Goals of biomedical investigation

- Understand pathophysiology of human disease
- Enable early diagnosis
- Enable prevention
- Enable new effective treatments
Three eras of disease gene discovery

- Discovery of genes for recognized Mendelian diseases
  - Driven by complete genetic maps

- Discovery of common variants in common disease
  - Driven by dense SNP genotyping

- Discovery of rare variants in not previously recognized Mendelian diseases and common diseases
  - Driven by high throughput sequencing
Pathophysiology transformed by genetics

- BRCA1 and breast cancer
- Fat-hypothalamic axis: Leptin, MC4R
- APP and $\gamma$-secretase mutations in Alzheimer’s
- Orexin system and sleep-wake regulation
- ApoL1 and African American ESRD
- IDH1 and glioblastoma multiforme
- Innate immunity and autophagy in IBD
- Nav1.7 and pain sensation
Hypertension

- Blood pressure > 140/90
- Affects 1.2 billion people
- Major risk factor for:
  - MI: 7.1 M deaths/year
  - Stroke: 5.5 M deaths/year
- Treatment:
  - 2/3 poorly controlled
  - Most require ≥ 3 drugs
- Pathogenesis unknown
Mutations that alter blood pressure

- Gitelman syndrome
- WNK1,4 PHA II
- Angiotensinogen
- Renin
- Al
- ACE
- All
- All receptor
- APA
- 1° aldo
- 17α-hydroxylase deficiency
- 11β-hydroxylase deficiency
- 21-hydroxylase deficiency
- Aldosterone
- DOC
- Gra
- Liddle Syndrome
- Recessive PHA 1
- Dominant PHA 1
- Hypertension exacerbated by pregnancy
- Cortisol
- AME
- Cortisone
- Bartter syndrome Type 1
- Bartter syndrome Type 2
Gain and loss of function mutations in the same gene drive bp across complete human spectrum.
Salt and blood pressure

- If salt is so important:
  - Why aren’t diuretics more effective as single agents?
  - Why is the epidemiologic data relating salt and BP so weak?
GIT140, a 9-Generation Gitelman's Syndrome Kindred
Genetic deficiency of Na-Cl cotransport induces marked increase in dietary salt intake

![Graph showing Urine Na/Cr (mmol/mmol) vs Na-Cl cotransporter genotype]
Common variants affecting intracranial aneurysm (6,000 cases, 14,000 controls from Europe, Asia, US)

These 6 loci explain 5% of the world-wide risk of hemorrhage from aneurysm

- Risk varies 4-fold across the top and bottom 5% of genetic risk

Significant loci
(p < 5 x 10^-8)

- CDKN2A/N2B
- Sox17
- RBBP8
- Endothelin receptor A
- 13q13.1
- 10q22.34

Nature Genetics, 2008, 2010
Common variants and blood pressure

- GWAS and follow-up in BPGen and CHARGE consortia (79,000 - 134,000 subjects per locus)

<table>
<thead>
<tr>
<th>Locus</th>
<th>Trait</th>
<th>mmHgΔ</th>
<th>Variance explained</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p36</td>
<td>SBP</td>
<td>-0.85</td>
<td>0.07%</td>
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<tr>
<td>10q24</td>
<td>SBP</td>
<td>1.16</td>
<td>0.08%</td>
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<td>SBP</td>
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<td>4q21</td>
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<tr>
<td>15q24</td>
<td>DBP</td>
<td>0.43</td>
<td>0.07%</td>
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Nature Genetics, 2009
Rare mutations in Framingham in genes in which homozygous mutations cause hypotension: **NCCT, NKCC2** and **ROMK**

- Identify all sequence variants in 3125 members of Framingham Heart Study

- Identify likely functional variants:
  - Variants at sites conserved from invertebrates to humans, function confirmed biochemically

Nature Genetics, 2008
• 1.6% of population heterozygous for mutations at completely conserved sites

• All are very rare, with allele frequency 1/2000 - 1/40000

• Half known LOF from prior genetics and biochemistry

• Virtually all of remainder since shown to be biochemical LOF
Heterozygous loss of function mutations in NCCT, ROMK and NKCC2 reduce blood pressure and protect from hypertension.
Whole exome sequencing

Single Illumina GAIIx lane:

- Mean 100x coverage of targeted bases
- 96% of heterozygous positions identified by SNP genotyping
- >99% of heterozygous calls validate by Sanger sequencing

Total direct cost (capture, sequencing, labor, machine and IT hardware) = $2500
Sequence production
Yale Center for Genome Analysis
Sequence production
Yale Center for Genome Analysis
Applications of exome sequencing

- Disease gene discovery
  - Previously unmappable Mendelian loci
    - Dominant reproductive lethals
    - Recessive traits with high locus heterogeneity
  - Somatic mutations in tumors
  - Rare mutations with moderate effect in common disease

- Clinical diagnosis
Clinical diagnosis by whole exome sequencing

- 5 month-old male with failure to thrive, volume depletion
- High renin, aldosterone
- Diagnosis?

Whole exome sequencing: Homozygous SLC26A3 mutation

![Genetic pedigree and sequence data](image)

PNAS, 2009
Cohort of subjects with malformation of cortical development from consanguineous union
Highly heterogeneous and unmappable
WDR62 mutations in 7 kindreds with microcephaly, migration defect and folding defects

Nature, 2010
Ichthyosis with confetti

Sporadic cases with defective barrier function and thousands of confetti-like spots

Science, 2010
De novo mutations in *Keratin 10* in IWC all result in frameshift into the same arginine-rich alternative reading frame.
Mutant K10 is mislocalized to the nucleolus
Aldosterone-producing adenoma (APA)

- Found in 5% of patients with severe hypertension
- Benign tumors, virtually never undergo malignant degeneration
- Are there mechanisms linking constitutive proliferation and constitutive hormone release?
Only 2.25 protein-altering somatic mutations per tumor; K⁺ channel *KCNJ5* is mutated twice

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Gene</th>
<th>Base change</th>
<th>Effect on protein</th>
<th># of reads from tumor</th>
<th># of reads from blood</th>
<th>p-value</th>
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<tbody>
<tr>
<td></td>
<td></td>
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<td>Ref. allele</td>
<td>Non-ref. allele</td>
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<td>KCNJ5</td>
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<td>T&gt;G</td>
<td>R3429S</td>
<td>60</td>
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<td>80</td>
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</table>
8 of 22 adosterone-producing adenomas have somatic G151R or L168R mutations in *KCNJ5* (p of occurrence by chance < $10^{-30}$)
G151R and L168R mutations lie in and near the KCNJ5 selectivity filter.
Membrane depolarization is the sufficient signal for both aldosterone secretion and cell proliferation.
Mendelian aldosteronism with massive adrenocortical hyperplasia: *KCNJ5 T158A* mutation
Past views on salt and blood pressure

• “One thing we know for certain. Salt does not cause high blood pressure.”
  - The Salt Institute
Changed views on salt and blood pressure

• Reduction in net salt balance now recognized as key goal of therapy by WHO and NHLBI Joint National Commission on Prevention, Diagnosis Evaluation and Treatment of Hypertension

• Early use of combination of diuretic + inhibitor of renin-angiotensin recognized as key combination
Impact on prevention:
Projected impact of 3g per day (25%) decrease in salt intake in US

# Strokes: \( \downarrow \text{32,000 - 66,000} \)
# Myocardial infarctions: \( \downarrow \text{54,000 - 99,000} \)
# Deaths from any cause: \( \downarrow \text{44,000 - 92,000} \)

Health care cost: \( \downarrow \text{$10B - $24B} \)

Goldman, NEJM, 2010
National Salt Reduction Initiative

Reduce dietary salt 25% by reducing salt in processed and restaurant foods
**Impact on new therapeutics**

Genetic targets for antihypertensive treatment

<table>
<thead>
<tr>
<th>Gene</th>
<th>Blood pressure</th>
<th>Serum K⁺</th>
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<tbody>
<tr>
<td>NCCT</td>
<td>↓</td>
<td>↓↓↓</td>
</tr>
<tr>
<td>MR</td>
<td>↓</td>
<td>↑</td>
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<tr>
<td>Aldo synthase</td>
<td>↓</td>
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<td>↓</td>
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<tr>
<td>ENaC</td>
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<td>↑↑↑↑</td>
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<tr>
<td>CLCNKB</td>
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<tr>
<td><strong>ROMK</strong></td>
<td>↓↓↓↓</td>
<td>←→</td>
</tr>
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</table>
Use of sequencing in clinical practice

- Why? Identify mutations that establish diagnosis or markedly change estimates of susceptibility or which dictate therapy
- Who? (Healthy or disease?)
- If healthy, when?
- How do we deal with incomplete understanding?
- How do we communicate results?
- Implications for education of health care professionals, patients, health and social policy
• Need to help industry focus on the best targets and prosecute them with passion!