



MASSACHUSETTS
GENERAL HOSPITAL

HEART CENTER

Using Genetics to Identify Novel Pathways for Atrial Fibrillation

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No relationships to disclose.



A Teaching Affiliate
of Harvard Medical School

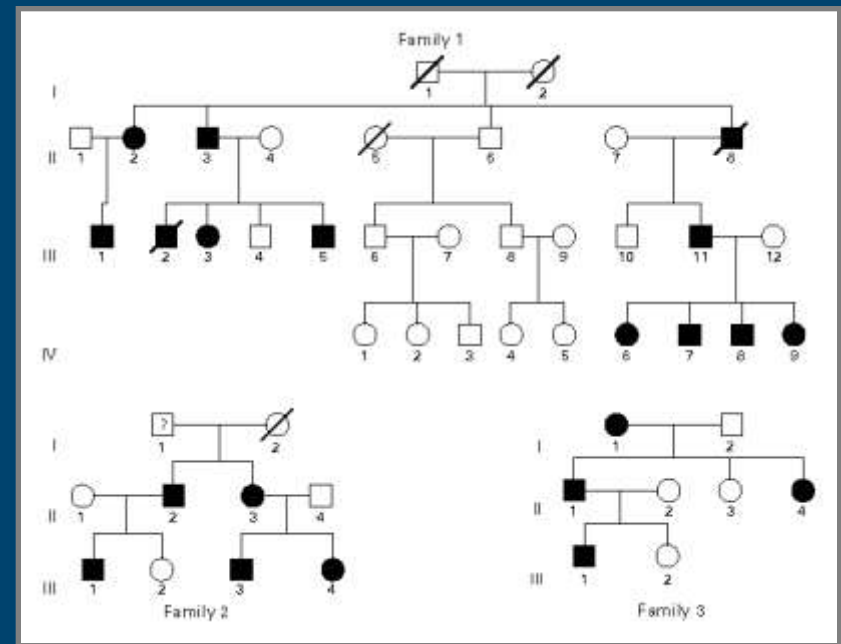
Prior reports of familial AF

Original description of a family of three brothers with AF dates to 1943.

Wolff, L. *NEJM* 229:10 396-8.

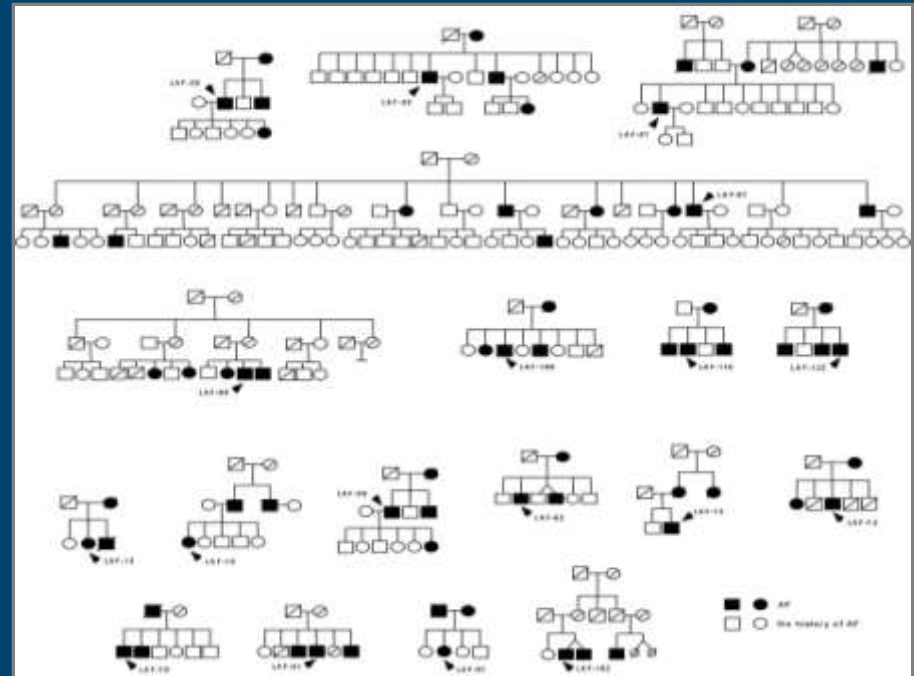
Three families with AF have been mapped to a locus on chromosome 10.

Brugada, R. *et al.*, *NEJM* 336:13 905-11.



MGH AF Study: Familial Clustering in Lone AF

- Early onset AF
- All subjects have:
 - Detailed questionnaire
 - Directed H & P
 - Blood samples for DNA & protein analyses
- >1,500 subjects enrolled



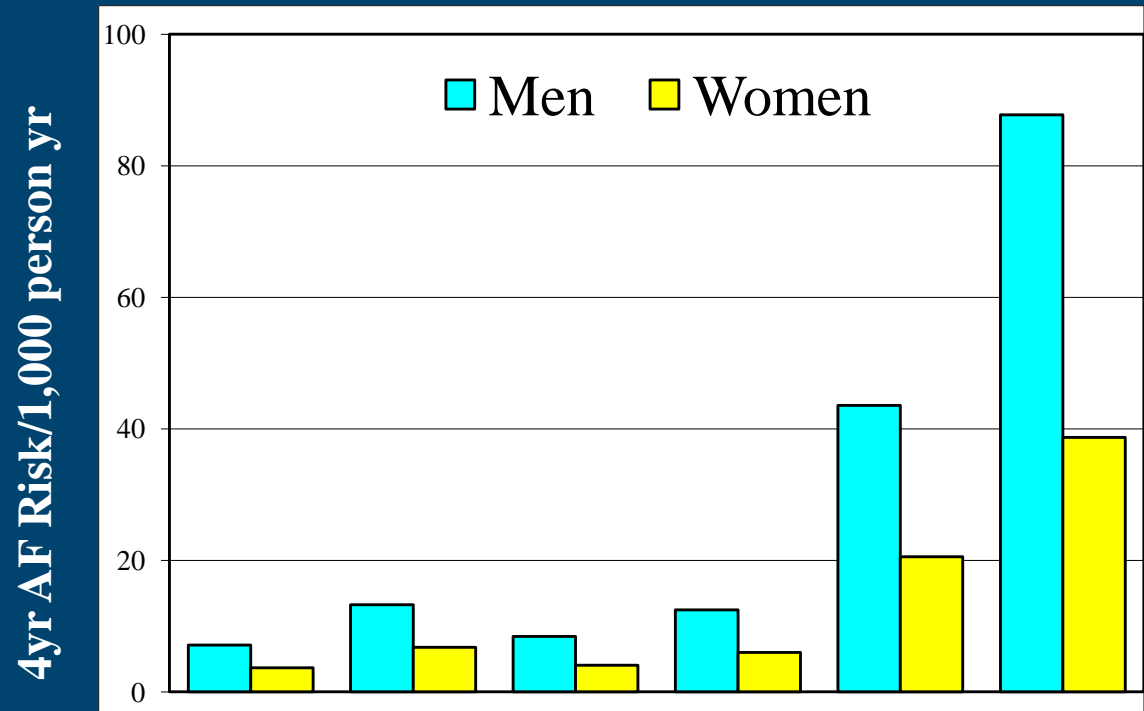
Ellinor et al. Human Genetics 2005.

Offspring AF risk by Parental h/o AF

AF \geq 1 parent
OR 1.9; (P=0.02)

<75yo, w/o h/o
heart disease
OR 3.2; (P< 0.001)

Fox...Benjamin JAMA
2004;291:2851



HTN	-	-	+	+	+	+
Diabetes	-	-	-	+	+	+
Heart Disease	-	-	-	-	+	+
Parent hx AF	-	+	-	-	-	+

Is premature AF a risk factor?

- Familial AF

 - Any documented occurrence in a first-degree relative

- Premature familial AF

 - Onset < 66 years of age

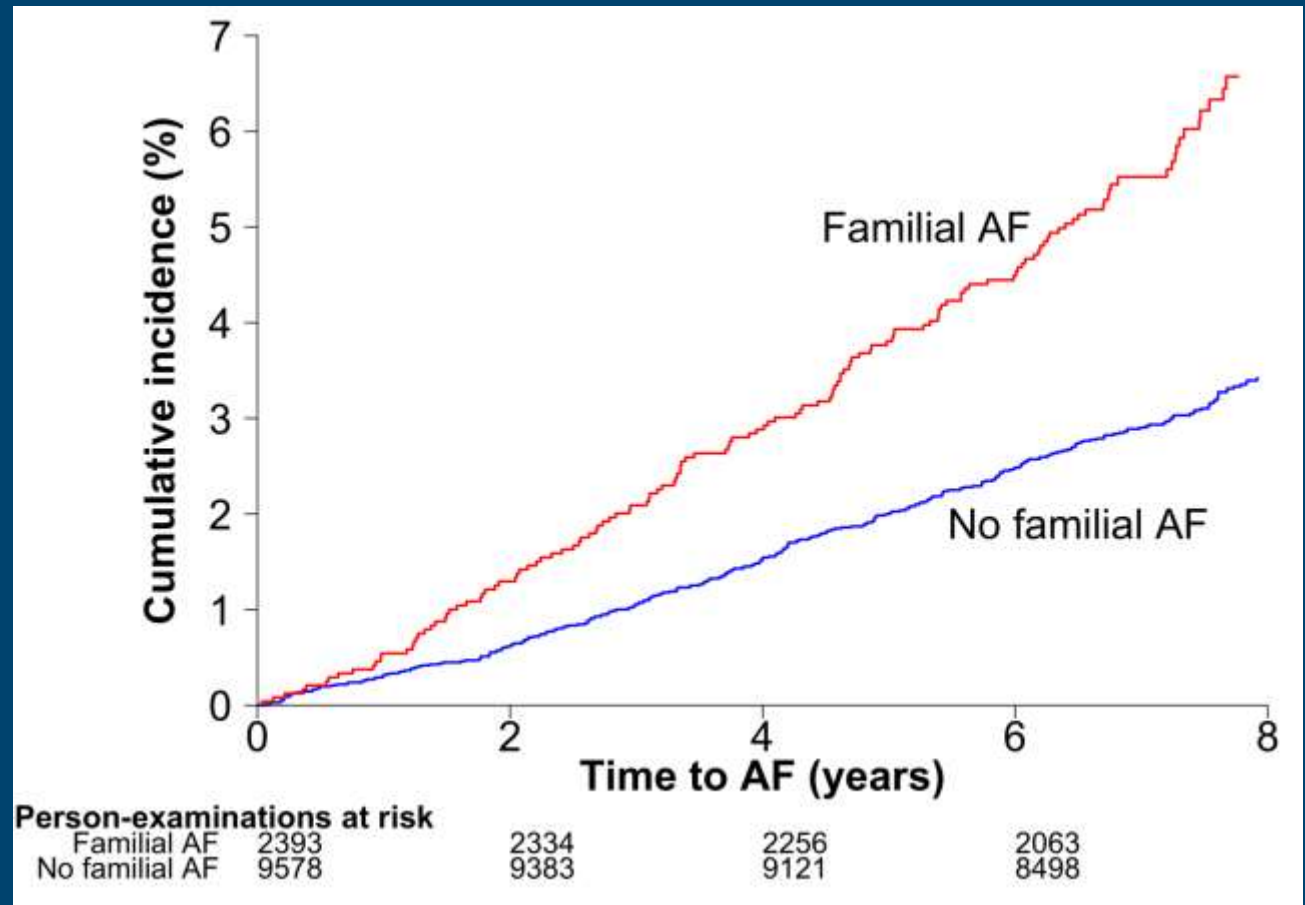
- Number of affected relatives

- Age of onset in youngest affected relative



AF in First Degree Relatives is Associated With an Increased Risk of AF

HR 1.39
(1.12-1.73)
P=0.003



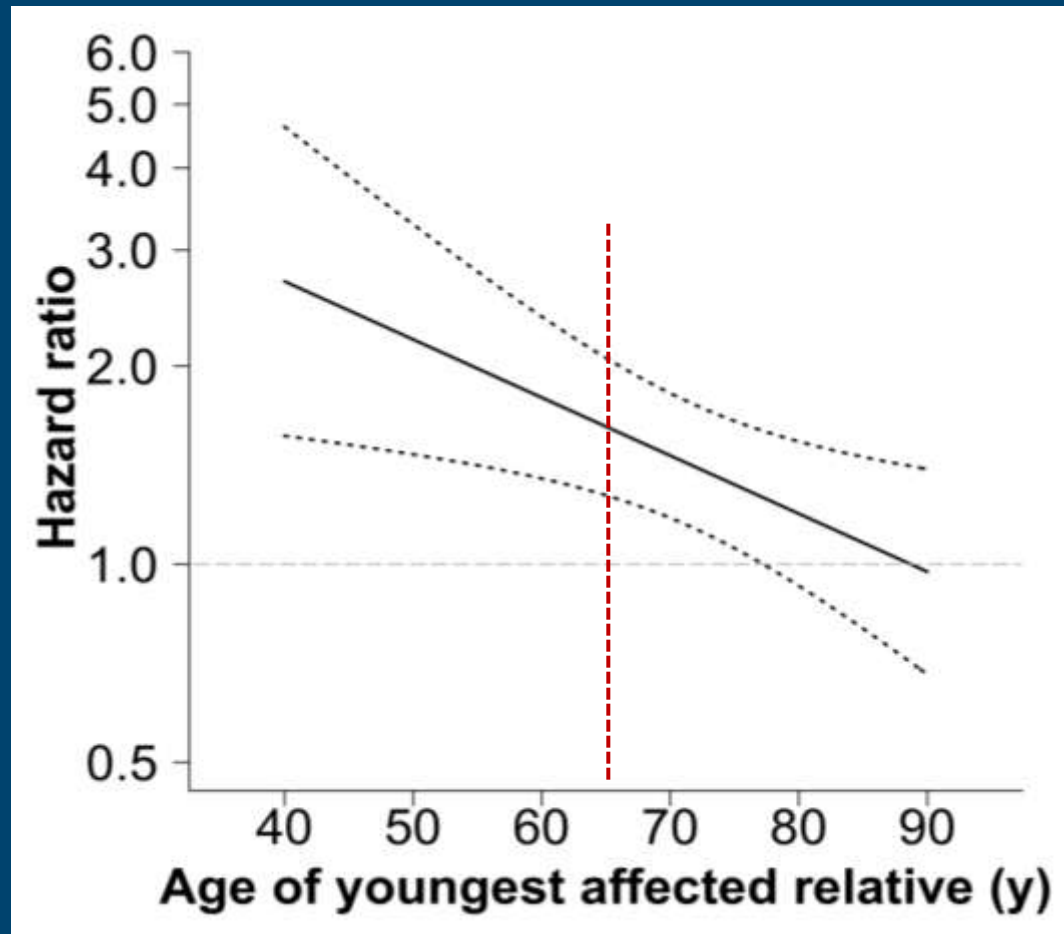
AF Risk by Age of Youngest Affected Relative

Premature
familial AF

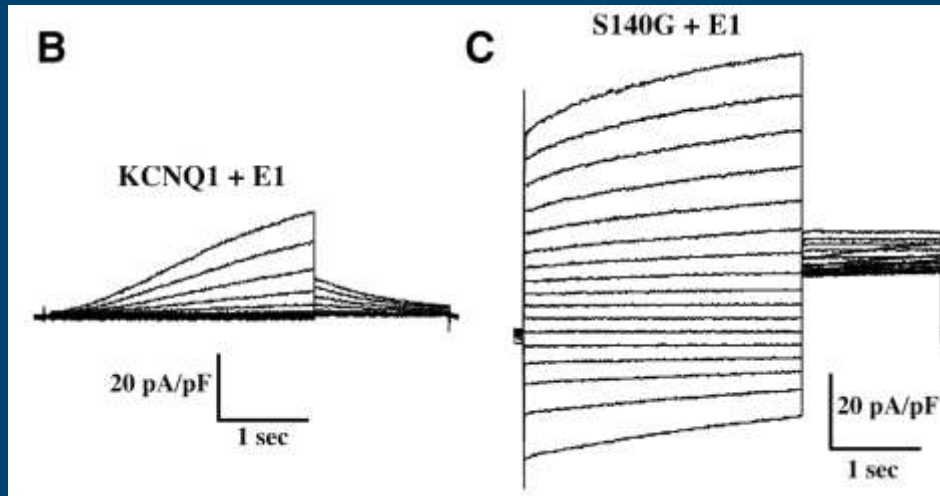
Onset <66 yrs

HR 2.01
(1.49-2.71)

$P < .001$



KCNQ1 (I_{Ks}) Mutation Leading to AF



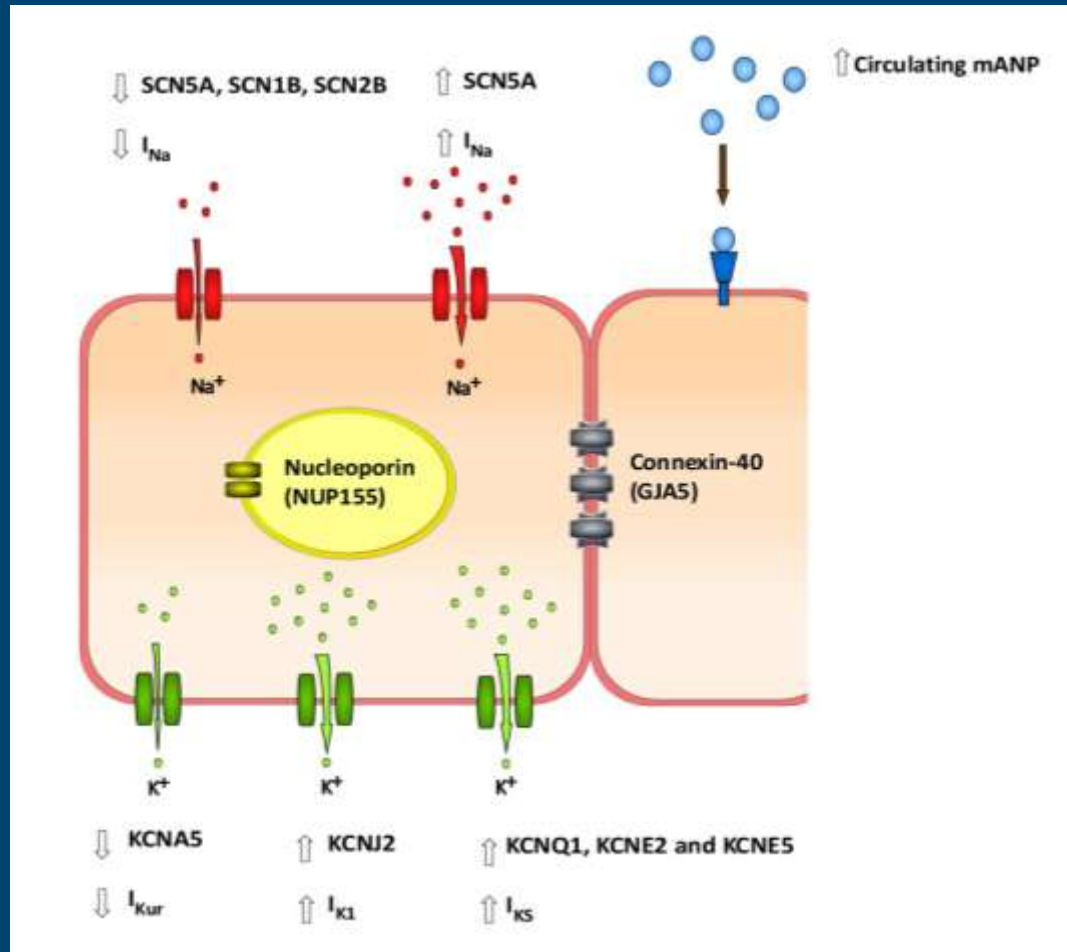
Gain of function mutation
in KCNQ1 can lead to AF.

Chen et al., *Science* Jan 10 2003: 251-254.

Notable features:

- 24 yrs of age mean onset
- Prolonged QT
- Three patients developed cardiomyopathy after AF

Mutations in Ion Channels & Signaling Molecules are Rare Causes of AF



Few additional variants identified by sequencing

Mutations are family specific

Mahida et al.
Card. Research 2010.

Simple versus complex traits

	Simple	Complex
Examples	HCM, FH, LQTS	HTN, CAD, AF
Disease prevalence	Rare	Common
Genes involved	Single	Many
Frequency of genetic variant	Rare / mutation	Common / polymorphism
Effect on protein function	Severe	Mild
Effect on phenotype	Large / causative	Small / susceptibility

CHARGE AF Consortium

AGES: Age, Gene, Environment Susceptibility
ARIC: Atherosclerosis Risk in Communities Study
CC: Cleveland Clinic
CHS: Cardiovascular Health Study
FHS: Framingham Heart Study
RS: Rotterdam Study
GHS: Gutenberg Heart Study
MONICA/KORA/German AF Network
MGH: Massachusetts General Hospital
Vanderbilt University
Women's Genome Health Study



Goal is to perform GWAS data for AF and the PR interval:

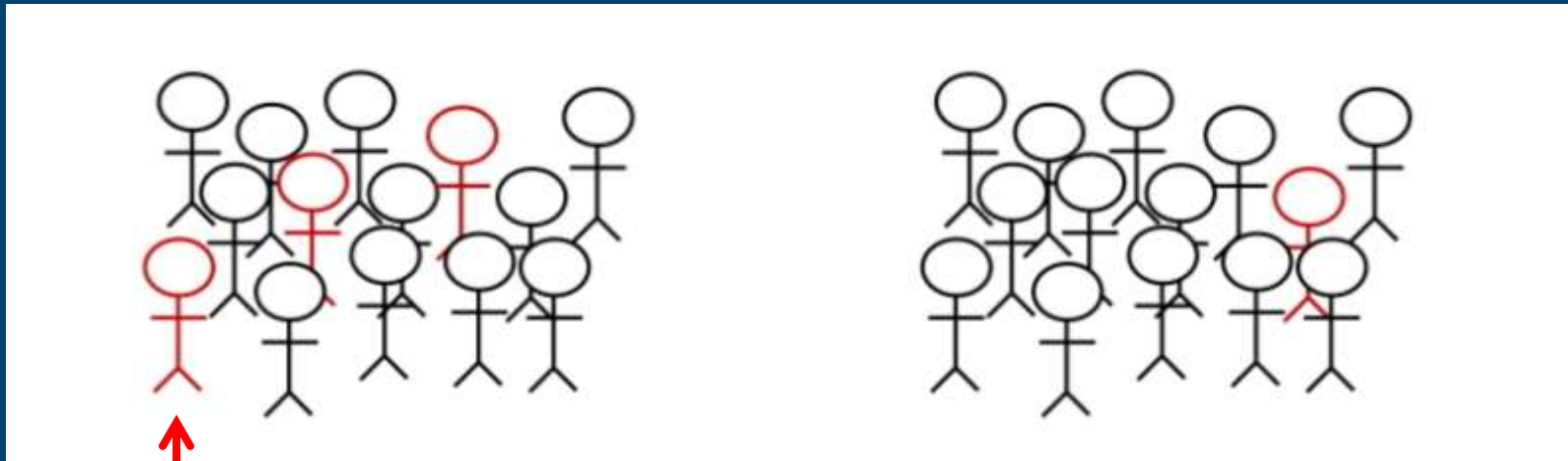
AF: ~6,600 with AF & ~56,000 without AF

PR: ~85,000 subjects

Genome wide association study

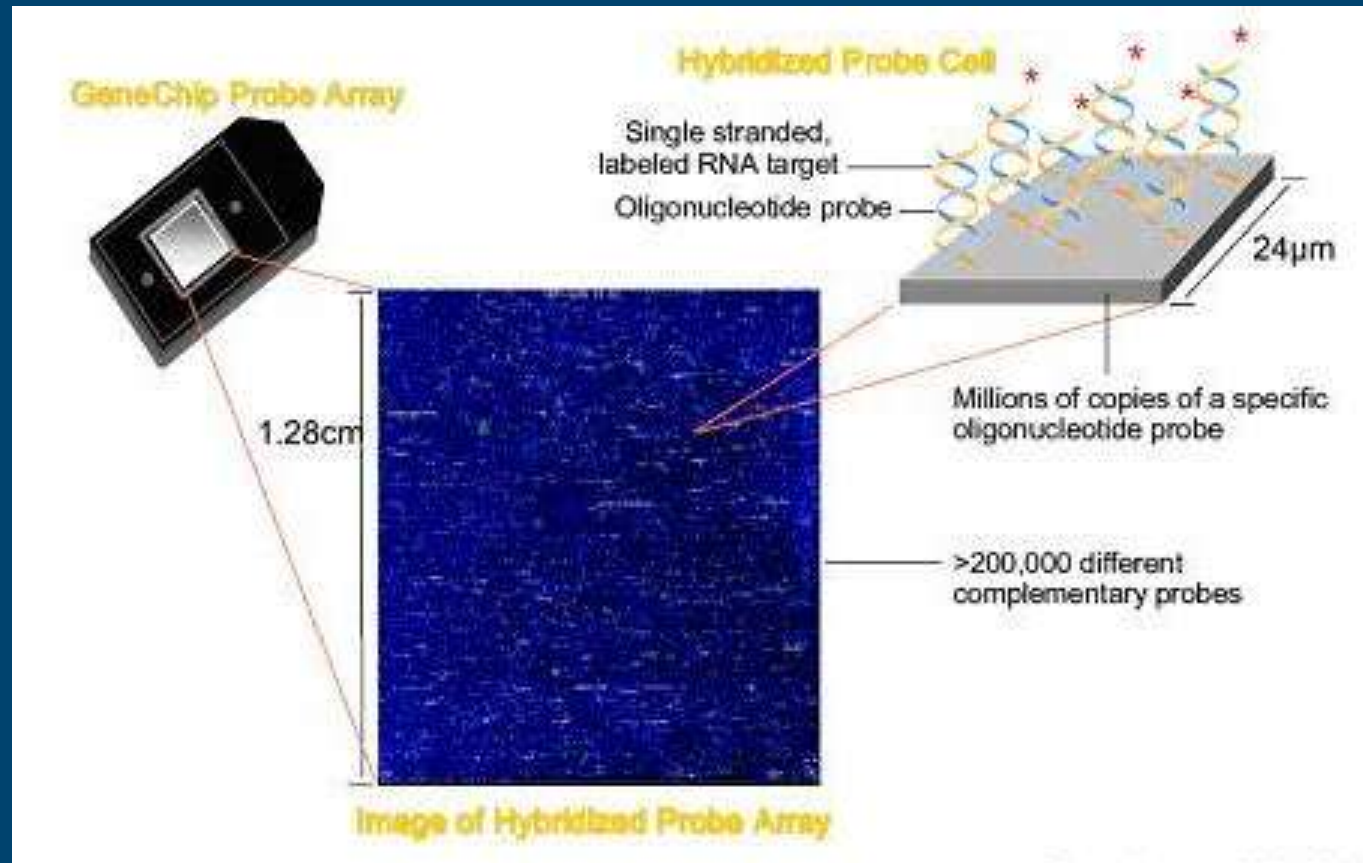
AF

No AF



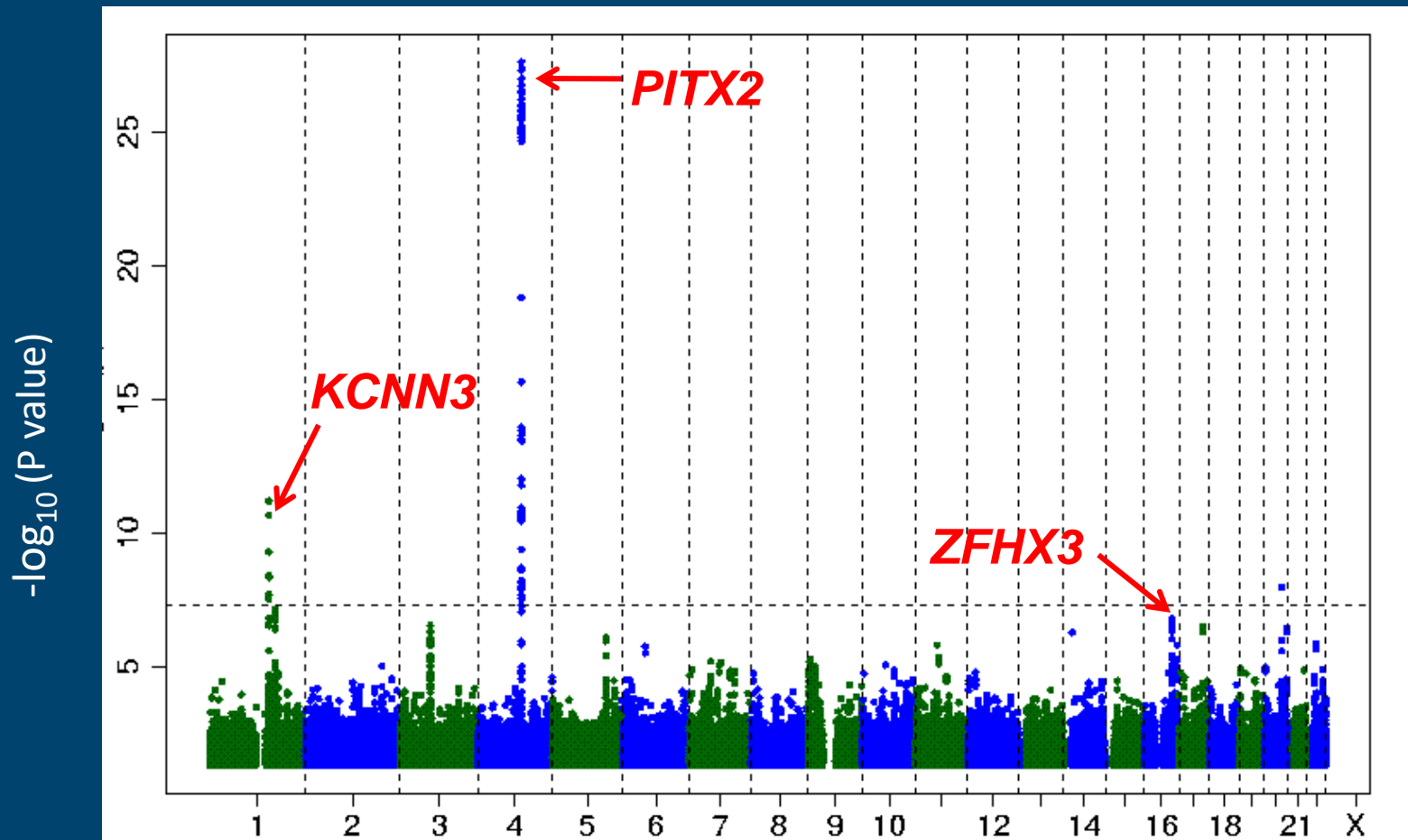
Genetic variant more common in those with AF

Genome wide association study – 2/3



“Fingerprint” each individual

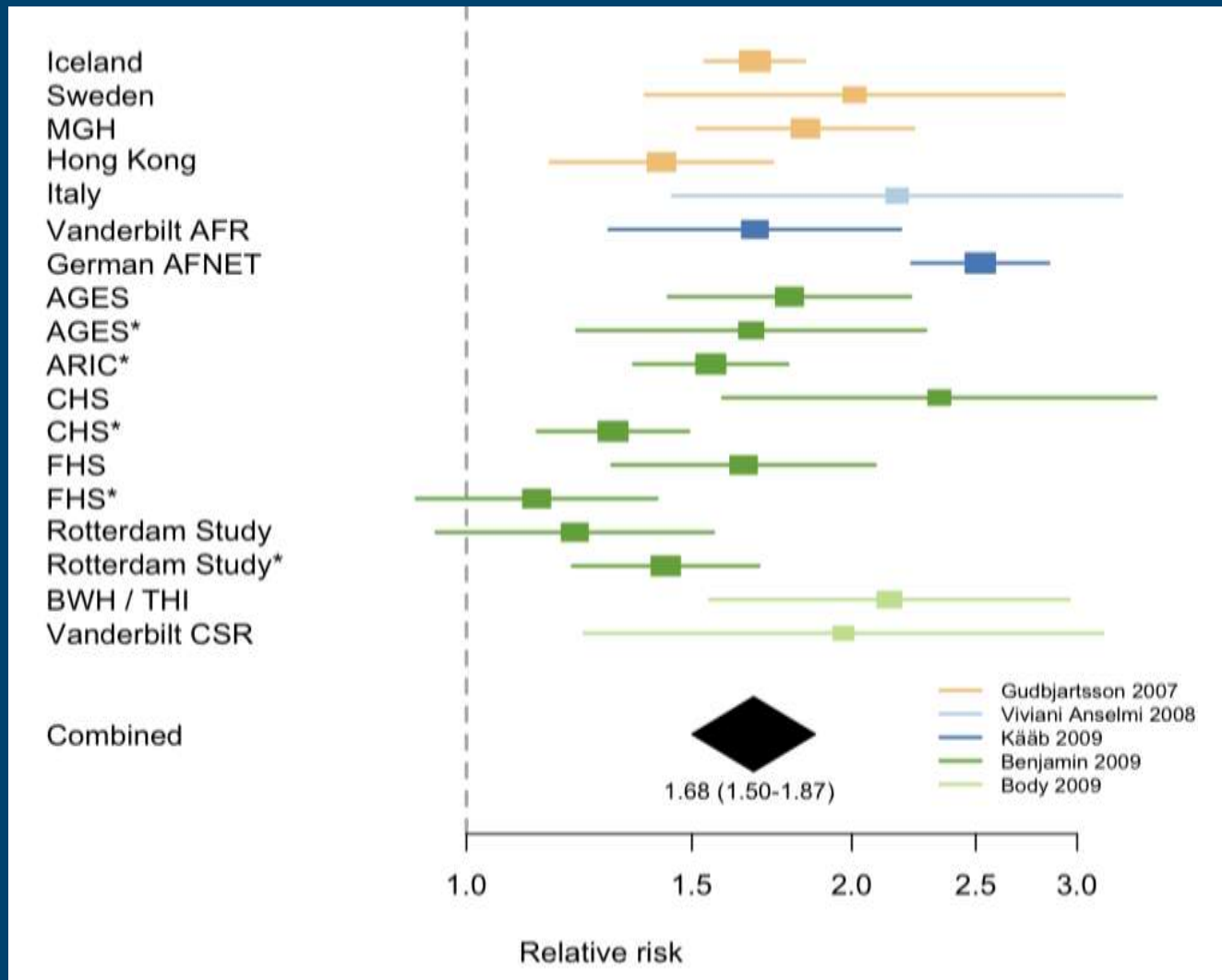
2011 - Three Genetic Loci for AF



Gudbjartsson et al. Nature, 2007
Gudbjartsson et al. Nature, 2009

Benjamin et al. Nature Genetics, 2009
Ellinor et al. Nature Genetics, 2010

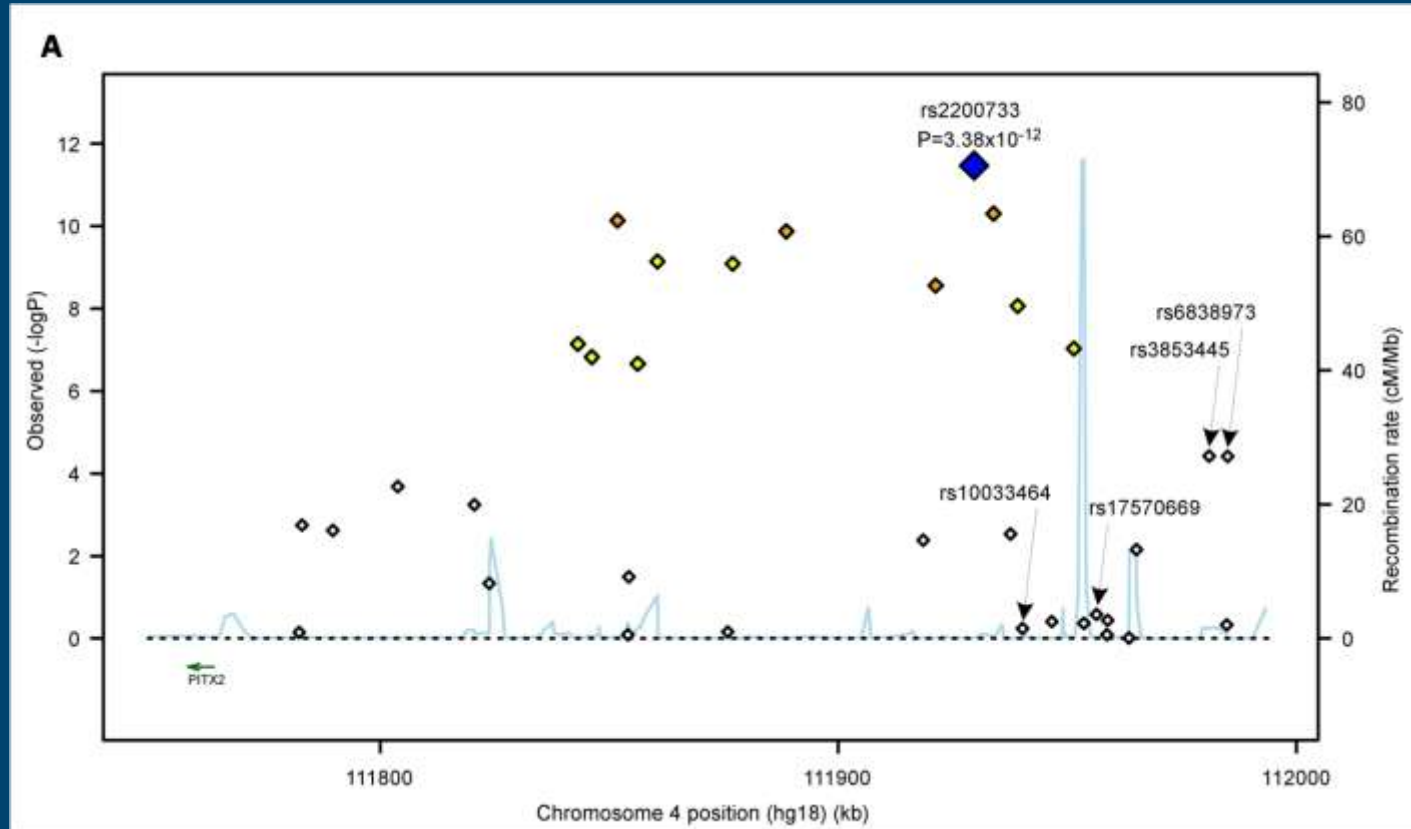
Variants on Chr 4q25 are strongly associated with AF



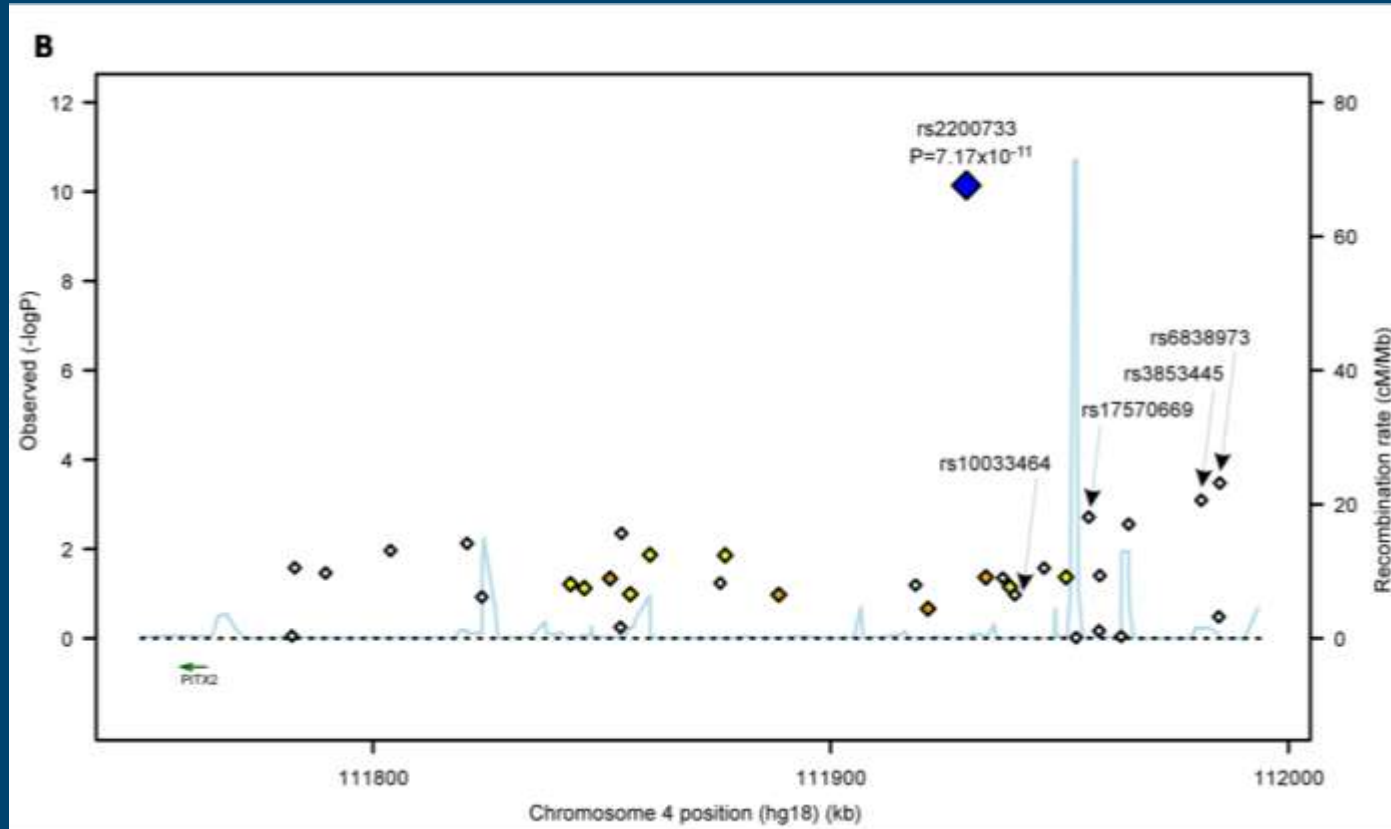
Fine mapping of Chr 4q25 locus: To identify additional susceptibility regions associated with AF

Stage	MGH		AFNet	
	Discovery		Replication	
Affection status	AF	No AF	AF	No AF
Number	790	1,177	2,145	4,073
Age (yrs)	63 ± 15	67 ± 13	49 ± 14	61 ± 12
Men	547 (69)	627 (53)	1,564 (73)	2,005 (49)
Hypertension	383 (49)	670 (57)	1,148 (56)	730 (18)

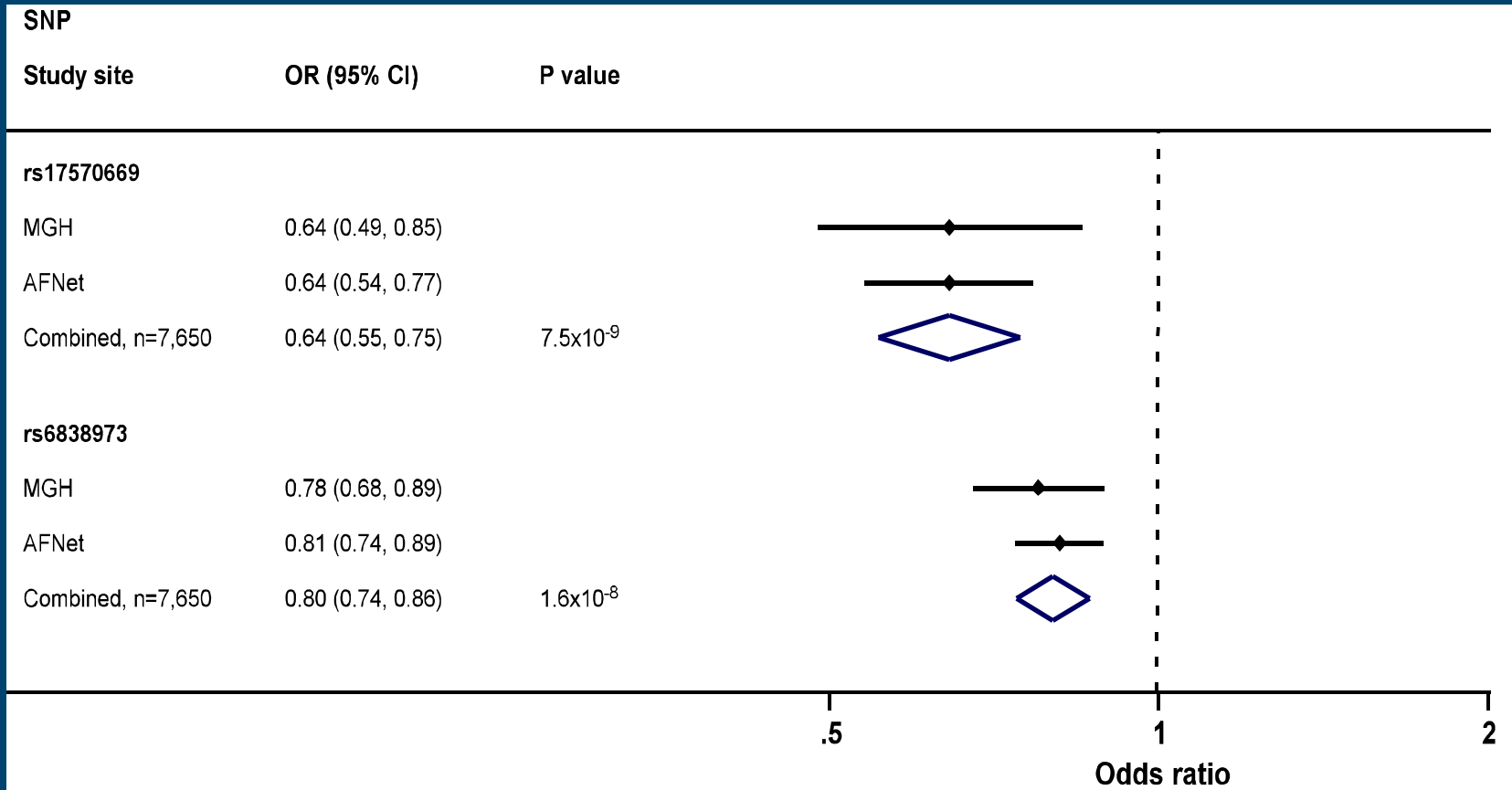
Chromosome 4q25 locus for AF



Adjusting for rs2200733 reveals 3 other SNPs associated with AF



Two Protective SNPs for AF

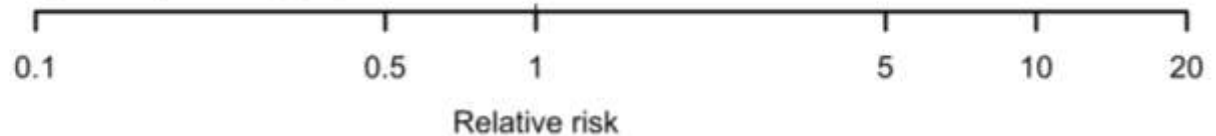


3 SNPs at 4q25 Predict High Risk of AF

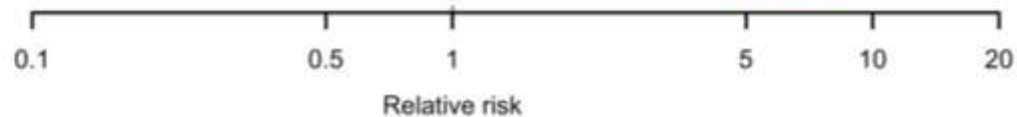
Genotype combination, (No. risk alleles)	Sample frequency (%)	RR (95% CI)	P Value
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TT / TA / CT	0.3	2.40 (1.36-4.23)	2.6e-03
CT / AA / TT	10.4	2.26 (2.02-2.53)	3.5e-46
TT / TA / TT	0.5	3.05 (1.95-4.78)	1.0e-06
TT / AA / CT	0.2	5.72 (3.08-10.64)	3.5e-08
TT / AA / TT	1.2	6.02 (4.56-7.96)	1.2e-36

4
5
5
5
6



Genotype combination	Sample frequency (%)	RR (95% CI)	P Value
CT / AA / CT (4)	4.5	1.74 (1.48-2.03)	6.2e-12
TT / TA / CT (4)	0.3	2.40 (1.36-4.23)	2.6e-03
CT / AA / TT (5)	10.4	2.26 (2.02-2.53)	3.5e-46
TT / TA / TT (5)	0.5	3.05 (1.95-4.78)	1.0e-06
TT / AA / CT (5)	0.2	5.72 (3.08-10.64)	3.5e-08
TT / AA / TT (6)	1.2	6.02 (4.56-7.96)	1.2e-36



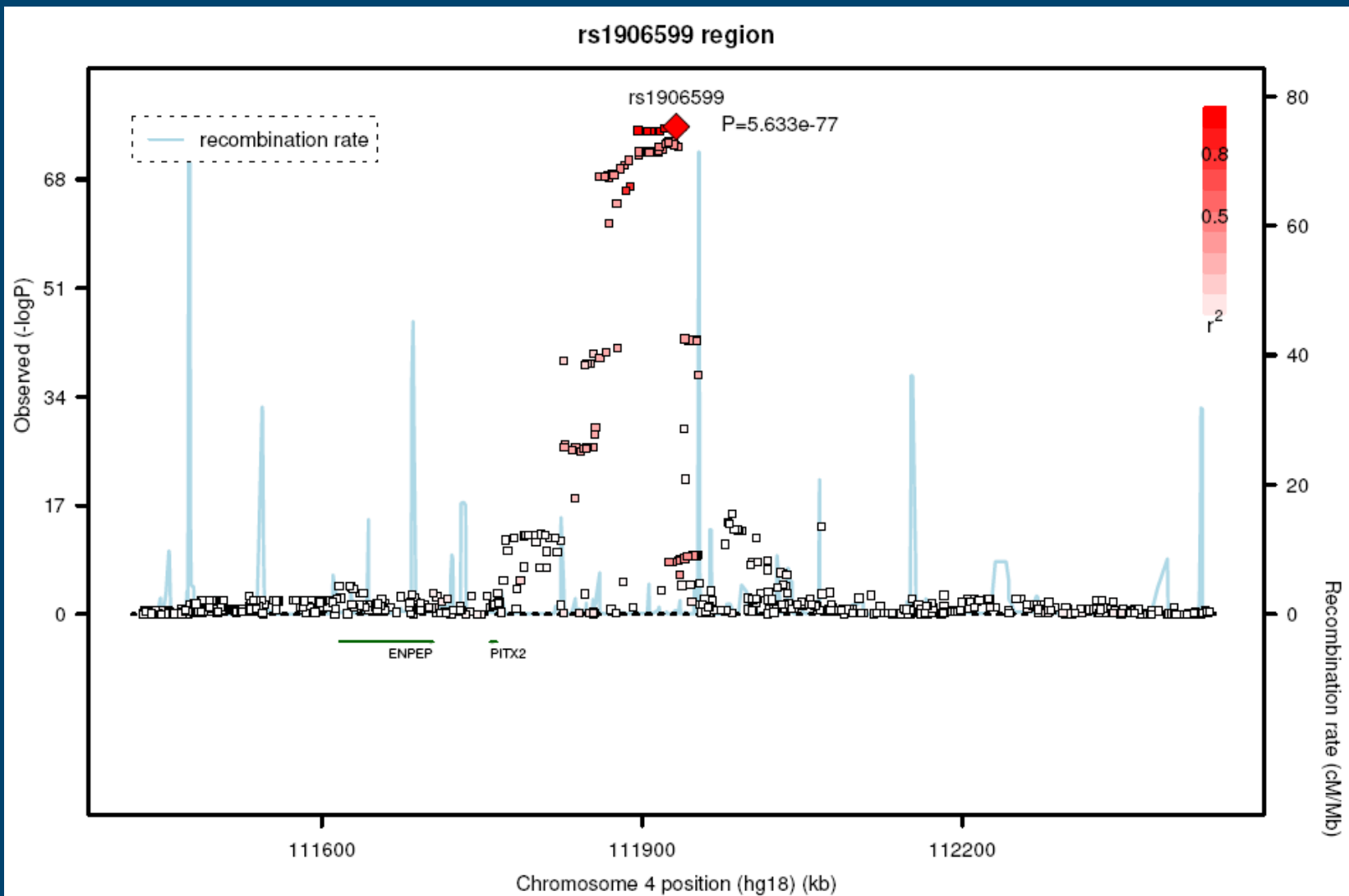
How do variants at 4q25 lead to AF?

- Non-coding variants in a genomic desert.
- Closest gene is PITX2.
- One isoform, Pitx2c:
 - Critical for left/right asymmetry
 - Including specification of the left atrium and pulmonary myocardium
 - Suppresses a default program for sinoatrial node formation in the left atrium

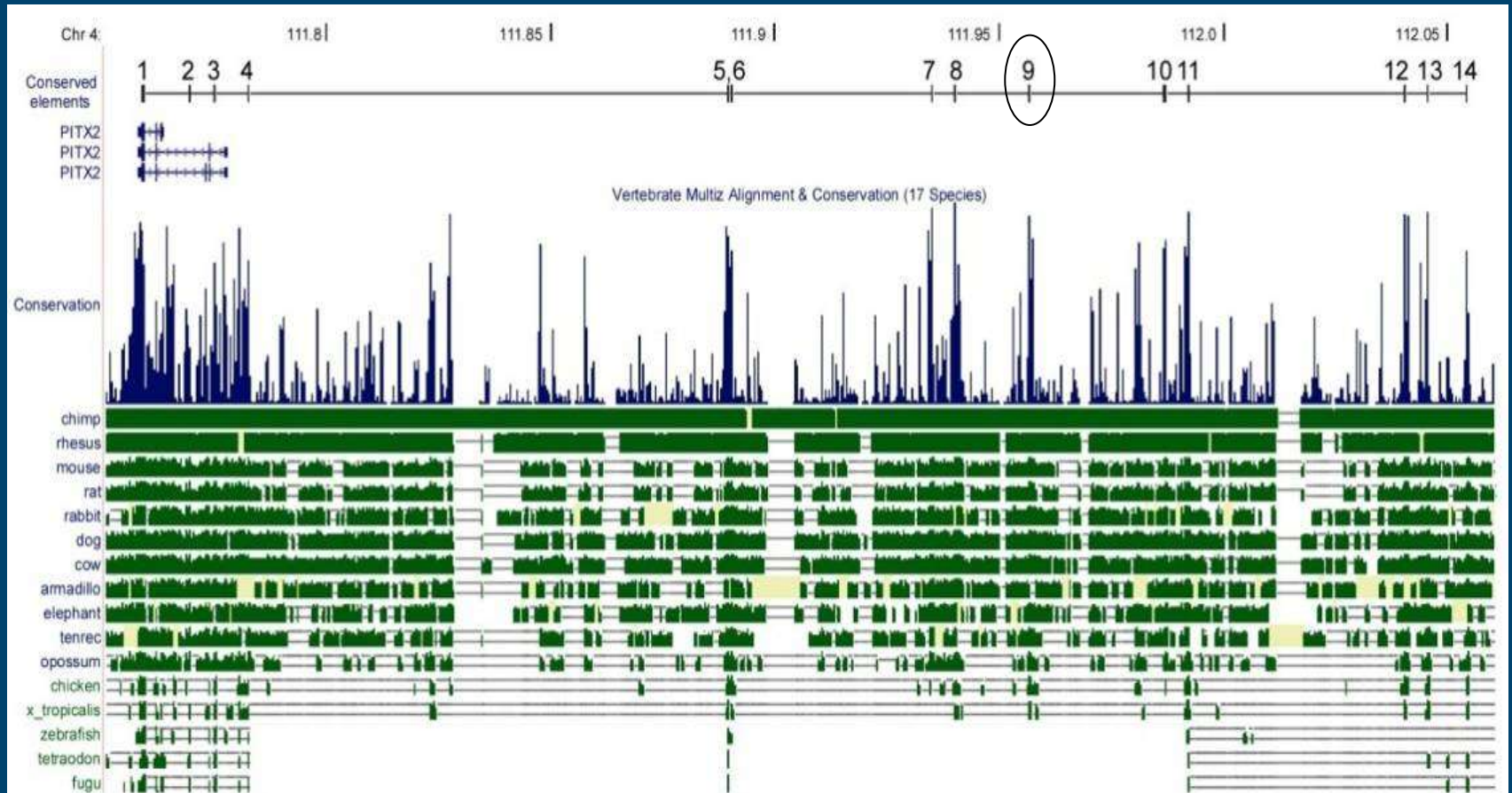


Mommersteeg et al, 2007

Regional plot of 4q25 locus for AF



Conserved non-coding regions around PITX2

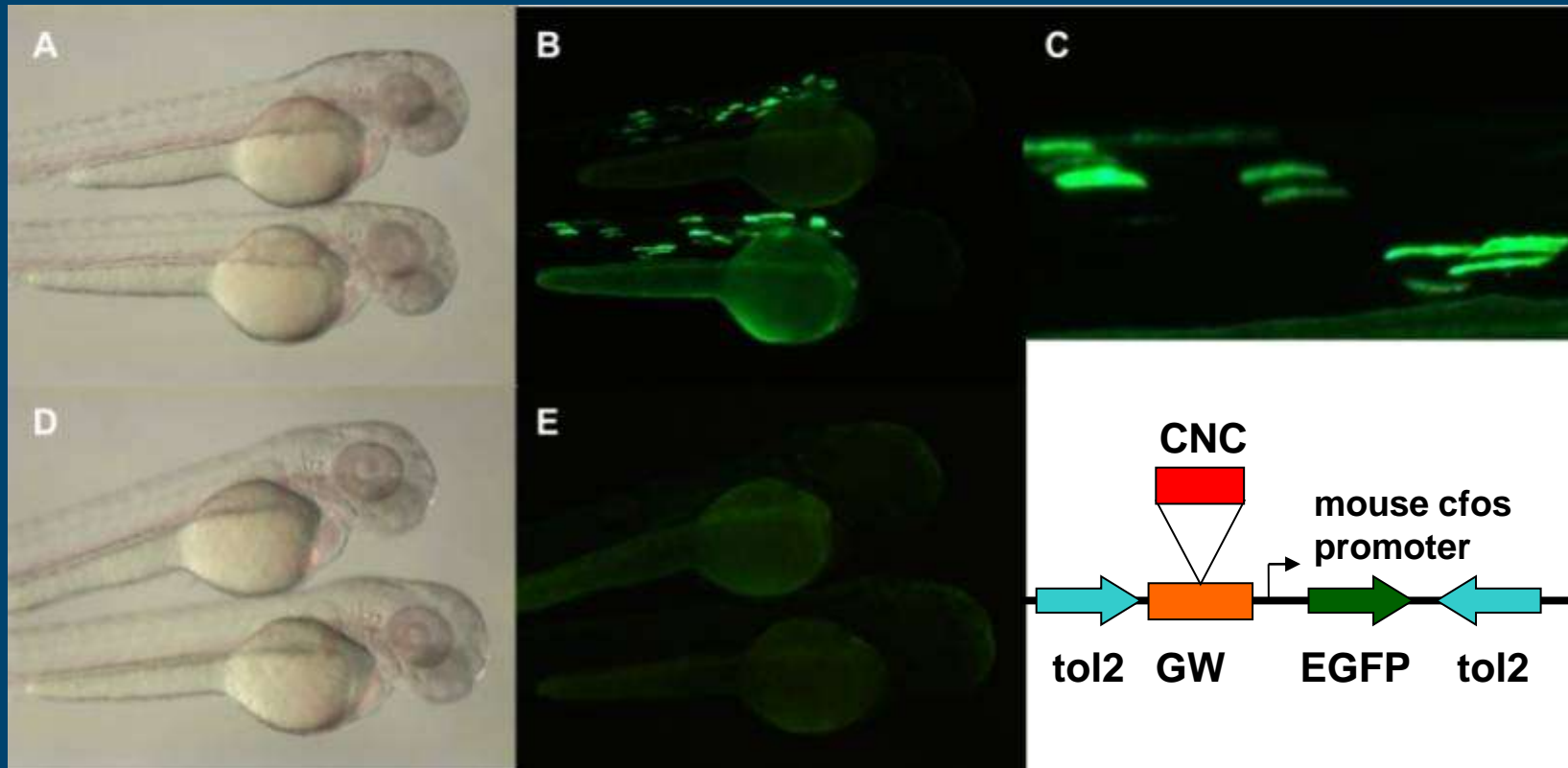


Why use zebrafish?

- Vertebrate
- Transparent
- Major organs have developed within first 48 hours
- Relatively inexpensive
- Rapid gene knockdown via morpholino injection



PC9 drives muscle specific expression in zebrafish



PC9 drives expression in limbs and somites

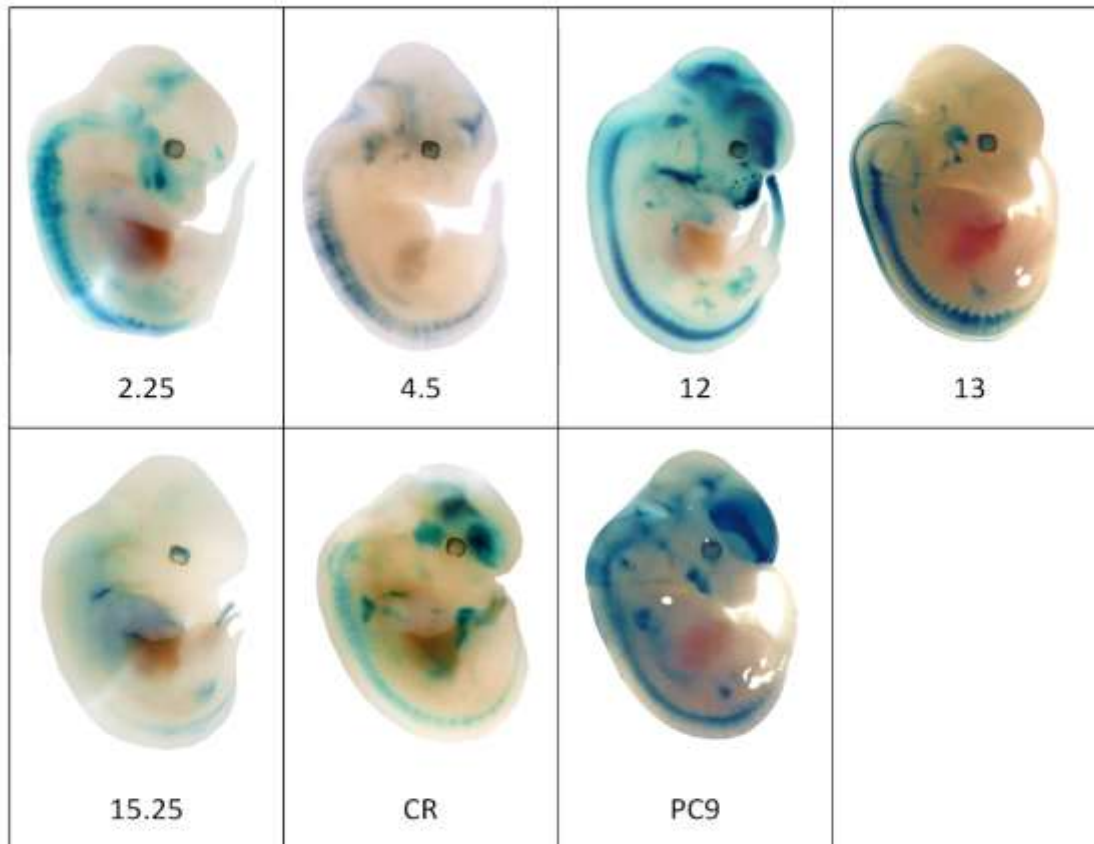
Embryo 5



Embryo 15



Six additional functional regions at 4q25 locus



Two additional genetic loci for AF

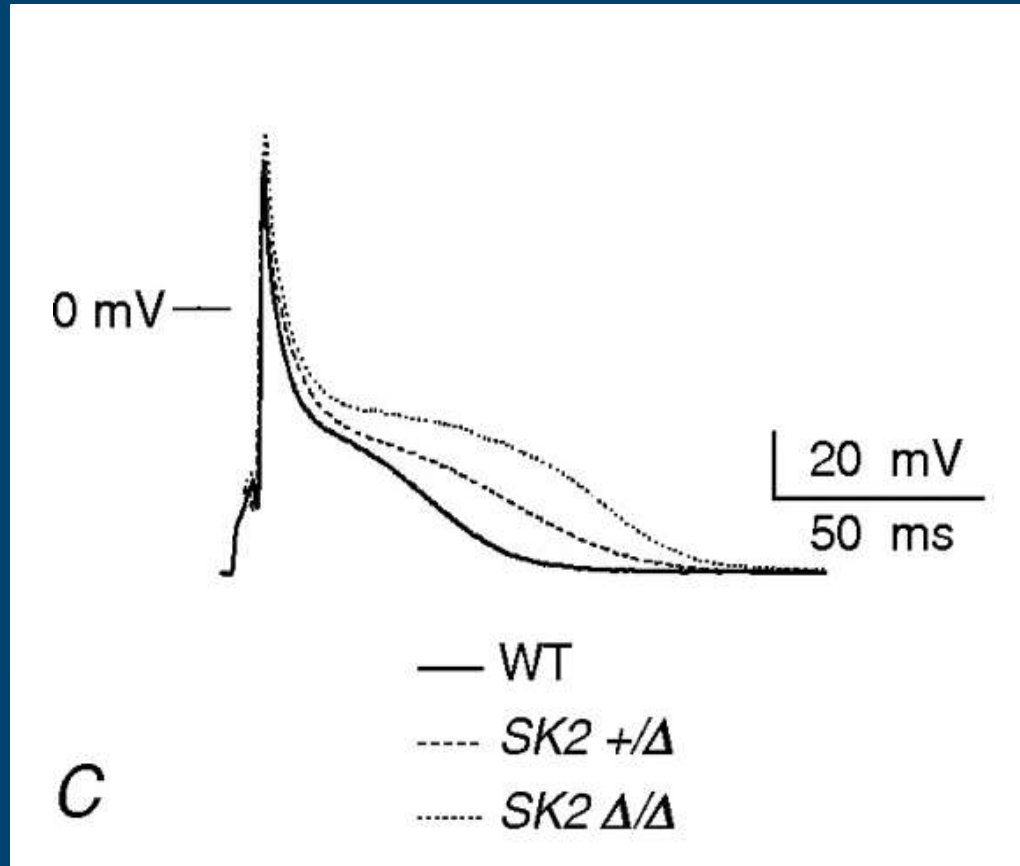
***ZFH3* Chr 16**

- Regulates myogenic and neuronal differentiation
- Function in cardiac tissue unknown

***KCNN3* Chr 1**

- Variants are within *KCNN3* or *SK3* gene
- Calcium activated potassium channel in the heart and brain

Modulation of KCNN2 alters cardiac repolarization

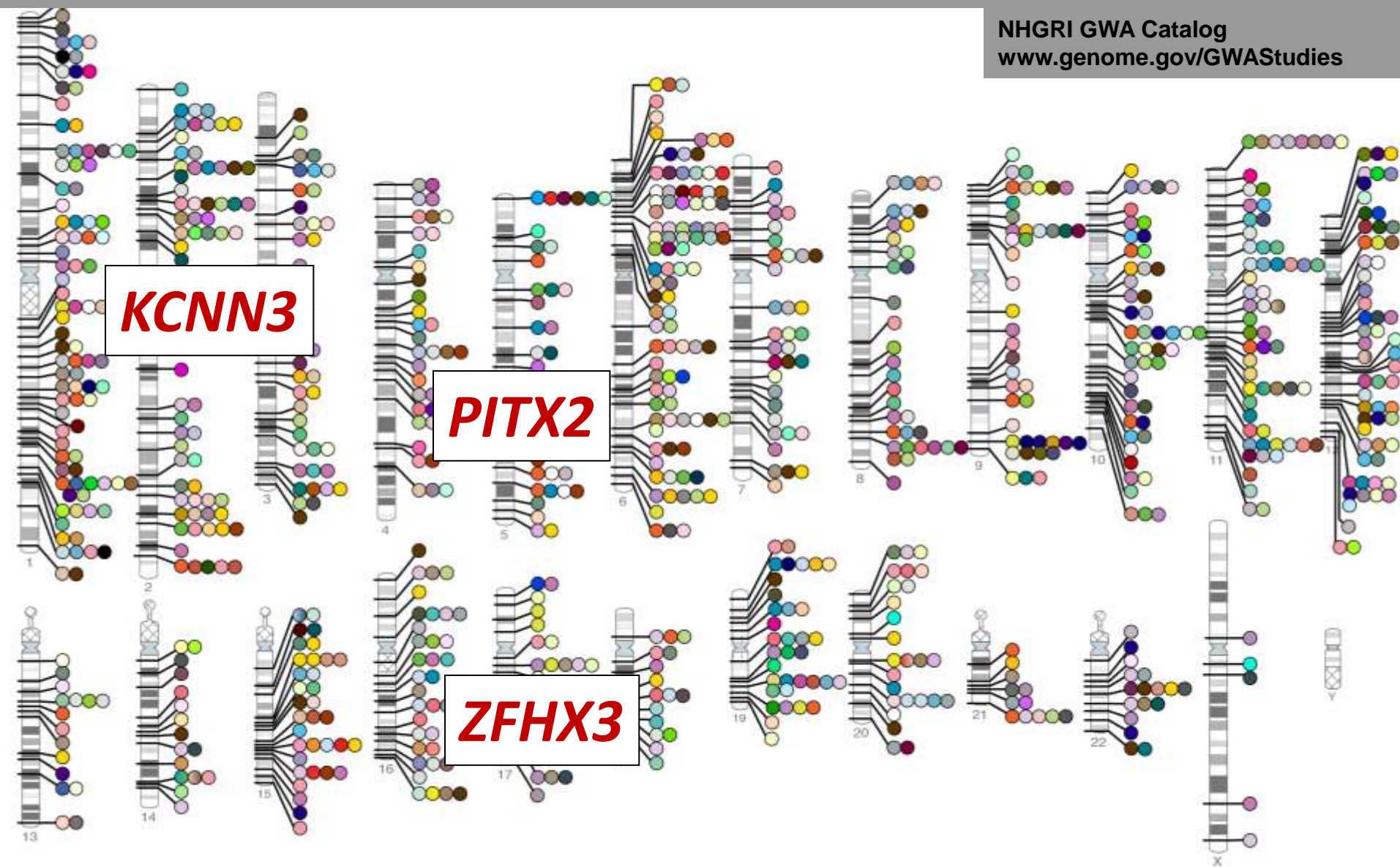


Published Genome-Wide Associations as of 6/2010

904 published GWA at $p \leq 5 \times 10^{-8}$ for 165 traits

NHGRI GWA Catalog

www.genome.gov/GWASudies



The New York Times

A Decade Later, Human Gene Map Yields Few New Cures

By NICHOLAS WADE

Published: June 12, 2010

“Medical Uses Limited”

“Despite early Promise, Diseases’
Roots Prove Hard to Find”

Familial AF is Associated with AF Risk

	HR (95% CI)	<i>P</i>
First-degree familial AF		
Age- and sex-adjusted	1.39 (1.12-1.73)	.003
Multivariable-adjusted	1.40 (1.13-1.74)	.002

Familial AF is Associated with AF Risk

	HR (95% CI)	<i>P</i>
First-degree familial AF		
Age- and sex-adjusted	1.39 (1.12-1.73)	.003
Multivariable-adjusted	1.40 (1.13-1.74)	.002
Multivariable- and SNP-adjusted*	1.38 (1.08-1.75)	.01

*N=2,861

Reality versus expectations of GWAS

- Small effects does not mean the pathway lacks importance
- Small effects does not mean manipulating the pathway will have a small effect
 - KCNN3 channel blockers are in development
- Science takes time

Future Directions for GWAS

Populations

Identify new loci

- GWAS of non-European ancestry
- Larger multi-center studies
- GWAS of sex chromosomes
- Copy number variants
- Gene-environment interactions

Causal variants

- Bioinformatic predictions of SNP function
- Correlation between SNP and gene expression
- Fine mapping of genetic loci
- Resequencing of candidate genes

Model systems

Pathogenesis

- Develop faithful animal and cell model systems
- Functional characterization of loci
- Regulation of gene expression
- Determine role of epigenetics

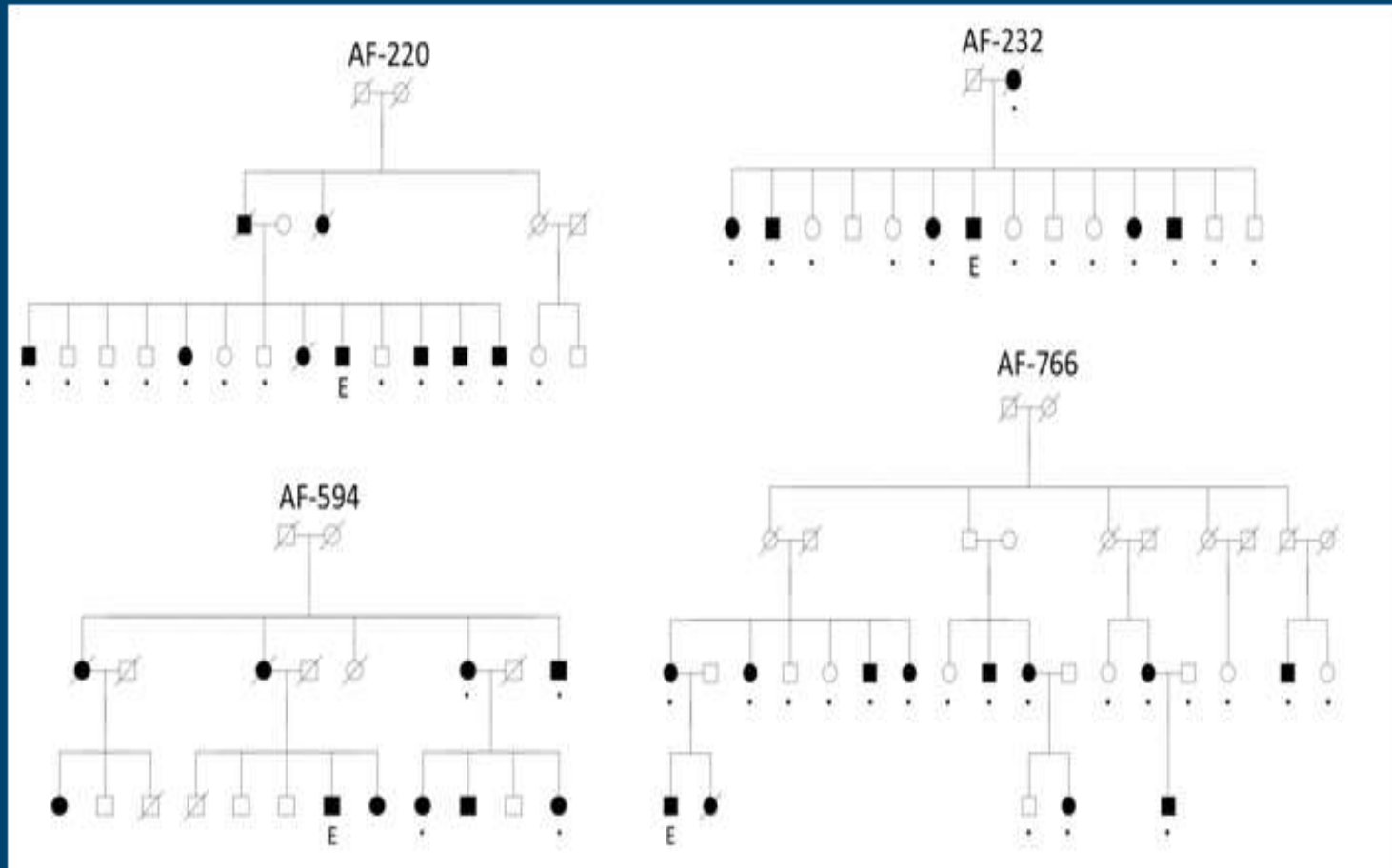
Individuals

Clinical application

Genotype-based prediction of:

- Disease risk
- Disease-related morbidity
- Response to treatment
- Prediction of toxicity
- And ultimately genotype-guided preventive strategies

Ongoing evaluation of large families with AF



Conclusions

- AF is heritable
- Familial AF and premature AF are risk factors for AF
- Genome wide association studies have identified common variants near *PITX2*, *ZFHX3* and *KCNN3* are strongly associated with AF
- Combination of SNPs at 4q25 are associated with a markedly increased risk of AF

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