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 and common diseases
  - **Driven by high throughput sequencing**

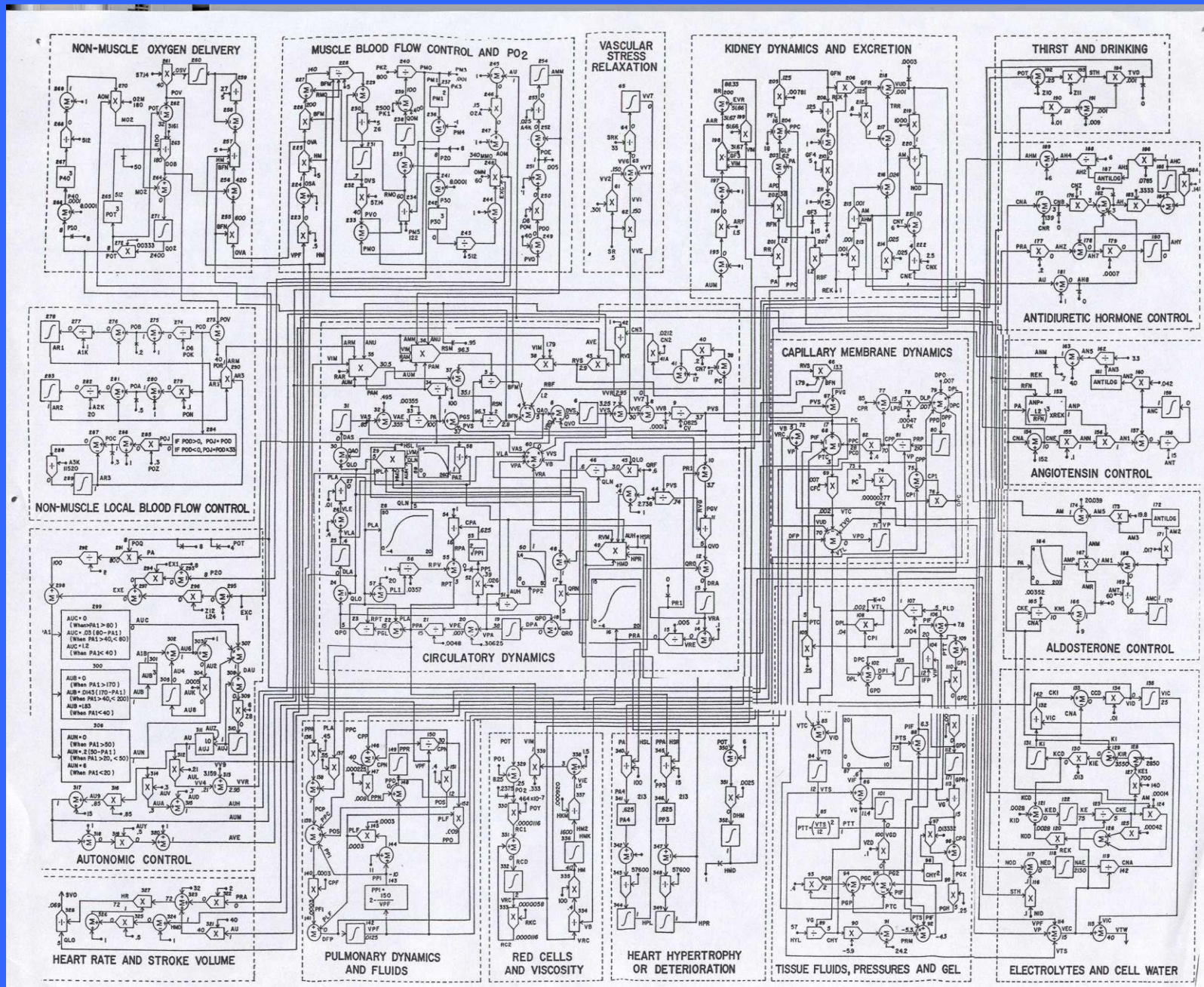
Pathophysiology transformed by genetics

- BRCA1 and breast cancer
- Fat-hypothalamic axis: Leptin, MC4R
- APP and γ–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

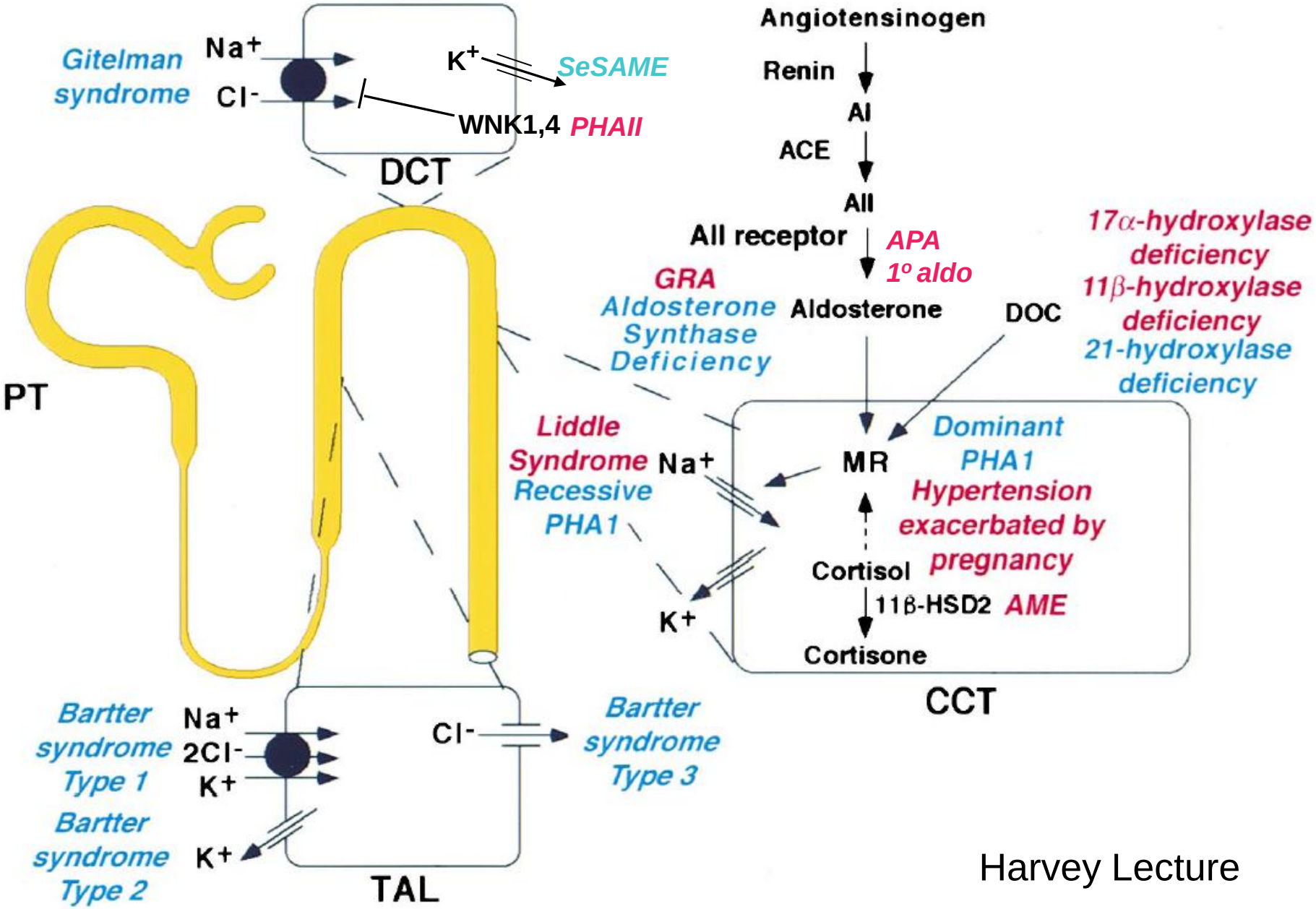
Hypertension

- 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

Integrated model of blood pressure homeostasis

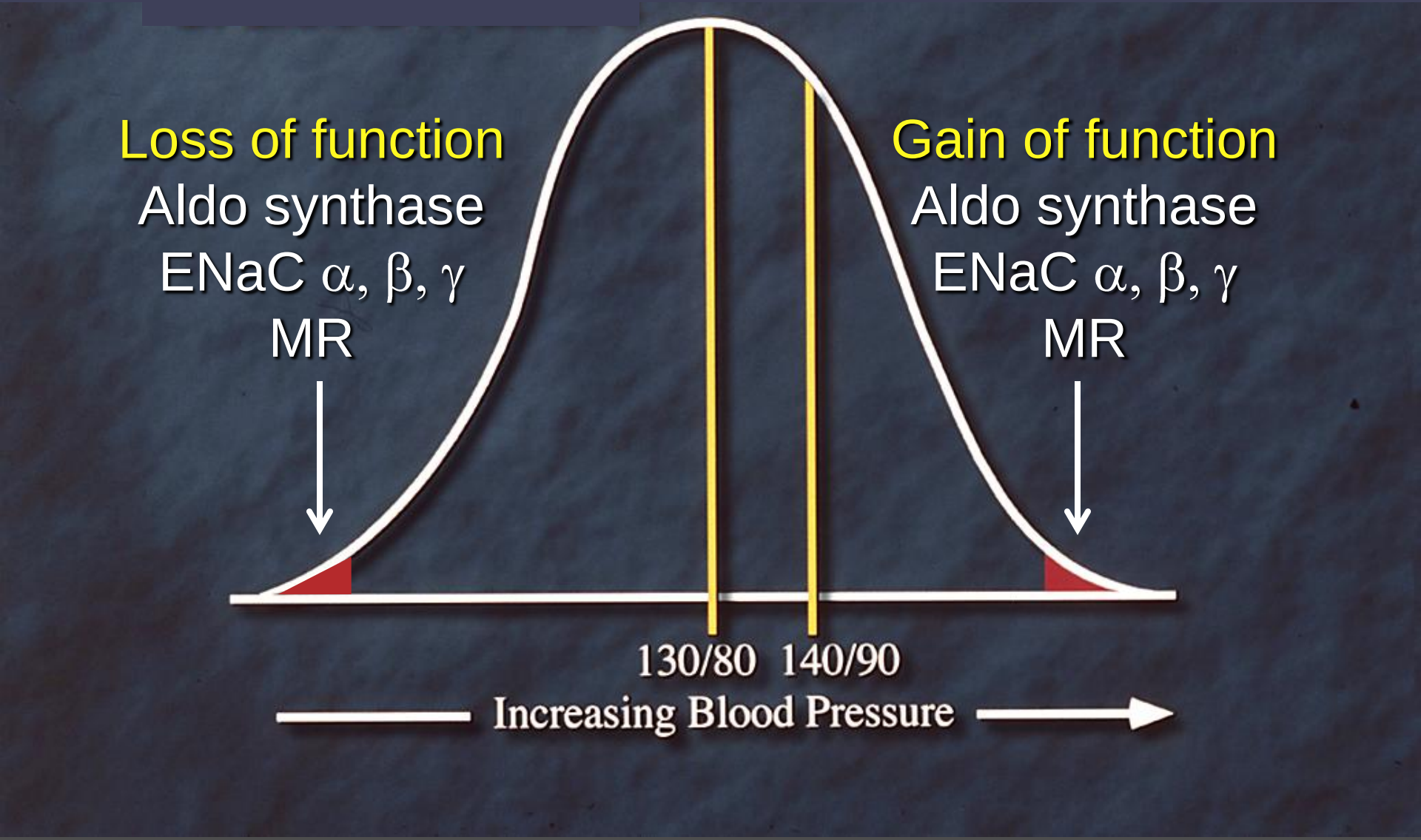


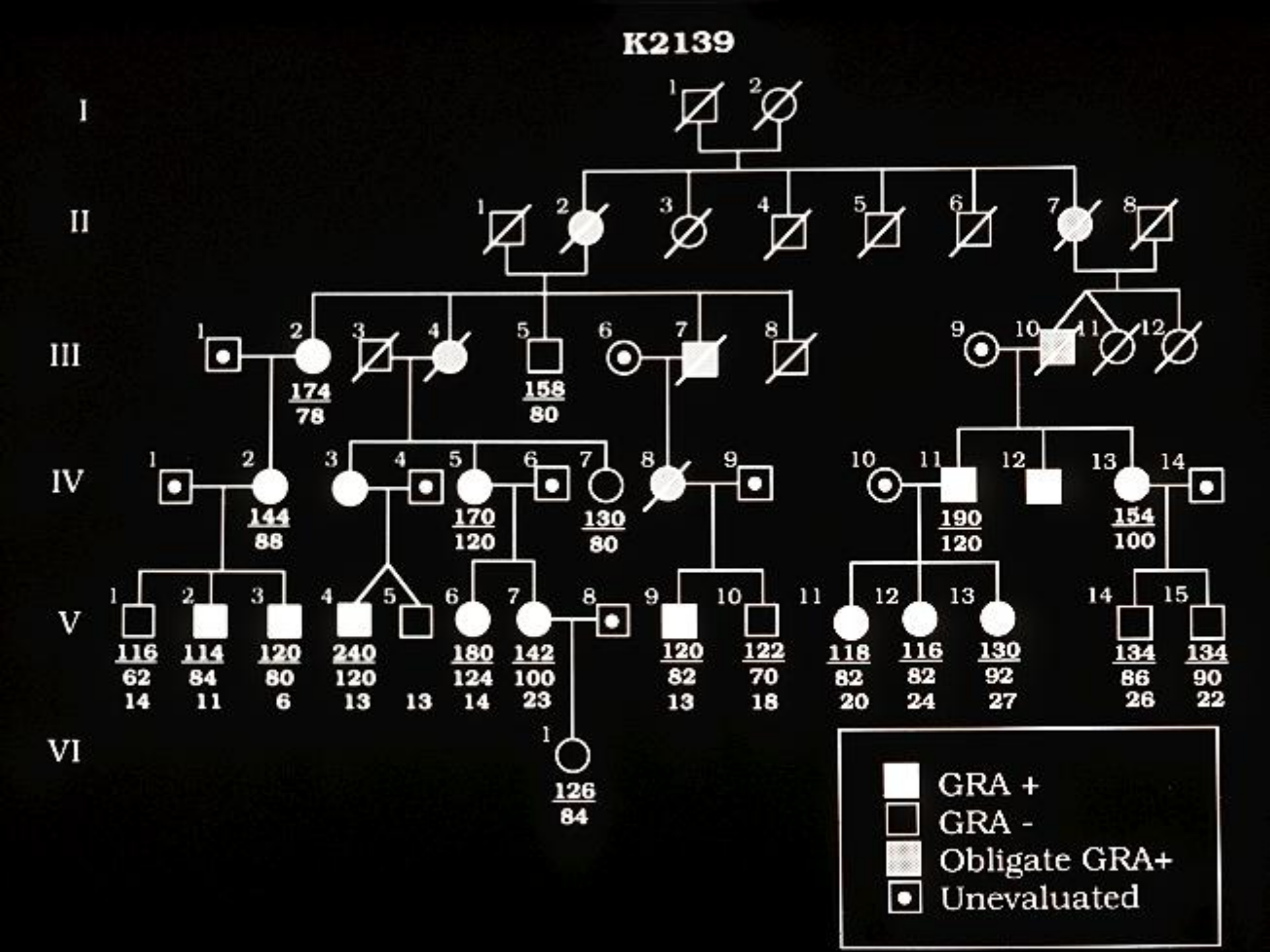
Mutations that alter blood pressure



Harvey Lecture

Gain and loss of function mutations in the same gene drive bp across complete human spectrum




K2139
I
II
III
IV
V
VI
174
78
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116
62
14
114
84
11
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80
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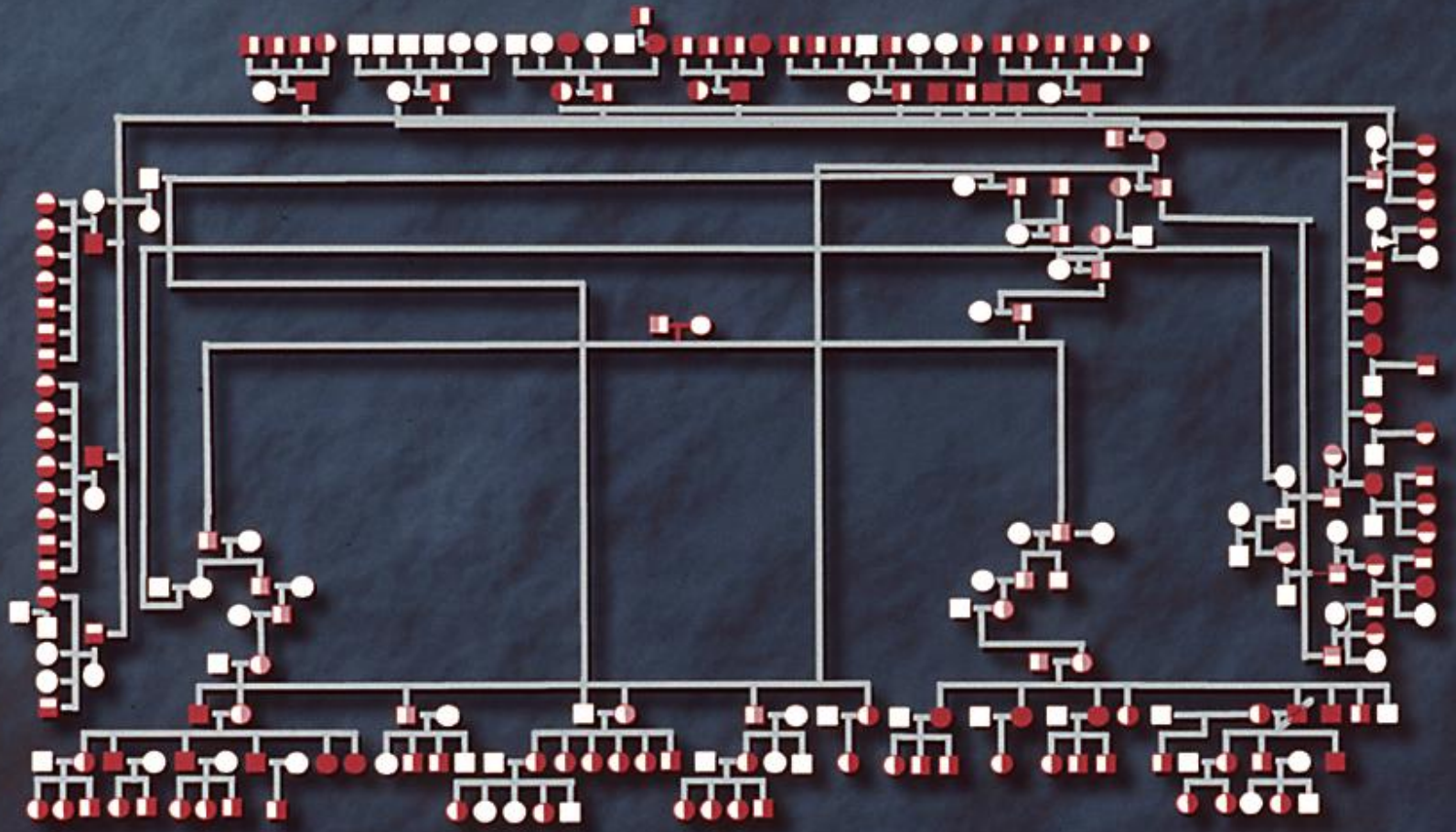
240
120
13
13
180
124
14
142
100
23
120
82
13
122
70
18
118
82
20
116
82
24
130
92
27
134
86
26
134
90
22
126
84
GRA +
GRA -
Obligate GRA+
Unevaluated

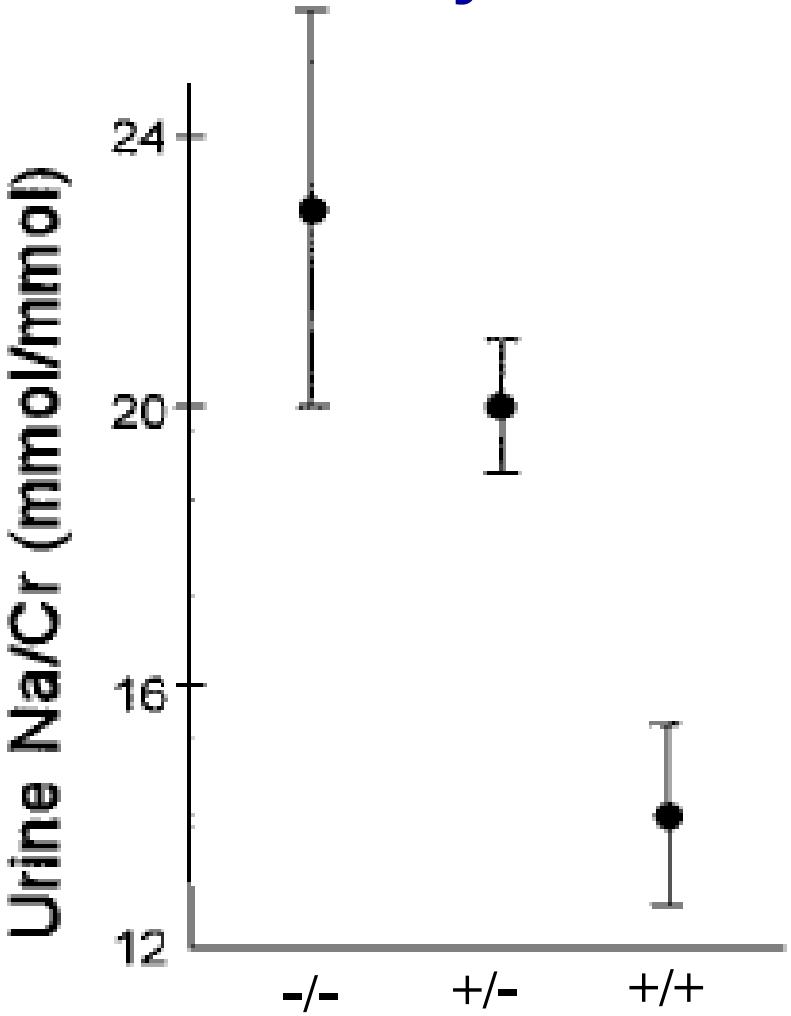
Salt and blood pressure

- 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?

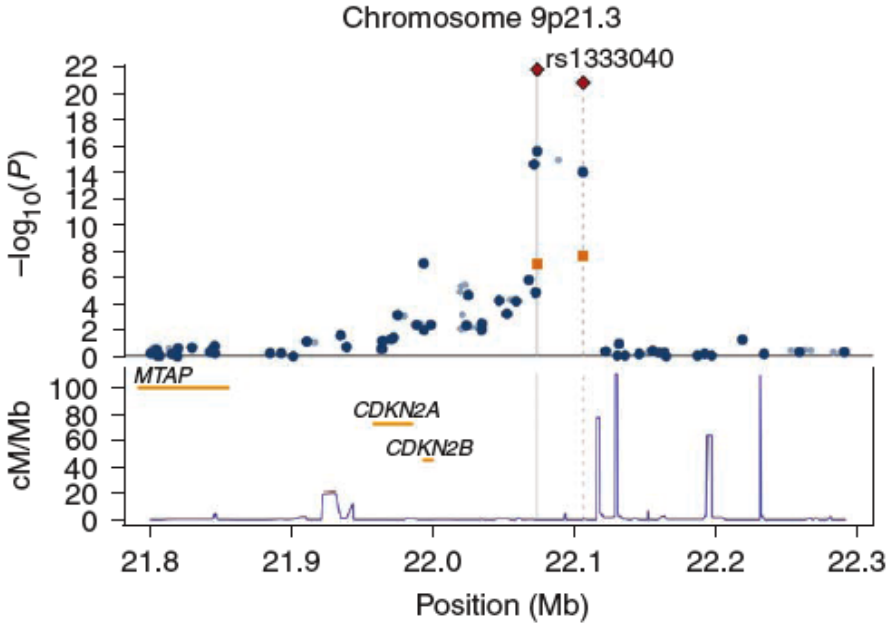
GIT140,a 9-Generation Gitelman's Syndrome Kindred



Genetic deficiency of Na-Cl cotransport induces marked increase in dietary salt intake



Common variants affecting intracranial aneurysm (6,000 cases, 14,000 controls from Europe, Asia, US)



Significant loci ($p < 5 \times 10^{-8}$)

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

- 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

Nature Genetics, 2008, 2010

Common variants and blood pressure

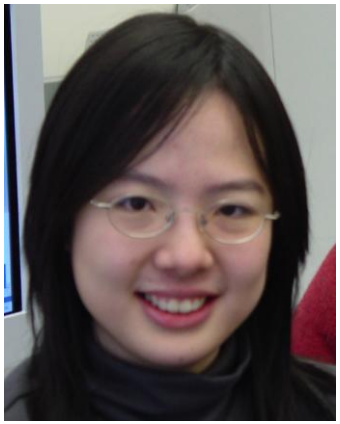
- GWAS and follow-up in BPGen and CHARGE consortia (79,000 - 134,000 subjects per locus)

Locus	Trait	mmHgΔ	Variance explained
1p36	SBP	-0.85	0.07%
10q24	SBP	1.16	0.08%
17q21	SBP	0.57	0.04%
4q21	DBP	0.50	0.09%
10q21	DBP	-0.39	0.04%
12q24	DBP	-0.46	0.09%
15q24	DBP	0.43	0.07%

Nature Genetics, 2009

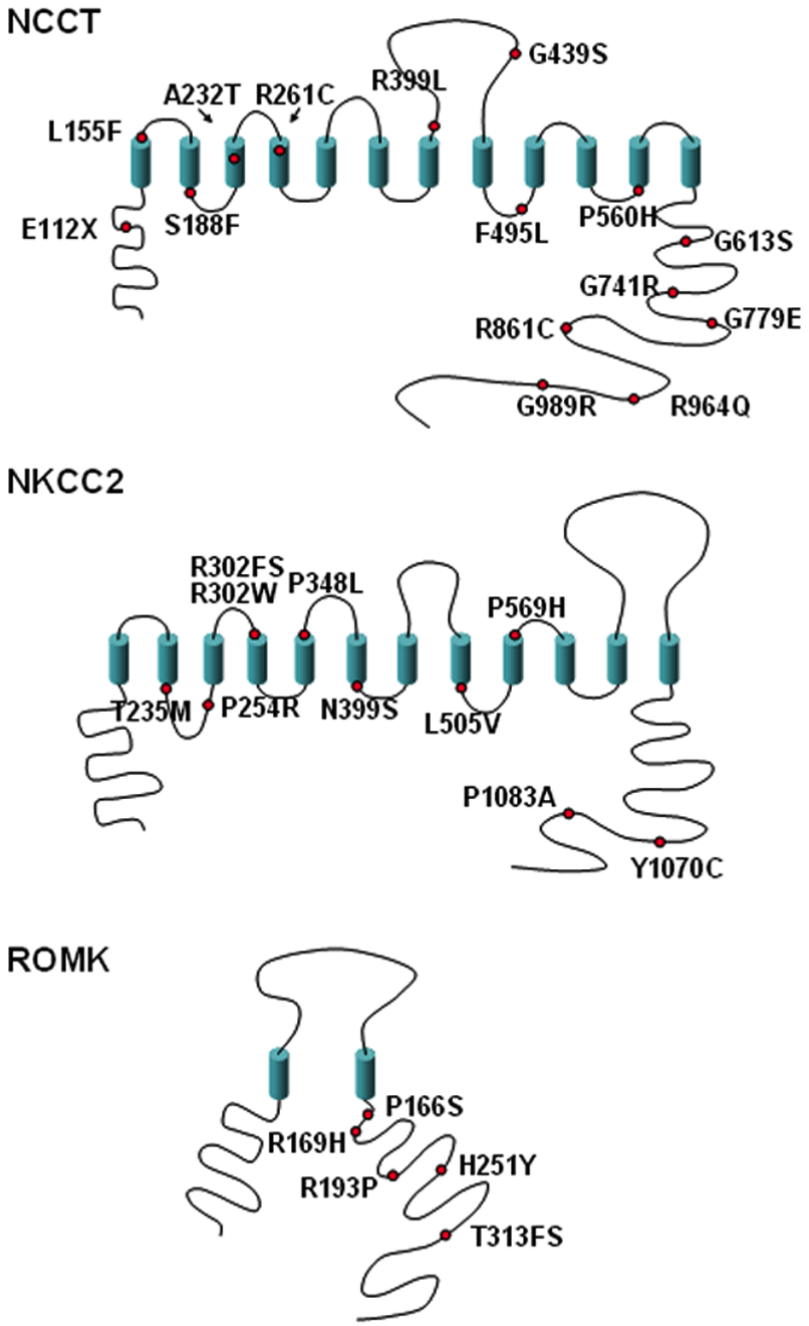
Rare mutations in Framingham in genes in which homozygous mutations cause hypotension: *NCCT*, *NKCC2* and *ROMK*

- 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

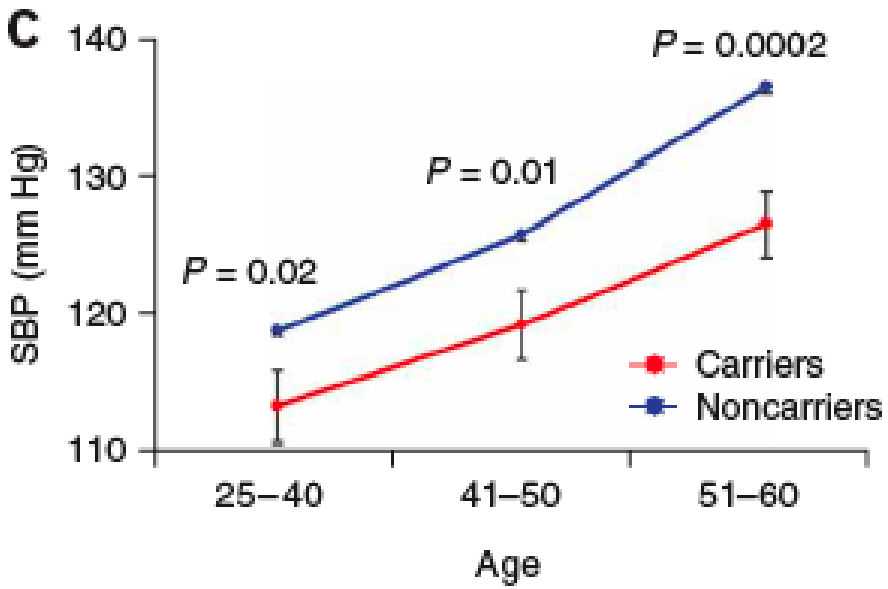


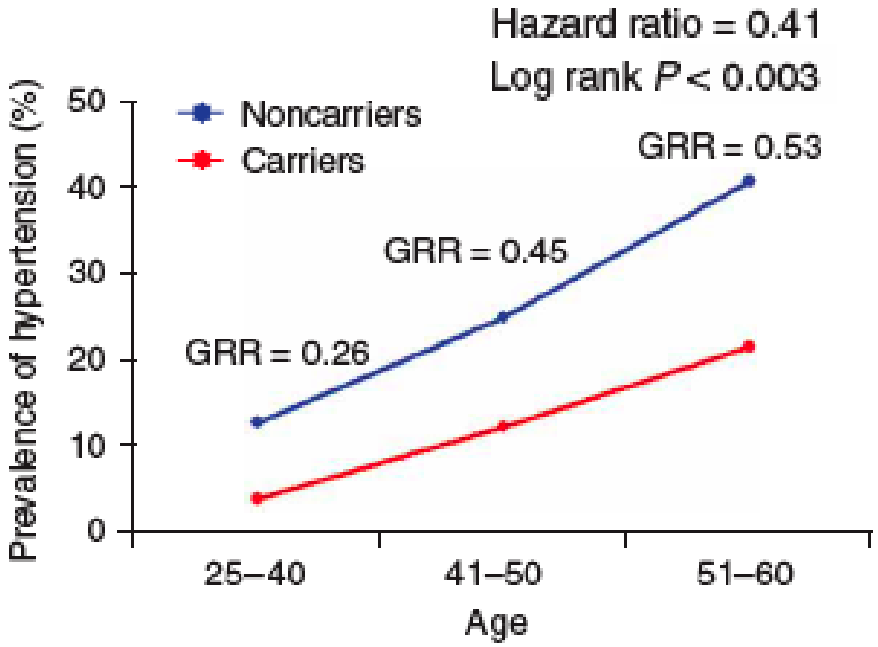
Nature Genetics, 2008

- **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**

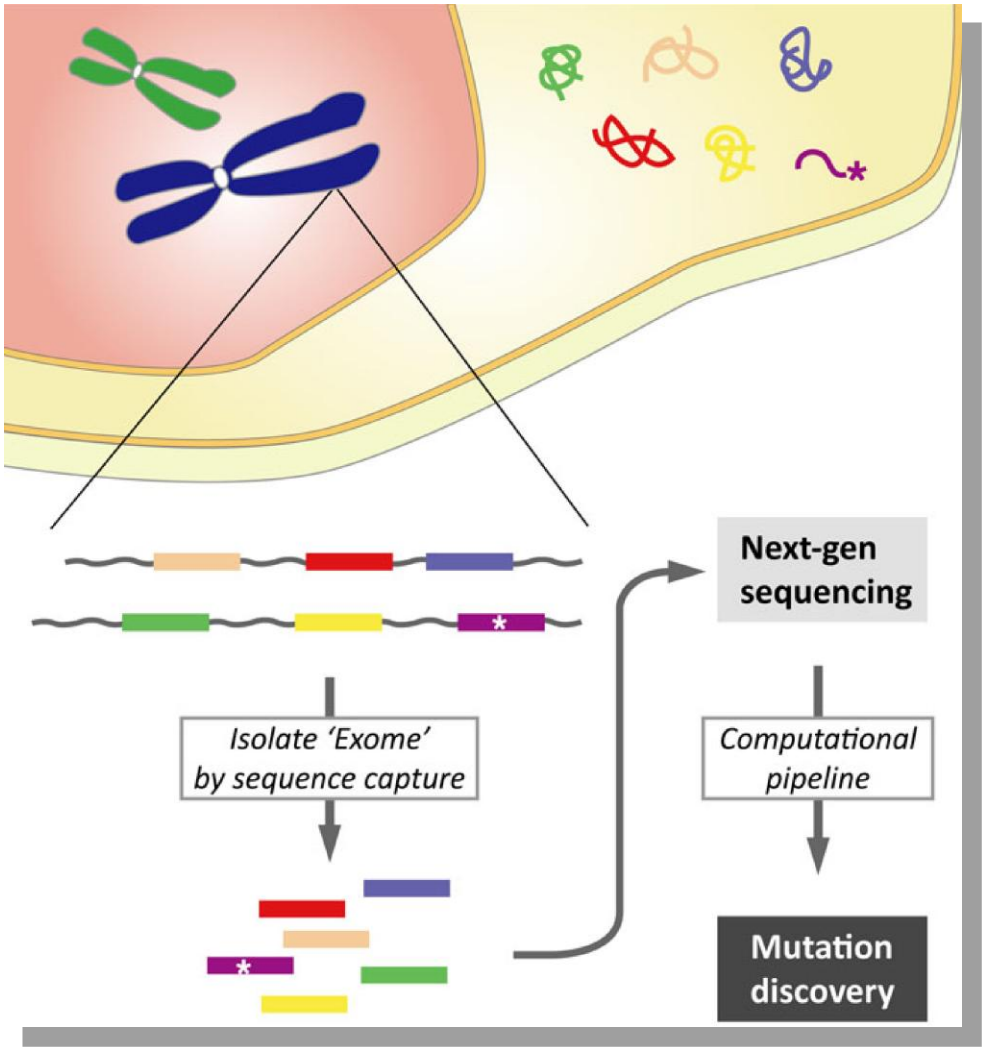


Heterozygous loss of function mutations in NCCT, ROMK and NKCC2 reduce blood pressure and protect from hypertension





Whole exome sequencing



Single Illumina GAIIx lane:

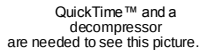
- Mean100x coverage of targeted bases
- 96% of heterozygous positions
  by SNP genotyping identified
- >99% of heterozygous calls
  validate by Sanger sequencing

→

- Total direct cost (capture, sequencing, labor, machine

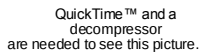
Sequence production

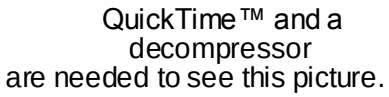
Yale Center for Genome Analysis



Sequence production

Yale Center for Genome Analysis



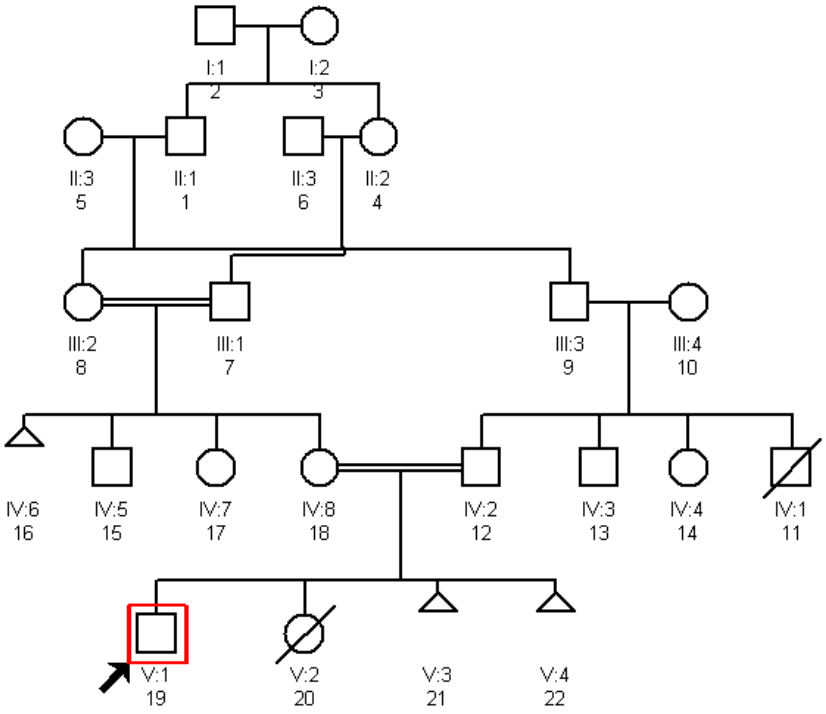


Applications of exome sequencing

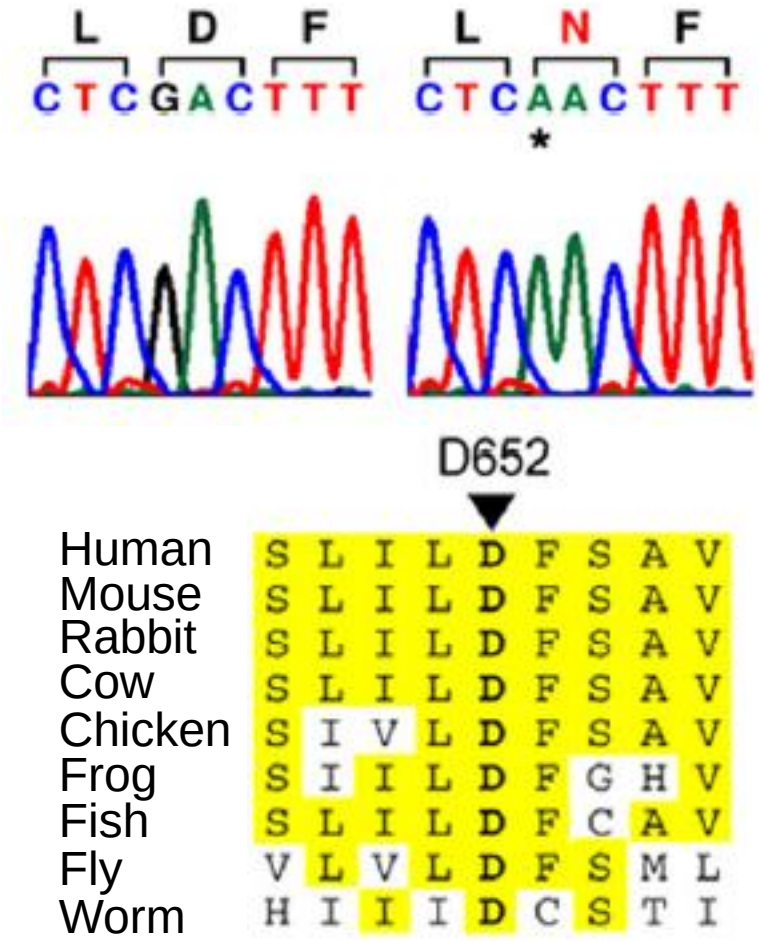
- Disease gene discovery
  - Previously unmappable Mendelian loci
    - Dominant reproductive lethals
    - Recessive traits with high locus heterogeneity
  - Somatic mutations in tumors
  - Rare mutations with moderate effect in common disease
- Clinical diagnosis

Clinical diagnosis by whole exome sequencing

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

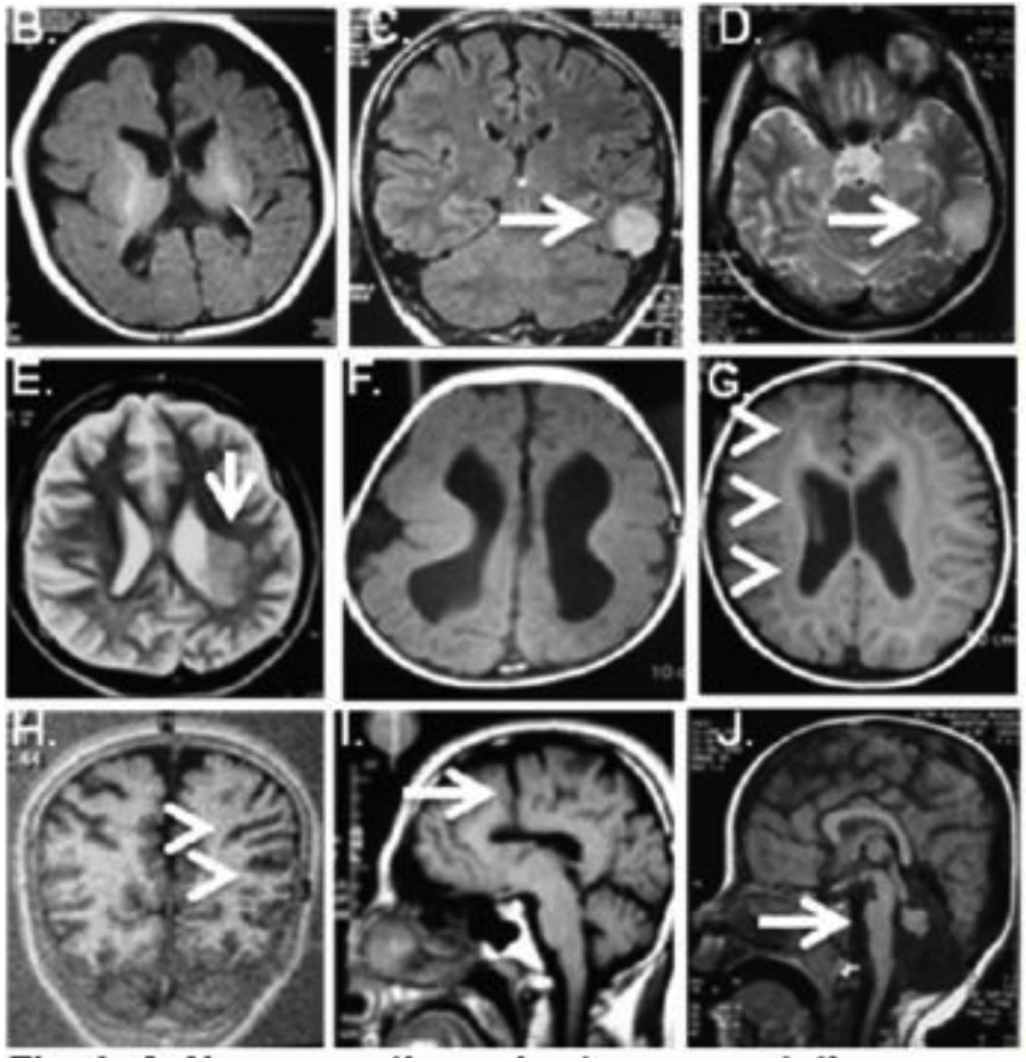


Whole exome sequencing:
Homozygous *SLC26A3* mutation

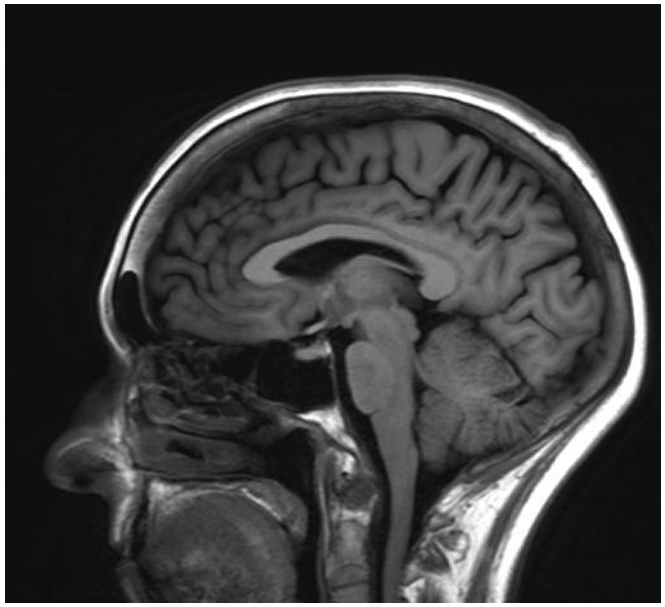


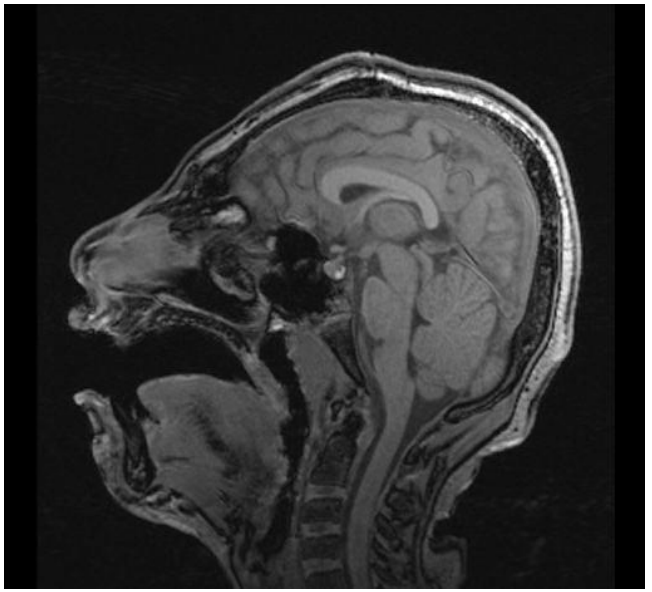
PNAS, 2009

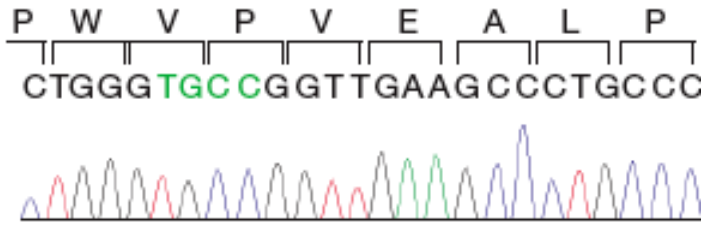
Cohort of subjects with malformation of cortical development from consanguineous union Highly heterogeneous and unmappable

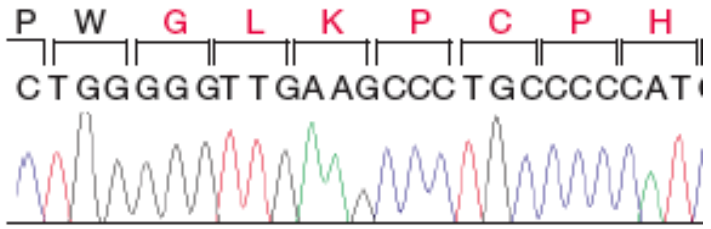


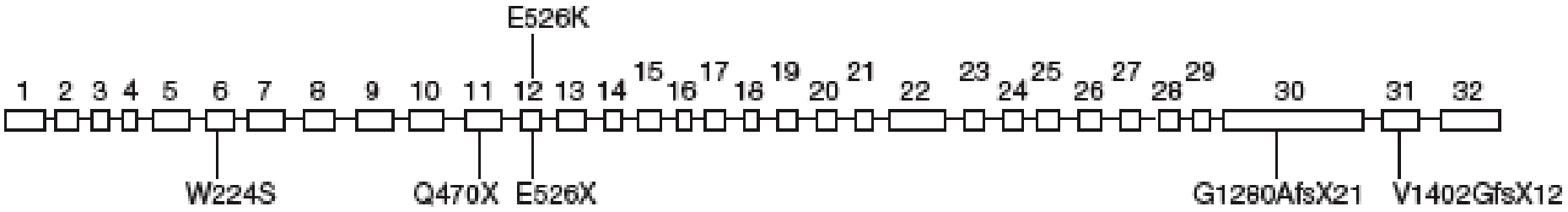
WDR62 mutations in 7 kindreds with microcephaly, migration defect and folding defects







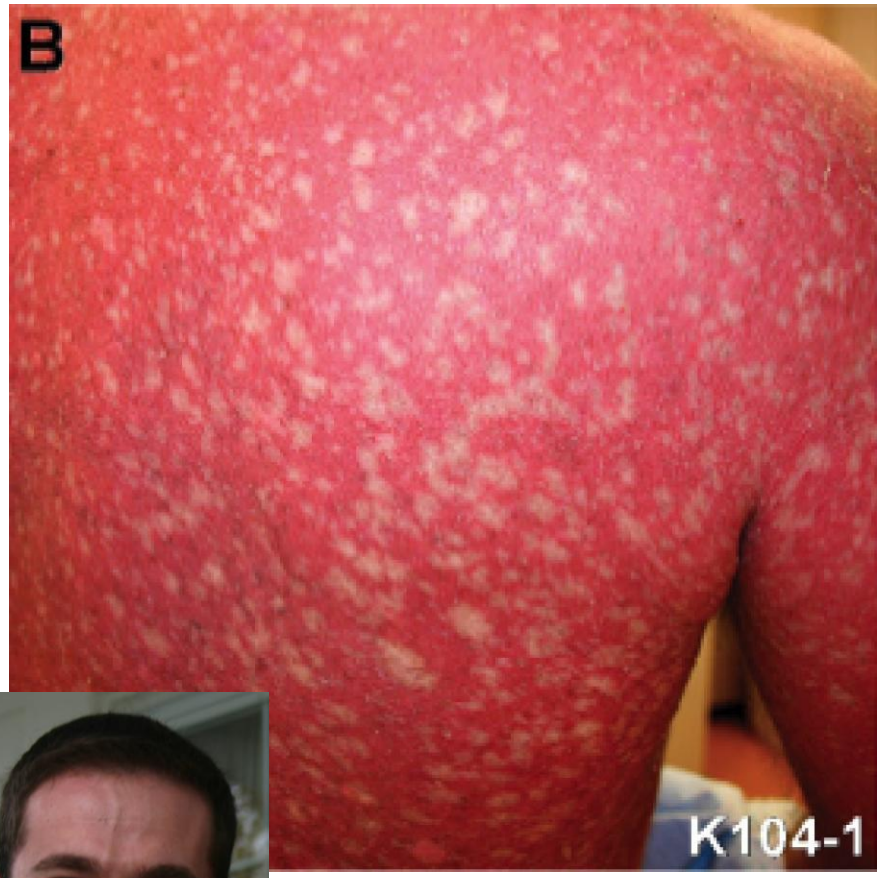


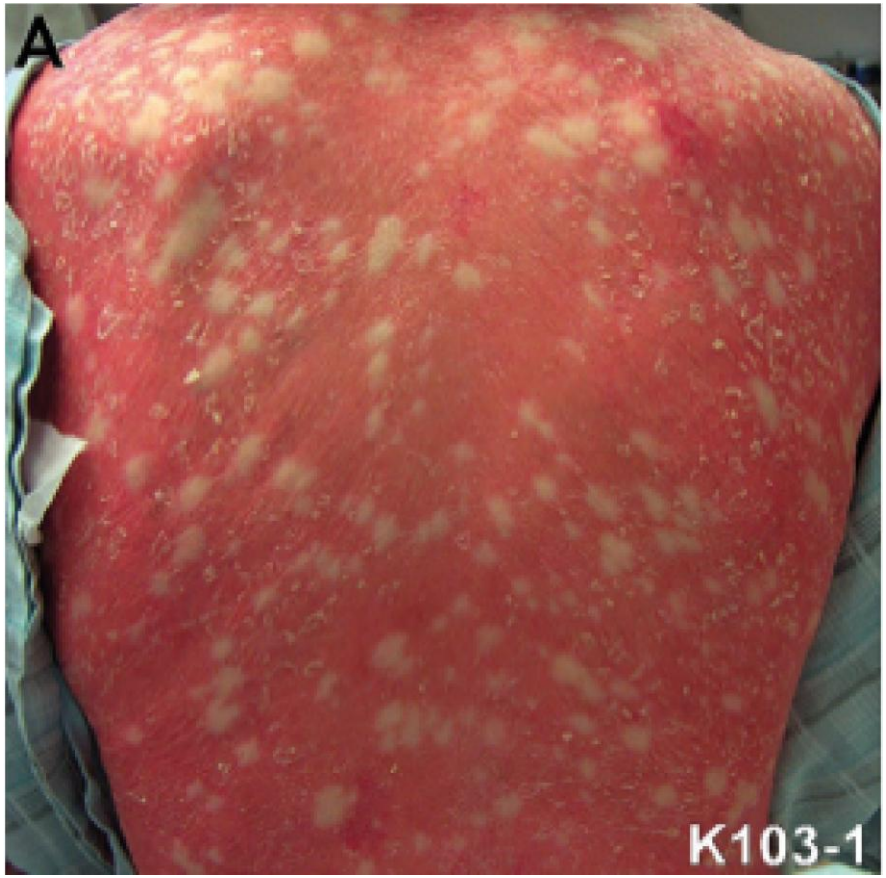


Nature, 2010

Ichthyosis with confetti

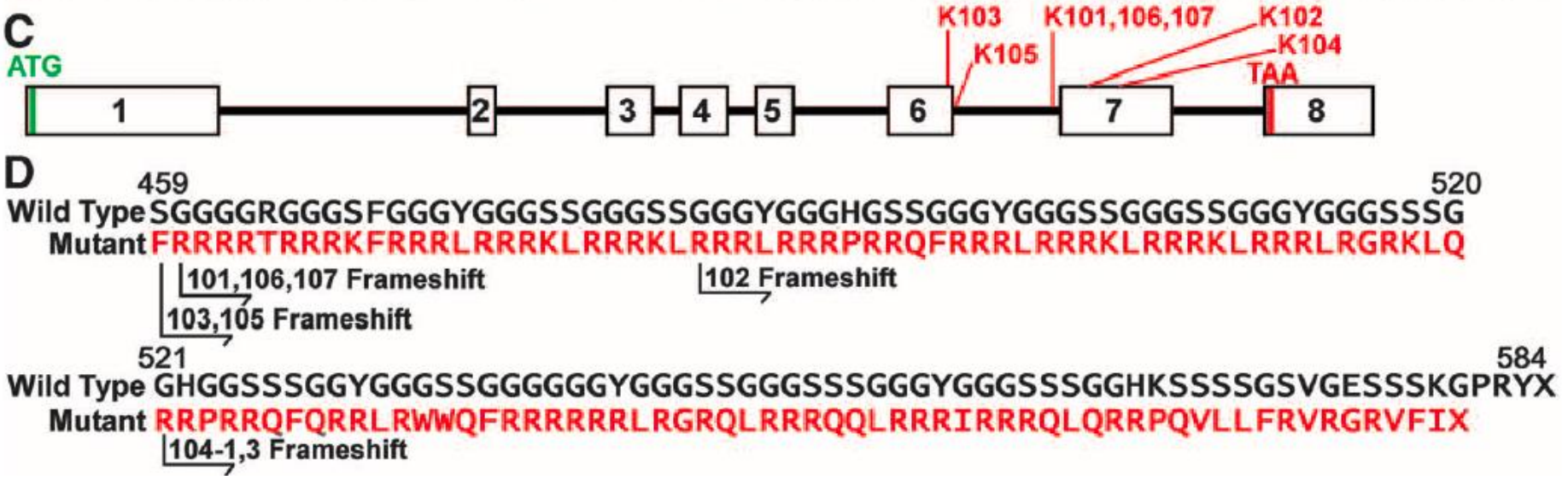
Sporadic cases with defective barrier function and thousands of confetti-like spots





Science, 2010

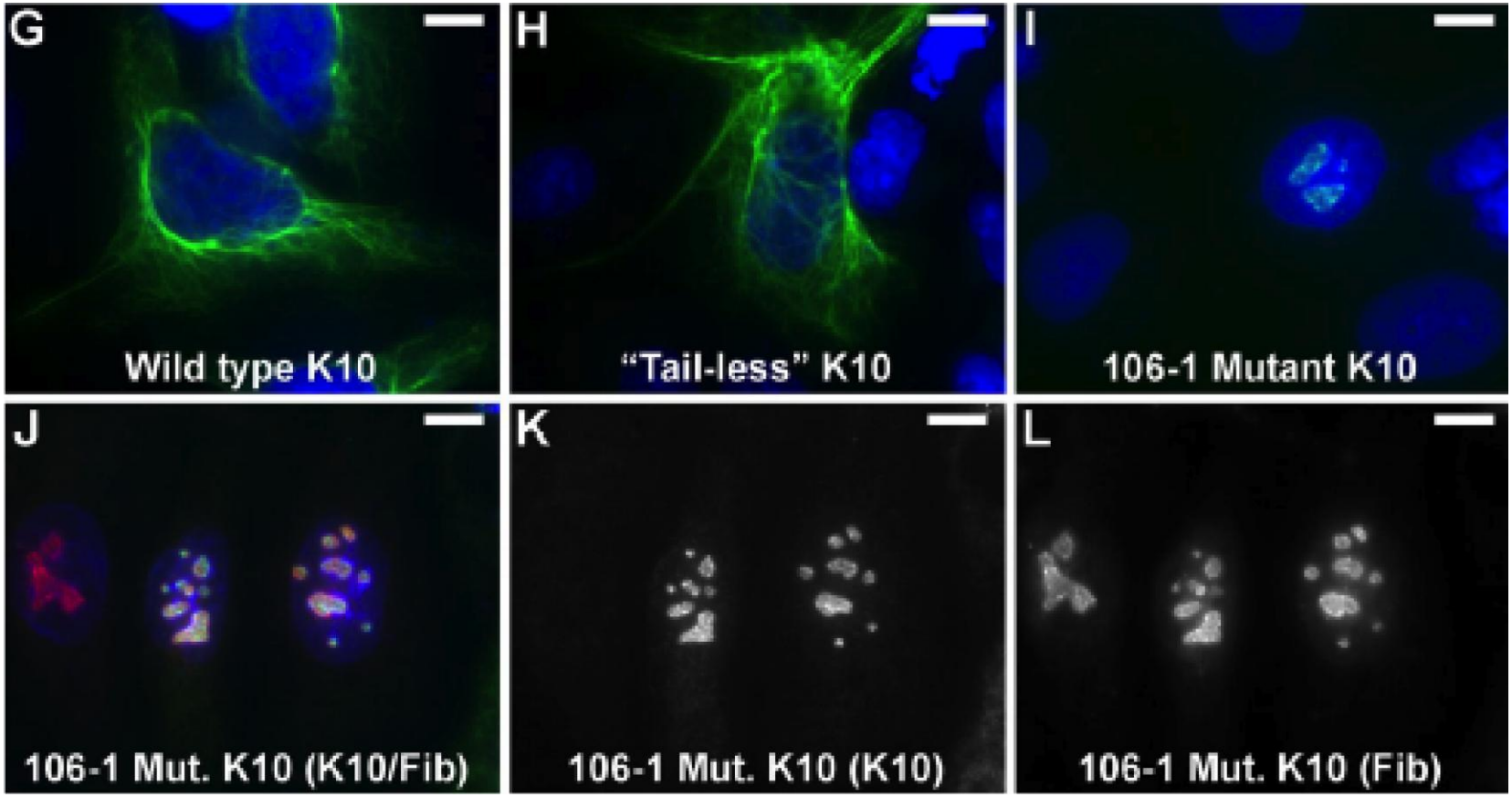
De novo mutations in *Keratin 10* in IWC all result in frameshift into the same arginine-rich alternative reading frame



Mutant K10 is mislocalized to the nucleolus

α–K10

α–K10,
α–fib



Aldosterone-producing adenoma (APA)

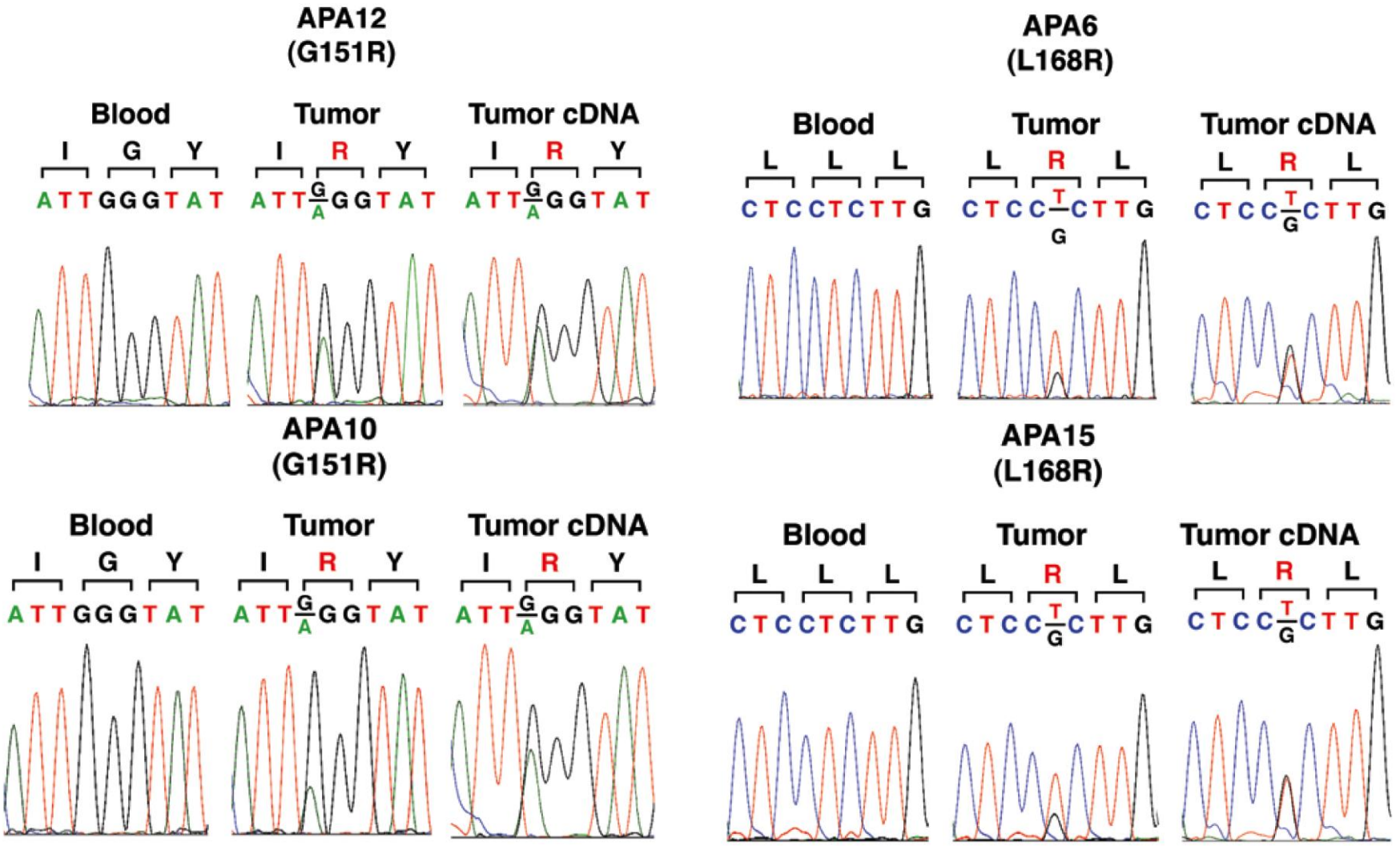
- Found in 5% of patients with severe hypertension
- Benign tumors, virtually never undergo malignant degeneration
- Are there mechanisms linking constitutive proliferation and constitutive hormone release?



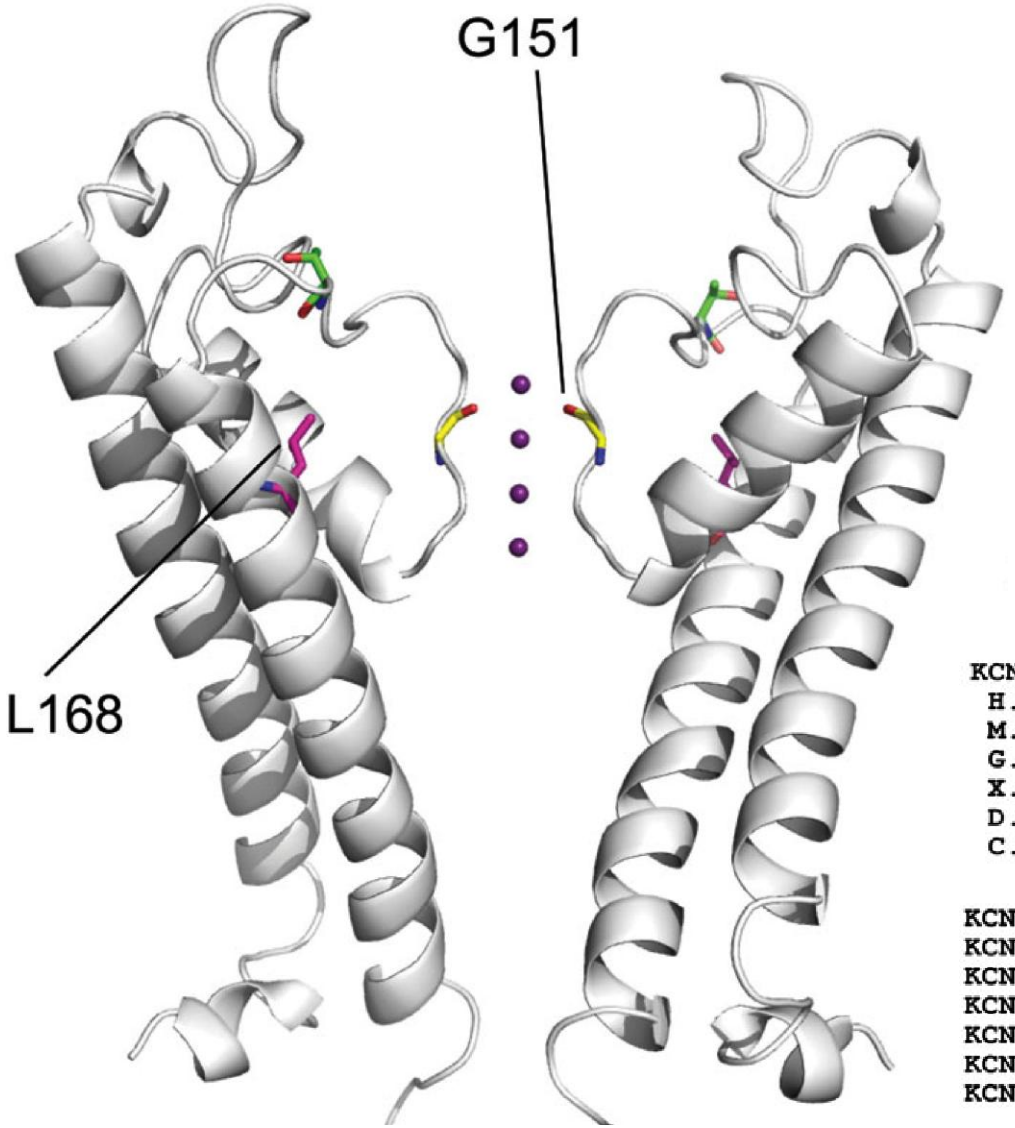
Only 2.25 protein-altering somatic mutations per tumor; K^+ channel *KCNJ5* is mutated twice

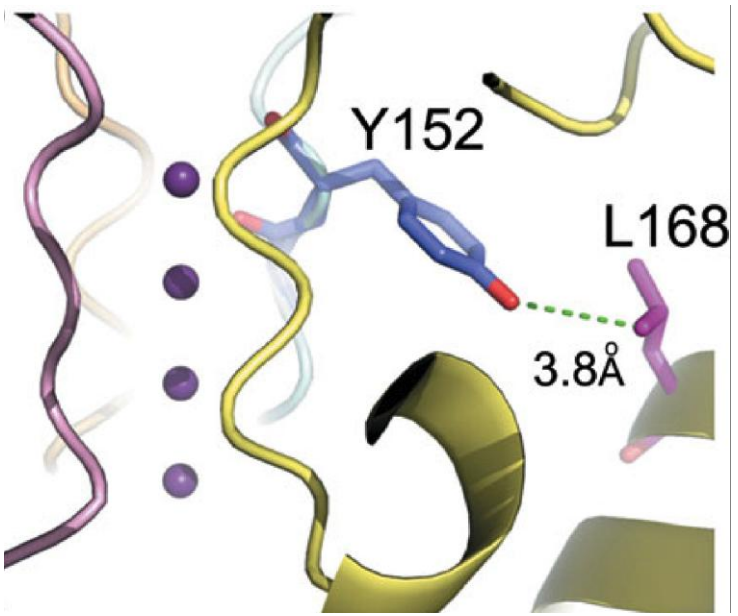
Tumor	Gene	Base change	Effect on protein	# of reads from tumor		# of reads from blood		p-value
				Ref. allele	Non-ref. allele	Ref. allele	Non-ref. allele	
APA9	*YY1*	C>G	T372R	115	69	184	0	1.3×10^{-24}
	ZFP37	C>G	V7L	47	23	77	0	4.0×10^{-9}
APA12	*FZD4*	C>A	C121F	491	139	872	0	1.6×10^{-55}
	KCNJ5	**G>A**	**G151R**	**120**	**59**	**290**	**0**	**1.9×10^{-28}**
	ARHGAP9	G>A	R66C	149	65	282	1	1.1×10^{-25}
APA15	***KCNJ5***	**T>G**	**L168R**	**159**	**65**	**456**	**0**	**3.5×10^{-35}**
	KDM5C	C>T	V1341M	30	30	54	0	7.6×10^{-11}
APA22	*PDE9A*	G>A	Exon 13 splice donor GT>AT	90	31	123	0	6.8×10^{-10}
	LRP1B	T>G	R3429S	60	14	80	0	1.7×10^{-5}

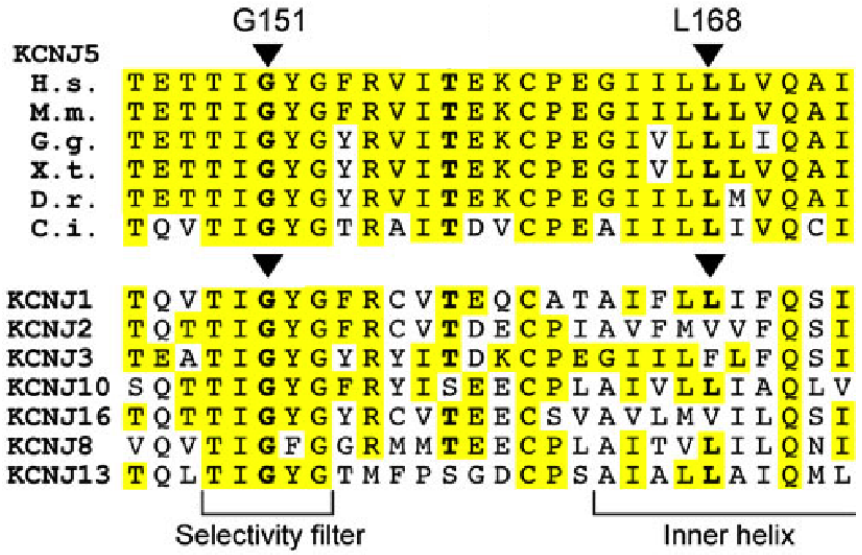
8 of 22 adosterone-producing adenomas have somatic G151R or L168R mutations in *KCNJ5* (p of occurrence by chance < 10^{-30})



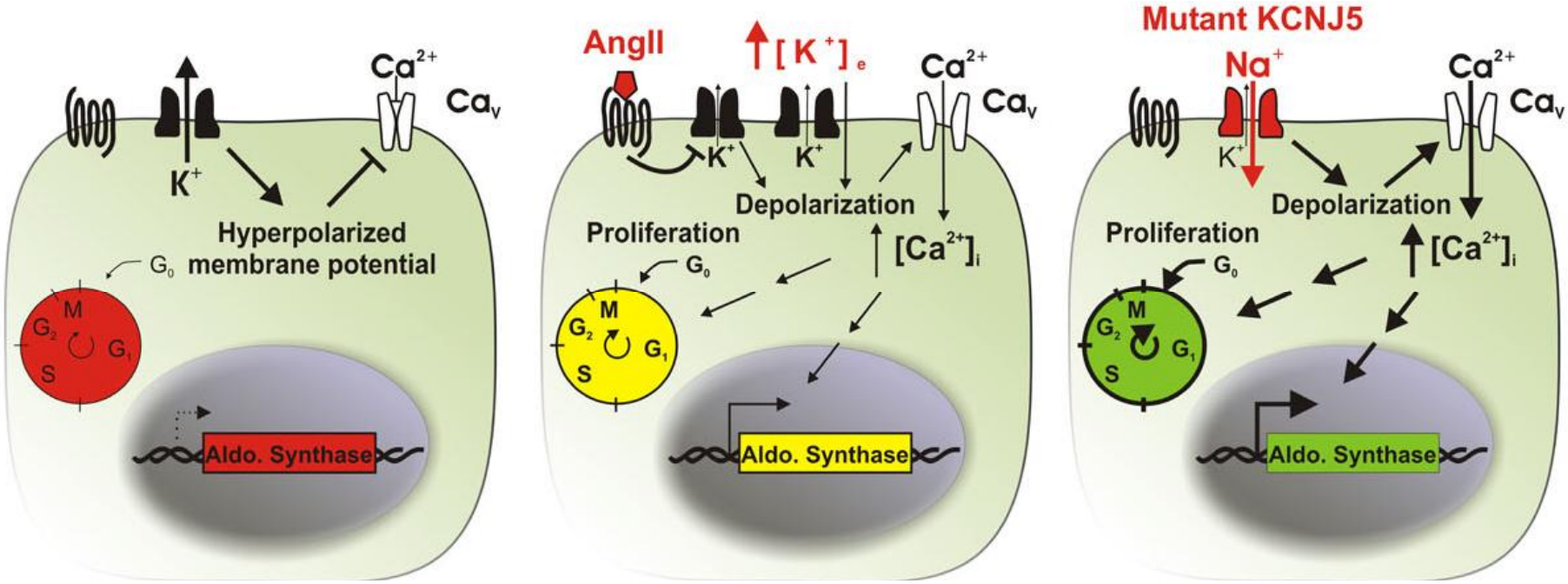
G151R and L168R mutations lie in and near the KCNJ5 selectivity filter



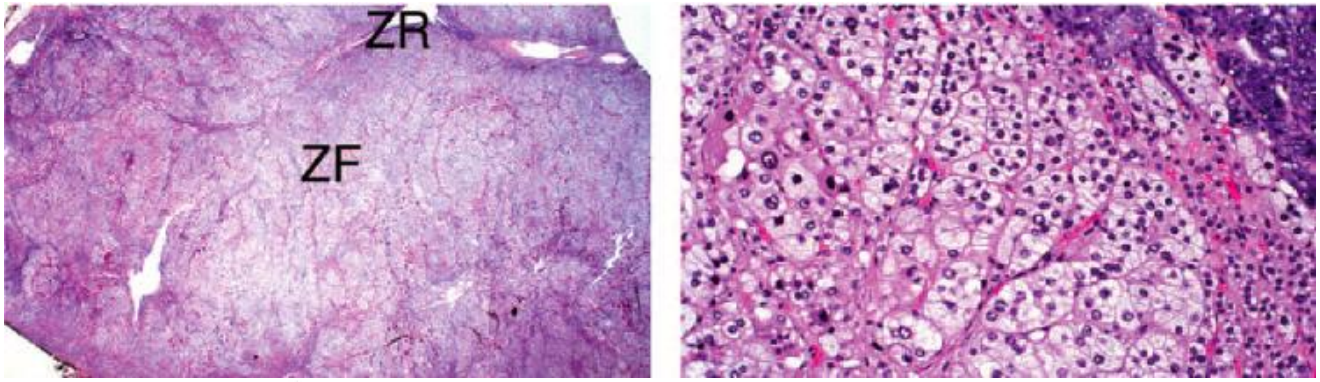


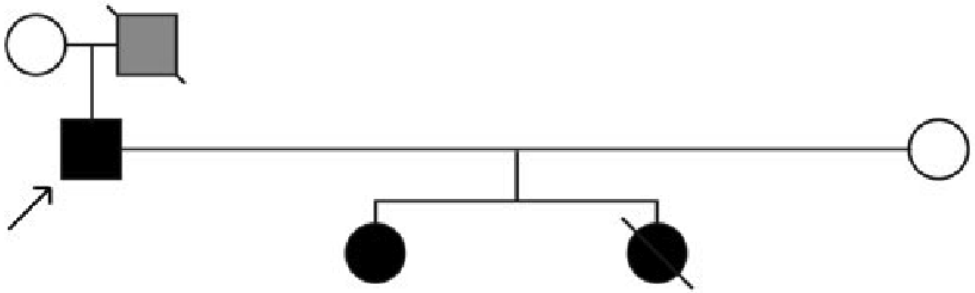


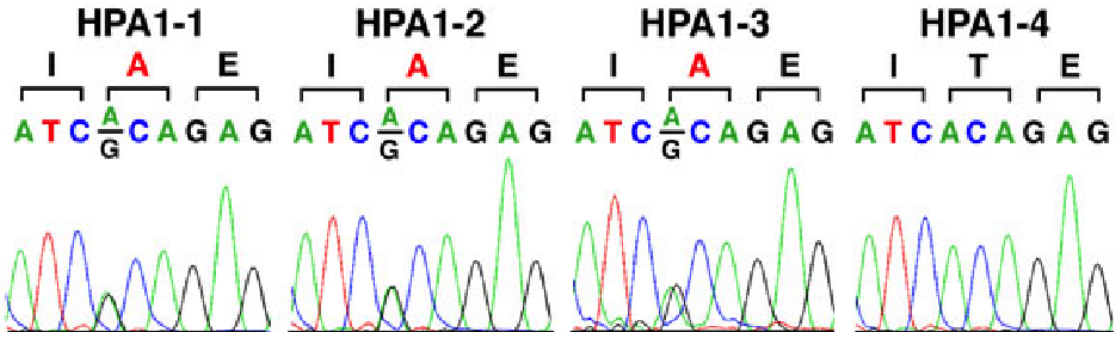
Membrane depolarization is the sufficient signal for both aldosterone secretion and cell proliferation



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

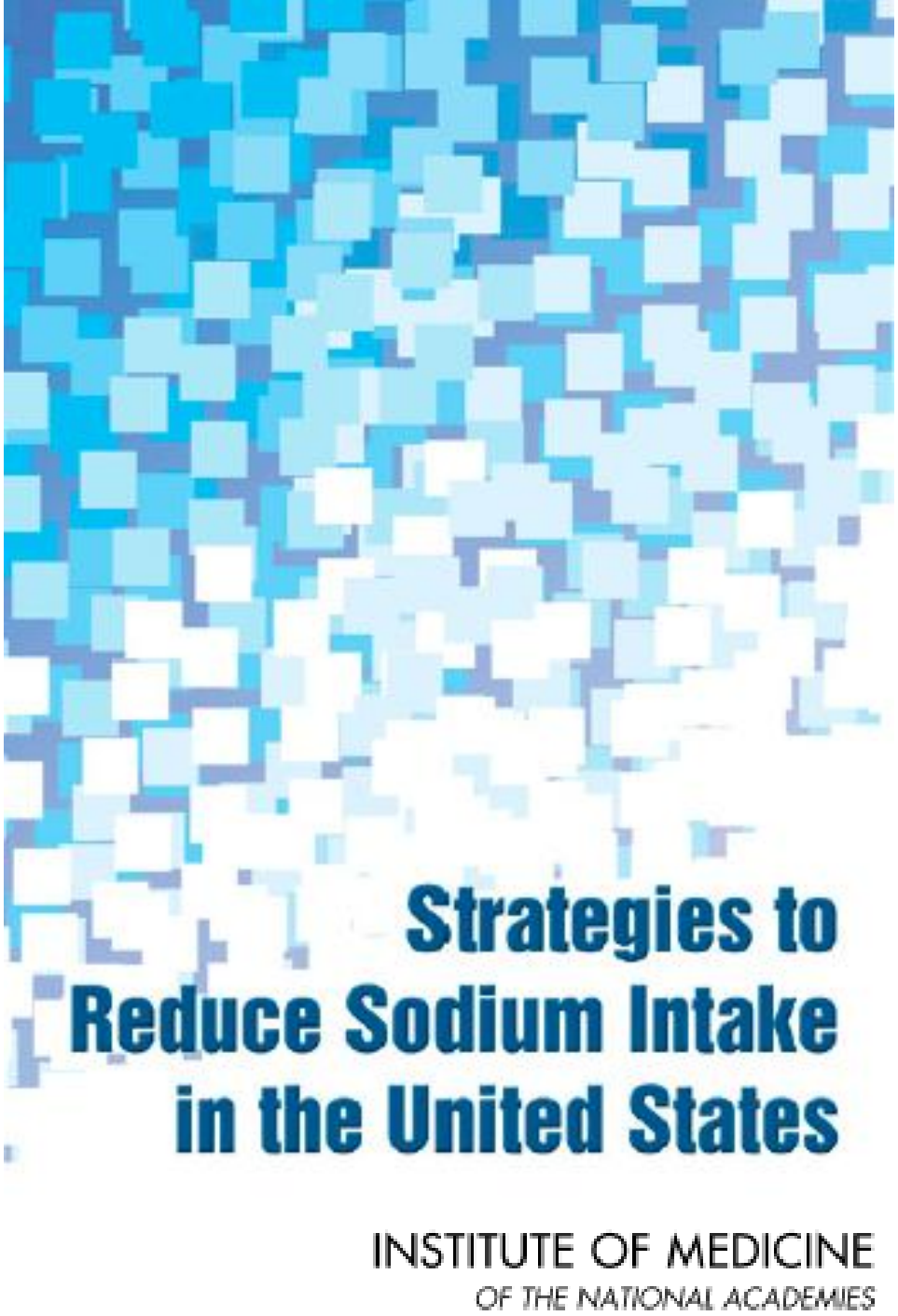
- Reduction in net salt balance now recognized as key goal of therapy by WHO and NHLBI Joint National Commission on Prevention, Diagnosis Evaluation and Treatment of Hypertension

- 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:	↓ 32,000 - 66,000
# Myocardial infarctions:	↓ 54,000 - 99,000
# Deaths from any cause:	↓ 44,000 - 92,000
Health care cost:	↓ $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

Effects of loss of function mutations on:

Gene	Blood pressure	Serum K^+
NCCT	↓	↓↓
MR	↓	↑
Aldo synthase	↓	↑
WNK1	↓	↓
ENaC	↓↓↓	↑↑↑
NKCC2	↓↓↓	↓↓↓
CLCNKB	↓↓↓	↓↓↓
ROMK	↓↓↓	↔

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

Therapeutics

- Need to help industry focus on the best targets and prosecute them with passion!