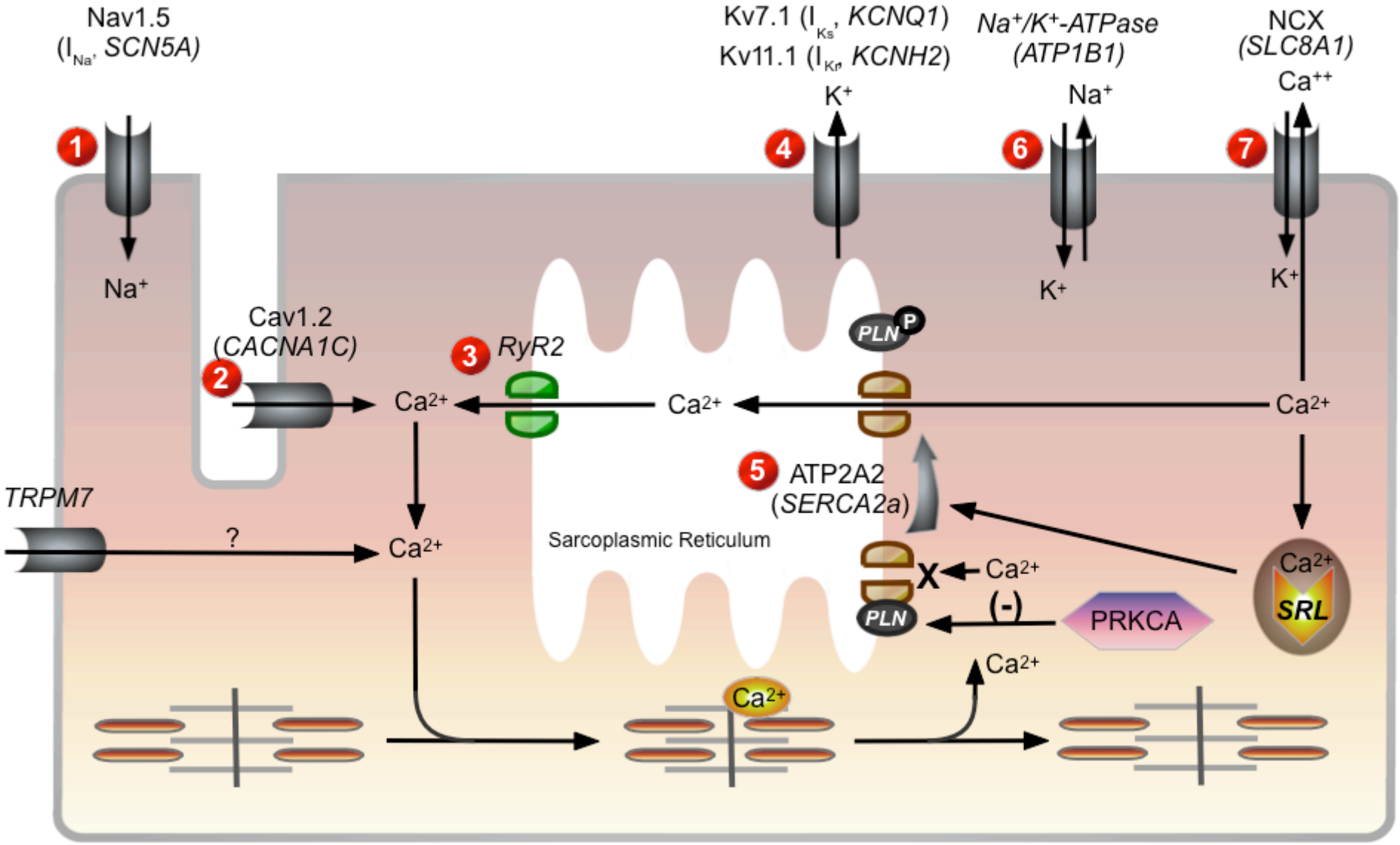
# genetically elusive Long QT Syndrome

- 298 probands free of *KCNQ1*, *KCNH2*, *SCN5A* mutations
- Amsterdam, London, Mayo, Nantes, Pavia, Toronto
- sequenced exons of *ATP2A2*, *CAV1*, *CAV2*, *SLC8A1*, *SRL*, *TRPM7*
- compared to controls, NHLBI ESP

# LQT proband mutations

| gene | amino acid change | # cases | in controls (yes/ no) | alt alleles in Exome Chip | in ESP | PolyPhen / SIFT |
|---|---|---|---|---|---|---|
| *ATP2A2* | p.Asn114Asp | 1 | no | no | no | BEN/TOL |
| ***ATP2A2*** | **p.Ile276fsX281** | **1** | **no** | **no** | **no** | **STOP** |
| *SLC8A1* | p.Ala368Val | 1 | no | no | 24/5379 | PROB/TOL |
| *SLC8A1* | p.Pro670Leu | 1 | no | no | no | BEN/DAM |
| *SLC8A1* | p.Val884Gly | 1 | no | no | no | PROB/DAM |
| *SRL* | p.Gly393Cys | 1 | no | no | no | PROB/DAM |
| *SRL* | p.Arg470Lys | 1 | no | no | no | BEN/TOL |
| *SRL* | p.Arg856Cys | 1 | no | no | 1/4915 | PROB/TOL |
| ***TRPM7*** | **p.Ile19fsX59** | **1** | **no** | no | **no** | **STOP** |
| *TRPM7* | p.Asp114Val | 1 | no | no | no | PROB/DAM |
| *TRPM7* | p.Ser1299Arg | 1 | no | no | no | BEN/TOL |
| *TRPM7* | p.Glu1342Asp | 2 | no | no | no | BEN/TOL |
| *TRPM7* | p.Leu1384Phe | 1 | no | no | no | POSS/TOL |

Arking...Newton-Cheh, *Nature Genetics* 2014


Nav1.5
(I$_{Na}$, SCN5A)
Kv7.1 (I$_{Ks}$, KCNQ1)
Kv11.1 (I$_{Kr}$, KCNH2)
Na+/K+-ATPase
(ATP1B1)
NCX
(SLC8A1)
Ca++
K+
Na+
K+
K+
1
2
3
4
5
6
7
Na+
Cav1.2
(CACNA1C)
RyR2
Ca2+
Ca2+
Ca2+
PLN
P
TRPM7
?
Ca2+
Sarcoplasmic Reticulum
ATP2A2
(SERCA2a)
X
Ca2+
(-)
PLN
PRKCA
Ca2+
SRL
Ca2+
Ca2+

# MOXIGEN
# genotype-directed physiology

- genotyped 1200 healthy volunteers
  - 18-40
  - no medications
  - normal electrolytes
- genotype score <20%ile vs >20%ile
- administer moxifloxacin (10msec ↑QT)
- assess whether genotype score modulates QT response

- Atrial natriuretic peptide & blood pressure
- cGMP regulating pathways emerge from BP GWAS
- QT interval variants & cardiotoxic drug response

# Why do clinical medicine and research?

- One informs the other
- Research questions posed can be framed in terms of clinical relevance
- Both are fun
- Caveat: strict discipline to manage time
  - Limit outpt panel
  - Skip inessential meetings
  - Prioritize, prioritize, prioritize
- You can't do both with equal focus

# Why do both basic and clinical research?

- As a physician-scientist, my target is human health
- Humans are good to study:
  - In vivo relevance of findings
- Humans are bad to study:
  - Close inbreeding frowned upon
  - Inability to control/standardize environmental exposures
- Let your findings drive the model

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Natriuretic peptide GWAS consortium

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