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 $\rightarrow$ Nt-ProANP + **ANP**

pro-BNP $\rightarrow$ Nt-ProBNP + **BNP**

corin / furin

corin

degradation by proteolysis

neprilysin
cGMP signaling cascade

- cytosolic [Ca^{+2}]
- cell permeability
- muscle contractility
Association of common variants in \textit{NPPA} and \textit{NPPB} with circulating natriuretic peptides and blood pressure

Christopher Newton-Cheh\textsuperscript{1–4}, Martin G Larson\textsuperscript{4,5}, Ramachandran S Vasan\textsuperscript{4,6,18}, Daniel Levy\textsuperscript{4,7,18}, Kenneth D Bloch\textsuperscript{2,8}, Aarti Surti\textsuperscript{3}, Candace Guiducci\textsuperscript{3}, Sekar Kathiresan\textsuperscript{1–4}, Emelia J Benjamin\textsuperscript{4,6}, Joachim Struck\textsuperscript{9}, Nils G Morgenthaler\textsuperscript{9}, Andreas Bergmann\textsuperscript{9}, Stefan Blankenberg\textsuperscript{10}, Frank Kee\textsuperscript{11}, Peter Nilsson\textsuperscript{12}, Xiaoyan Yin\textsuperscript{4}, Leena Peltonen\textsuperscript{13–15}, Erkki Vartiainen\textsuperscript{13}, Veikko Salomaa\textsuperscript{13}, Joel N Hirschhorn\textsuperscript{3,16,17}, Olle Melander\textsuperscript{12,19} & Thomas J Wang\textsuperscript{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 ($P = 8 \times 10^{-70}$)
  - decreased systolic blood pressure ($P = 2 \times 10^{-6}$)
  - decreased diastolic blood pressure ($P = 1 \times 10^{-6}$)
  - reduced odds of hypertension ($OR = 0.85$)

Newton-Cheh et al, Nature Genetics 2009
Genotype-directed physiologic study

Genotyping for rs5068
n=699, minor allele frequency 4%, healthy, European-derived, normotensive volunteers

AA genotype (low ANP) (n=23)

AG genotype (high ANP) (n=8)

High-sodium diet (4.6 g/day for 1 week)

Low-sodium diet (0.23 g/day for 1 week)

and

Overnight stay, 2 liters saline infusion over 2 hours with serial Nt-proANP measurements
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

Plasma Nt-proANP levels (nmol/L)

p<0.02 for genotype
p<0.001 for diet

~50%

Low salt High salt

Arora, JCI 2013
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.
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. Wang1,2,18

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 x 10^{-18}).

Arora, JCI 2013
miRNAs predicted to target the major A but not minor G allele of rs5068

Expressed in cardiac tissues:

rs5068

\[
\text{NPPA}
\]

no

\[
\text{miR-196a*}
\]

yes

\[
\text{miR-425}
\]

no

\[
\text{miR-4770}
\]

yes

\[
\text{AUGACACAAAUUGCAGCAGAGACC}
\]

\[
\text{CGGCAACAGAAACUGCCUGAG}
\]

\[
\text{AUGACACAAAUGCAGCAGAGACC}
\]

\[
\text{AAUGACACGAUCACUCCCGUUGA}
\]

\[
\text{AUGACACAAAUGCAGCAGAGACC}
\]

\[
\text{UGAGAUGACACUGUAGCU}
\]
Validation of the predicted binding of miR-425 with the *NPPA* 3’UTR

```
Luciferase  3’UTR with major allele

miR-425

Luciferase  3’UTR with minor allele

x

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

relative luciferase activity

Arora, JCI 2013
AntimiR-425 reverses *NPPA* 3’UTR inhibition
Anti-miR-425
(major allele vs. minor allele)

relative luciferase activity

Control  Major-LUC  Minor-LUC

Arora, JCI 2013
Induced pluripotent stem cell-derived human cardiomyocytes (AA genotype)

Relative NPPA mRNA levels

Nt-proANP levels (nmol/L)

p=0.0005

p=0.00001

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
• Atrial natriuretic peptide & blood pressure
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LETTER

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

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

\[
\text{pro-ANP} \xrightarrow{\text{corin}} \text{Nt-ProANP} + \text{ANP} \xrightarrow{\text{neprilysin}} \text{degradation by proteolysis} \\
\text{pro-BNP} \xrightarrow{\text{corin / furin}} \text{Nt-ProBNP} + \text{BNP} \xrightarrow{\text{corin / furin}} \text{degradation by proteolysis}
\]
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)
Riociguat for the Treatment of Pulmonary Arterial Hypertension

Hossein-Ardeschir Ghofrani, M.D., Nazzareno Galiè, M.D., Friedrich Grimminger, M.D., Ekkehard Grüning, 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

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
**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 ($2 \times 10^{-9}$)
- mouse KO $\rightarrow$ 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
• $NPPA-NPPB \rightarrow$ ANP/BNP production
• $NPR3 \rightarrow$ ? ANP/BNP clearance vs signalling
• furin $\rightarrow$ BNP activation
• eNOS $\rightarrow$ NO production
• $GUCY1A3-GUCY1B3$ $\rightarrow$ SGC $\alpha_1, \beta_1$ prodn
• $PDE3A$ (cGMP/cAMP)
Soluble guanylate cyclase

- Allele-specific enhancer assays luciferase constructs
- Recruiting healthy volunteers by sGC genotype
- Administering iNO, measuring plasma cGMP
- Platelet aggregation by ADP, NO donor
- Expression α1, β1 subunit
• Atrial natriuretic peptide & blood pressure
• cGMP regulating pathways emerge from BP GWAS
• QT interval variants & cardiotoxic drug response
• Atrial natriuretic peptide & blood pressure
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QT prolongation & sudden cardiac death

general population
Schouten 91
Algra 91
Siscovick 96
Straus 06

CAD
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
## left ventricular expression QTLs

<table>
<thead>
<tr>
<th>QT SNP</th>
<th>chr</th>
<th>position</th>
<th>transcript</th>
<th>best eSNP for transcript</th>
<th>$r^2$ between QT SNP &amp; eSNP</th>
<th>direction of eSNP effect for QT increasing allele</th>
<th>Transcript association of QT SNP ($P$)</th>
<th>Transcript association of QT SNP with adjustment for best eSNP ($P$)</th>
<th>attenuated signif</th>
<th>QT association of QT SNP ($P$)</th>
<th>QT association of best eSNP ($P$)</th>
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<td>rs17457880</td>
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<td>160,434,778</td>
<td>FCGR2B</td>
<td>rs17457880</td>
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<td>$\uparrow$</td>
<td>1x10^{-5}</td>
<td>0.99</td>
<td>YES</td>
<td>3x10^{-10}</td>
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<td>1x10^{-7}</td>
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<td>109,207,586</td>
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<td>1x10^{-6}</td>
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<td>13</td>
<td>73,411,123</td>
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<td>rs1886512</td>
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<td>4x10^{-8}</td>
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<td>16</td>
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<td>3x10^{-14}</td>
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</table>

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

<table>
<thead>
<tr>
<th>gene</th>
<th>amino acid change</th>
<th># cases</th>
<th>in controls (yes/no)</th>
<th>alt alleles in Exome Chip</th>
<th>in ESP</th>
<th>PolyPhen / SIFT</th>
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<tr>
<td>ATP2A2</td>
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<td>BEN/TOL</td>
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<td><strong>no</strong></td>
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<tr>
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<td>no</td>
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<td>no</td>
<td>no</td>
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<td>no</td>
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<td>POSS/TOL</td>
</tr>
</tbody>
</table>

Arking...Newton-Cheh, *Nature Genetics* 2014
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
Queen Mary University of London
Patricia Munroe
Mark Caulfield

International Consortium of Blood Pressure GWAS

Natriuretic peptide GWAS consortium

QT Interval – International GWAS consortium

Johns Hopkins
Aravinda Chakravarti
Georg Ehret

Erasmus Medical Center, Netherlands
Mark Eijgelsheim
Bruno Stricker
Cornelia van Duijn

Helsinki, Finland
Veikko Salomaa
Aki Havulinna

Malmö Diet and Cancer
Gustav Smith
Olle Melander
Cristiano Fava

Vanderbilt University
Thomas Wang

Supported by the
MGH Clinical Research Center, Catalyst Pilot
MGH Interim Support Fund
National Institutes of Health (HL098283, HL113933)
NHLBI Exome Sequencing Project
Doris Duke Charitable Foundation
Burroughs Wellcome Fund