2025 Howard B. Burchell Memorial Lecture

Dr. Burchell is widely recognized as one of the foremost authorities in cardiology during the 1950s and 1960s. He is considered to have set the stage, with his colleagues, for the ablation of accessory AV connections, which ultimately led to the current era of interventional cardiac electrophysiology. The annual Burchell lecture is a tradition that was created over twenty years ago as a way to honor Dr. Burchell and his contributions to the world of medicine.

Minneapolis Heart Institute Foundation | GRAND ROUNDS

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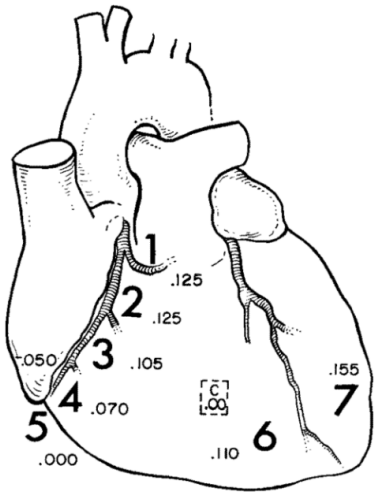


FIGURE 3 The epicardial map recorded by Burchell during MaGoon's surgery for an atrial septal defect. The large numbers are for location identification only. The smaller numbers indicate that the earliest site of pre-excitation is at the extreme lateral portion of the right atrio-ventricular (AV) groove. This was the first successful attempt to localize an accessory AV connection in a patient at surgery. Lidocaine was injected at the suspected site of the pathway, resulting in temporary elimination of the ventricular pre-excitation. See text for further discussion. (Reproduced by permission from Reference[4]).

2

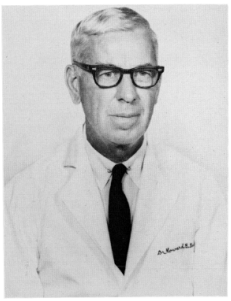
Circulation
JANUARY 1971
VOL. XLIII NO. 1
AN OFFICIAL JOURNAL of the AMERICAN HEART ASSOCIATION

EDITORIAL

Howard B. Burchell, M.D.

WITH THIS ISSUE, CIRCULATION begins its twenty-second year. One of the major contributions of the American Heart Association, CIRCULATION has occupied an internationally recognized position of leadership since its inception.

This eminent position did not just happen. The vision, care, and work of many contributed to it. Over and above administrative support from the American Heart Association and the efforts of the Publications Committees, three factors stand out as vital to the success of CIRCULATION. One is the painstaking work of authors leading to carefully prepared manuscripts. The second is the collective contribution of the Editorial Boards which have suggested means of improving the quality of papers submitted for publication. The third, and perhaps one of the most vital factors, has been the work, intelligence, and dedication of the Editor.



The retirement of Dr. Burchell from the Editorship with the last issue makes this the occasion for all of us in cardiology to express our deep gratitude for the service he has rendered. To understand the deep measure of that service, it is appropriate to consider the qualities which have made Howard Burchell a great Editor.

In my view, among Howard's many attributes, several are of paramount importance to a successful Editorship—his high professional standing, ceaseless striving for perfection, and compassion. Dr. Burchell came to the Editorship with exceptional stature in cardiology. This quality had been based upon a sound education not only in the field of his choice, but in pathology and physiology. With a keen mind and persuasive personality, he has continued to use his basic science background, not only in unraveling the complexities of clinical cardiology as they apply to the patient, but also in teaching the developing cardiologist. Significant of his Editorship was

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Burchell Lecture Past Presentations

- **2024: Duke Cameron, MD** *The Marfan Syndrome*
- **2023: Douglas Mann, MD** *Heart Failure with an Improved Left Ventricular Ejection Fraction: Mechanics, Models and Management*
- **2022: Clyde Yancy, MD** *Heart Failure; a new coming of age for an old disease*
- **2021: Mathew Maurer, MD** *Cardiac Amyloidosis: Transition from rare, underdiagnosed and untreatable to an increasingly and easily recognized and treatable disorder*
- **2019: Navin Kapur, MD** *Ventricular Unloading: State of the Art and Future Directions*
- **2018: Anne Marie Valente, MD** *The STORCC Initiative (Standardized Outcomes in Reproductive Cardiovascular Care)*
- **2017: Robert Harrington, MD** *Rethinking Randomized Clinical Trials*
- **2016: Carl Pepine, MD** *Emergence of Nonobstructive Coronary Artery Disease in Women*
- **2013: Richard Asinger, MD** *Stroke Prevention in Atrial Fibrillation: An Overview and Future Directions*
- **2012: David Holmes, Jr, MD** *Global Cardiovascular Disease*
- **2010: Bernie Gersh, MD** *The Epidemic of Cardiovascular Disease in the Developing World: Global Implications*

Minneapolis Heart Institute Foundation | GRAND ROUNDS

4

Minneapolis Heart Institute Foundation®
Cardiovascular Grand Rounds

Burchell Lecture: Insights from Atrial Fibrillation Genomics: From Bedside to Bench and Back Again

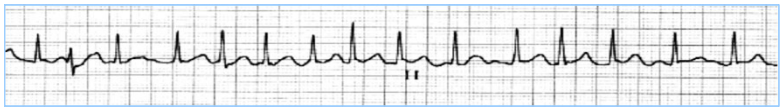
Speaker: Mina K. Chung, MD
President Elect, Heart Rhythm Society
Professor of Medicine, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
Staff, Department of Cardiovascular Medicine, Heart, Vascular & Thoracic Institute, Cleveland Clinic
Staff, Department of Cardiovascular and Metabolic Sciences, Lerner Research Institute, Cleveland Clinic

April 14, 2025 | 7:00 – 8:00 AM

Minneapolis Heart Institute Foundation | GRAND ROUNDS

5

Insights from Atrial Fibrillation Genomics: From Bedside to Bench and Back Again



Mina K. Chung, M.D.
Section of Cardiac Electrophysiology and Pacing
Department of Cardiovascular Medicine
Heart, Vascular and Thoracic Institute
Department of Cardiovascular and Metabolic Sciences
Lerner Research Institute

Atrial Fibrillation Innovation at the Cleveland Clinic

Cleveland Clinic

No Relevant or Financial Disclosures

CLEVELAND AF

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Howard B. Burchell, MD: A Renaissance Cardiologist

- Vital role in introducing modern cardiology to Mayo Clinic
- Chief of Cardiology at University of Minnesota
- Editor-in-Chief of Circulation

A portrait at 95 by Frits Meijler in 2002
Am J Cardiol 2010;105:566 –569

Atrioventricular and Ventriculoatrial Excitation in Wolff-Parkinson-White Syndrome (Type B)

Temporary Ablation at Surgery

By Howard B. Burchell, M.D., Robert L. Frye, M.D., Milton W. Anderson, M.D., and Dwight C. McGoon, M.D.

Circulation 1967, 36::663-672

Launching modern interventional EP

Howard Burchell—Personal Reminiscences – Frits Meijler:

How can you call atrial fibrillation "The grandfather of cardiac arrhythmias?" Does celibacy protect against atrial fibrillation?

7

AF Genomics: From Bedside to Bench and Back Again

- AF epidemiology and pathophysiology
- AF genetics and genomics
- Association with structural variation
- The age paradox
- The pathway to a genomics-based drug discovery
- Integrating imaging and genomics for interventional targeting

8

Prevalence, Incidence, Morbidity and Mortality of AF

Prevalence and Incidence of AF is increasing and projected to double between 2010 and 2030

50 million
estimated individuals with AF worldwide in 2020

At least **5.6 million** individuals with AF in USA in 2015

- about 11% estimated cases were undiagnosed

Overall lifetime risk:
- 30-40% in White individuals
- 20% in African American individuals
- 15% in Chinese individuals

In Medicare beneficiaries, the most frequent outcome in 5-yrs after AF diagnosis was death (19.5% at 1-yr; 48.8% at 5-yrs)

AF is associated with increased risks:
- 1.5-to 2-fold risk of death
- 2.4-fold risk of stroke
- 1.5-fold risk of CI/ dementia
- 1.5-fold risk of MI
- 2-fold risk of SCD
- 5-fold risk of HF
- 1.6-fold risk of CKD
- 1.3-fold risk of PAD

AF accounted for **$28.4 billion/ year** in US healthcare spending in 2016

Abbreviations: AF indicates atrial fibrillation; CI, cognitive impairment; CKD, chronic kidney disease; HF, heart failure; MI, myocardial infarctions; PAD, peripheral arterial disease; SCD, sudden cardiac death; yr, year; and yrs, years.

American Heart Association.

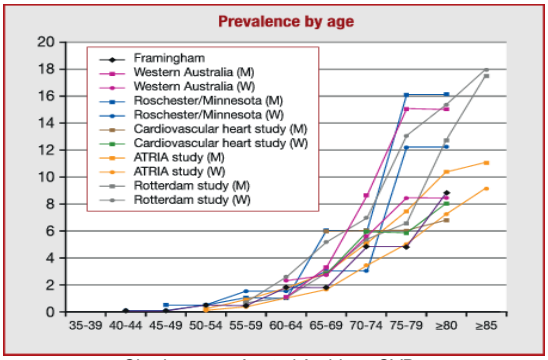
Joglar, J. A. et al., 2023 ACC/AHA/ACCP/HRS Guideline for the Diagnosis and Management of Atrial Fibrillation. *Circulation.*

9

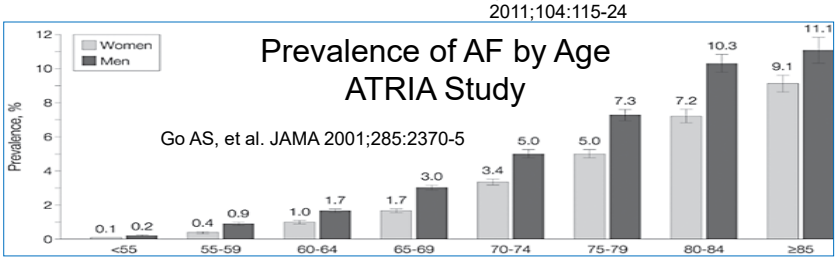
9

Atrial Fibrillation

- Most common sustained arrhythmia
  - –50 million worldwide
  - –5 million new cases/year
  - –6 million in the US; 12 million by 2050
- Associated with:
  - –Stroke
  - –Atrial cardiomyopathy
  - –Heart failure
- Associated with heterogeneous CV stresses
  - –Older age
  - –Obesity
  - –HTN, DM, CAD, valve ds
  - –Heart failure



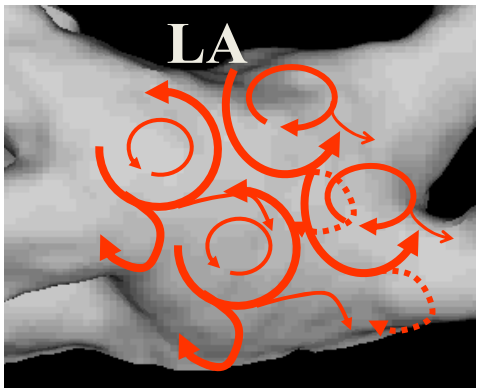
Charlemagne A, et al Archives CVD 2011;104:115-24



10

1950s-1990s: AF is a *Reentrant Arrhythmia*

- **Multiple Wavelet Hypothesis**
- **Structural substrates**



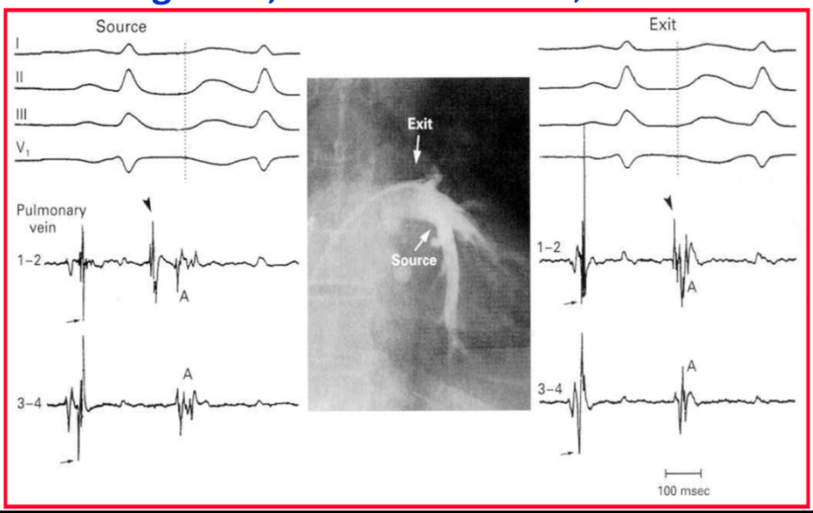
Moe – 1950s

11

1998: AF Mechanisms – Focal

Spontaneous Initiation of AF by Ectopic Beats Originating in the Pulmonary Veins

Haissaguerre, et al. NEJM 1998;339:659-666



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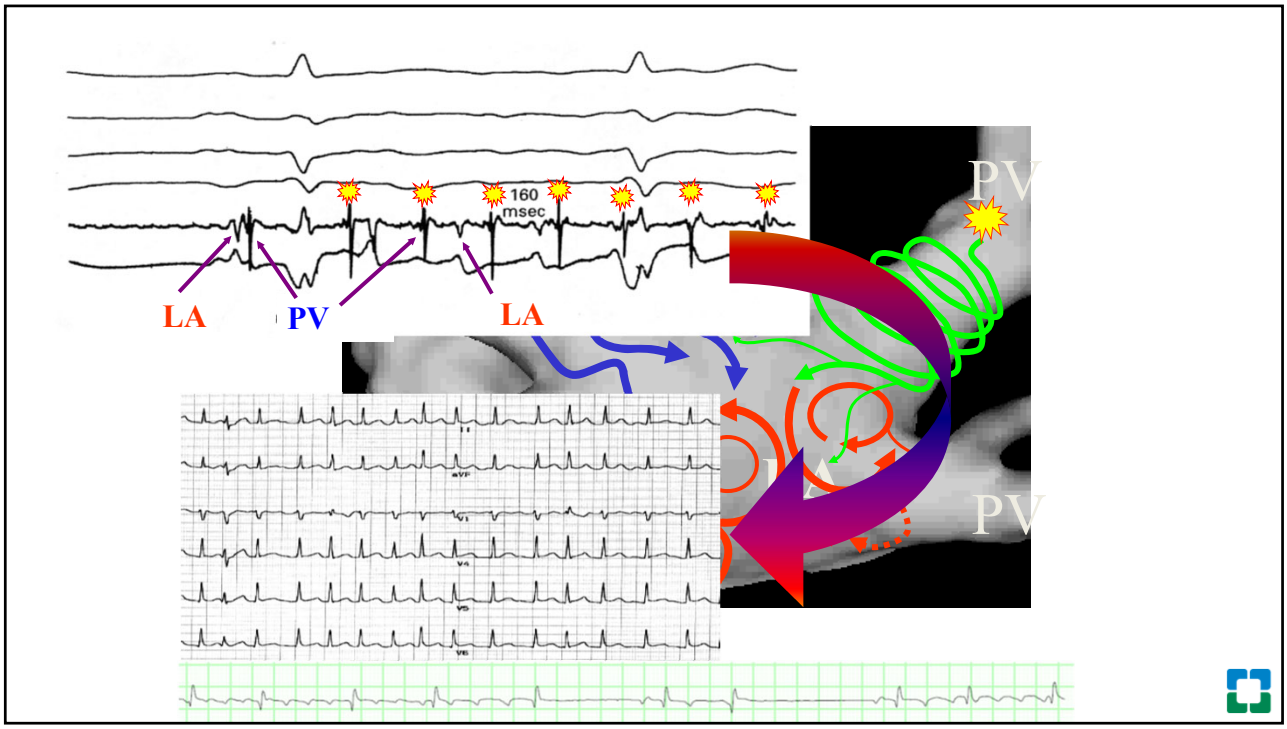


13

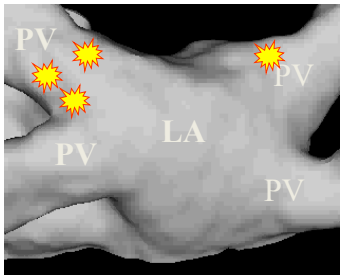
Initiation and Maintenance of AF
Dual Substrate Model

Substrate for AF Initiation

Electrical Remodeling

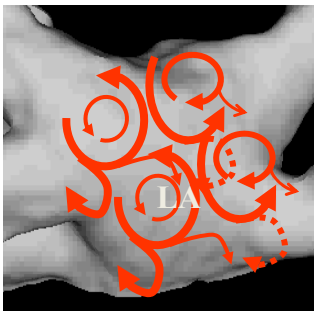
Structural Remodeling

Substrate for AF Maintenance



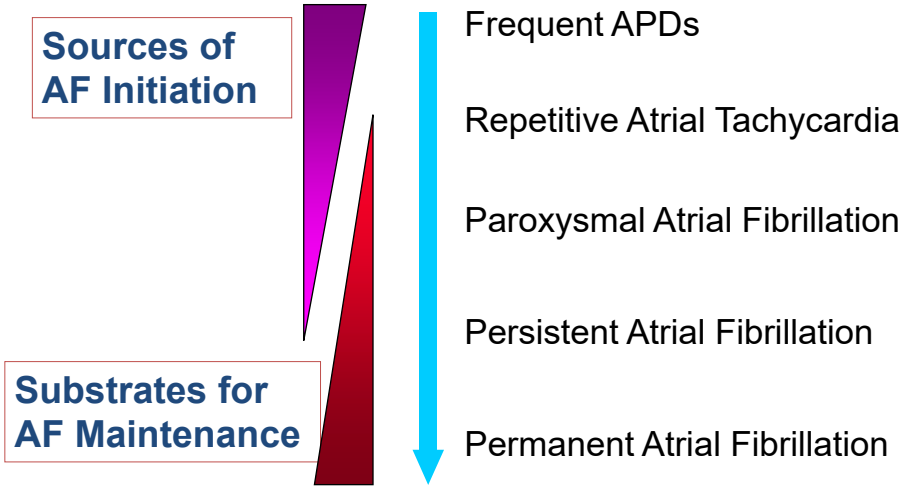
Modified from Lesh, M

- Stretch
- Calcium overload
- LVH
- Ventricular dysfunction
- Valve disease
- Autonomic tone
- Inflammation
- Fibrosis
- Toxins



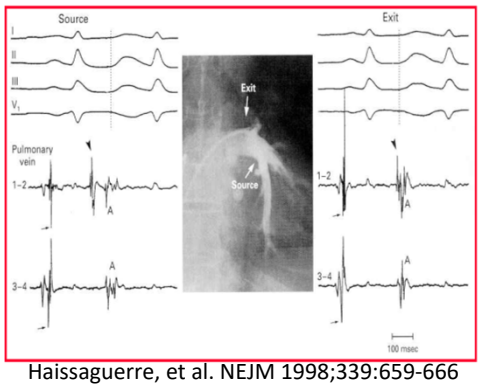
14

Natural History of AF
Dual Substrate Model

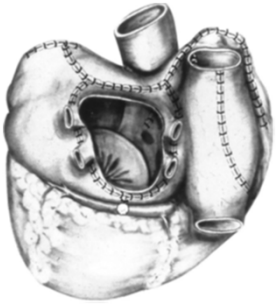


15

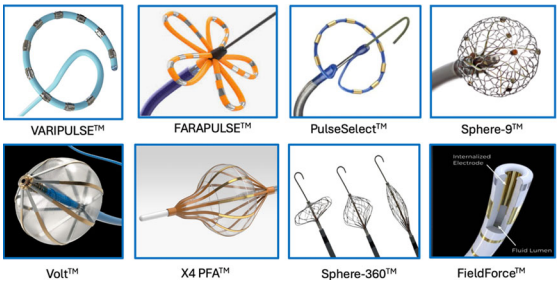
Cornerstone of AF ablation:
PV Isolation



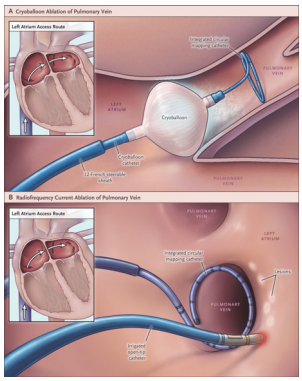
Haissaguerre, et al. NEJM 1998;339:659-666



Cox JL. The Journal of Thoracic and Cardiovascular Surgery, 141:1093-7, 2011



Yavin et al J Cardiovasc Dev Dis 2025; 12, 10.



Kuck KH et al FIRE AND ICE Investigators, NEJM 2016;374:2235-2245

16

Some remaining issues in AF ablation

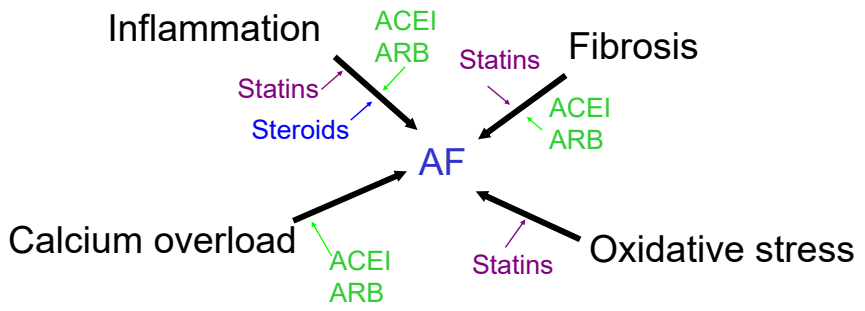
- Ceiling of success rates, even in paroxysmal AF
- Recurrence even after successful PVI – improving durability
  - recovery of conduction
  - new atypical atrial flutters
- Poor success rates in persistent AF
- Role of substrate ablation in persistent AND paroxysmal AF
- Reducing complication rates
  - PV stenosis, AE fistula, atypical flutters; hemolysis, coronary spasm
- Are there upstream therapies that can target AF substrates?

17

Traditional "Upstream Therapies for AF"

Pathways leading to atrial remodeling and promotion of AF

ACEIs, ARBs, Statins, Steroids, N-3 FAs



However, with the exception of steroids and statins for postop AF and ACEI/ARBs in HF, RCTs in AF have been disappointing

18

Role of genetics in AF

AF is heritable

- **Framingham** – Fox et al JAMA 2004
  - *Parental AF ↑s risk of AF in offspring*
  - Parent with AF → OR 1.85
  - Parent with AF <75 y/o → OR 3.23
- **Iceland** (DeCODE genetics, Arnar et al EHJ 2006)
  - *AF ↑s risk of AF in 1st degree relatives* → RR 1.77
  - Early onset AF <60 y/o → RR 4.67
- **MGH** (Ellinor et al Hum Genet 2005)
  - *Lone AF ↑↑s risk of AF in family members*
  - RR → Sons 8.1, Daughters 9.5, Brothers 70, Sisters 34
  - 30% of LAFs have FH of AF
- **Danish Twin study** – AF heritability 62%
- **UK Biobank** – AF heritability 22%

19

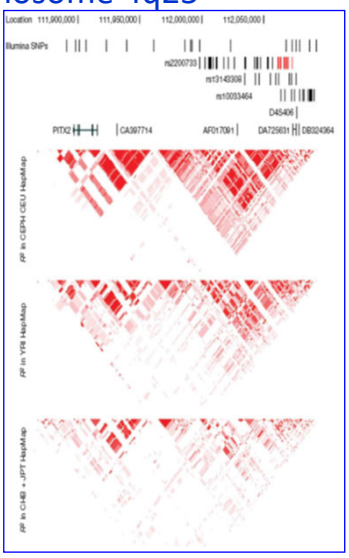
Genetics: The "ultimate" upstream approach

2007: AF is Heritable and has a Genetic component

DeCODE (Iceland): GWAS of AF – chromosome 4q25

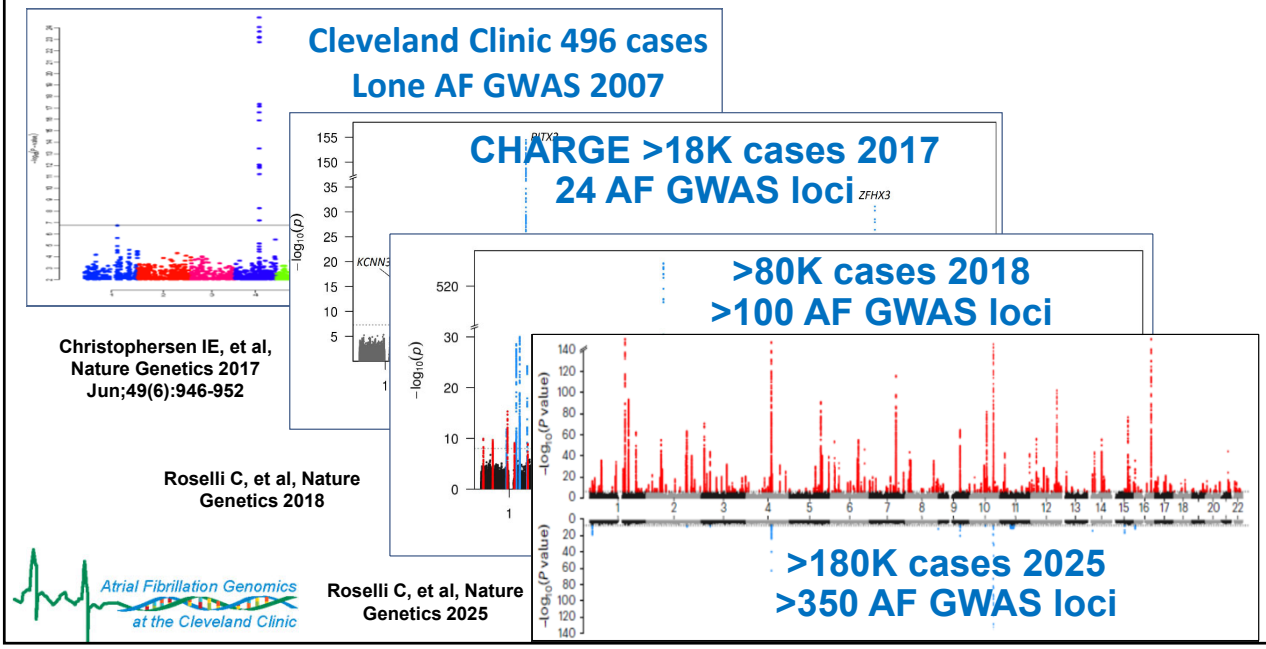
Gudbjartsson, et al Nature 2007

- Top 3 SNPs - Chromosome 4q25
- ORs 1.3 – 2.0
- Replicated in several cohorts
  - Replication cohort
  - Cohorts from Sweden, USA, Hong Kong



20

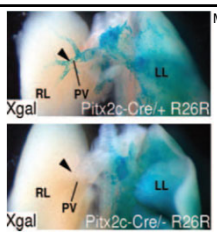
Genome-wide association studies in AF



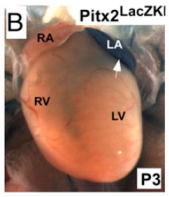
21

PITX2c is the top candidate gene nearest the top AF locus at Chr. 4q25

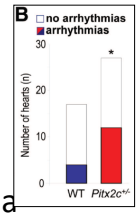
- Directs asymmetric cardiac morphogenesis
- Involved in formation of PVs[1]
- Expressed in the human and mouse LA[2,3]
  - Essentially absent in other parts of the heart
- Suppresses a left atrial sinus node program[2,3]
- Pitx2 deficient mice are
  - more susceptible to pacing induced atrial arrhythmia
  - Do not develop a pulmonary myocardial sleeve[1]
- Complex developmental TF interactions[7]
- Role in early cardiac regeneration and repair[8]



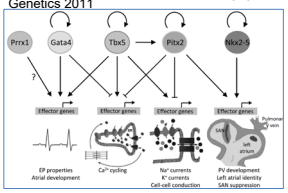
Mommersteeg, et al Circ Res 2007



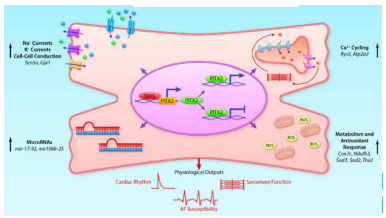
Wang *et. al.*, PNAS 2010



Kirchhof, et al. Circ CV Genetics 2011



van Ouwerkerk, et al. Circ Res 2020

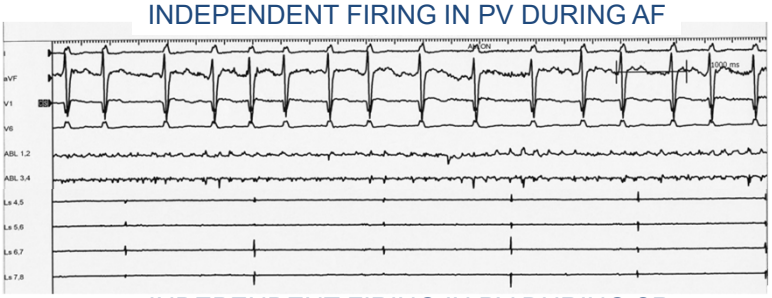


[1]Mommersteeg, et al Circ Research 2007;101:902-9; [2]Wang *et. al.*, PNAS 2010;107:9753-8; [3]Mommersteeg, et al Circ Res 2007:100:354-62; [4]Kirchhof, et al. Circ CV Genetics 2011;4:123-33; [7]van Ouwerkerk Circ Res 2020;127:34-50; [8]Tao et al. Nature 2016; 534:119-123.

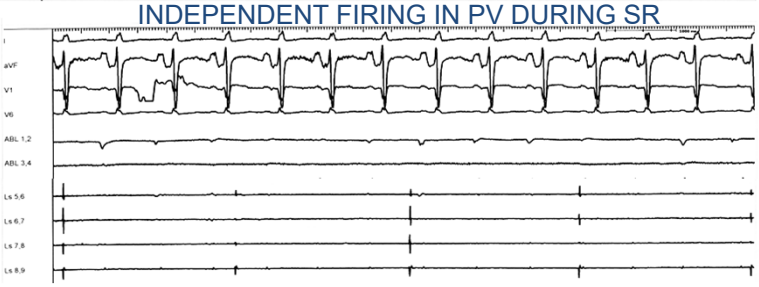
22

Could this explain PV triggers?
Post-PVI Dissociated Firing

INDEPENDENT FIRING IN PV DURING AF



INDEPENDENT FIRING IN PV DURING SR



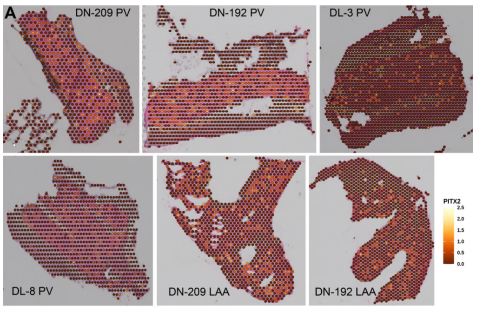
23

Spatial RNA Seq from donor heart tissues

- 3 heart, 5 lung donors; 3 AA, 5 EA
- 10x Genomics Vizium platform

Regional and cellular differential expression

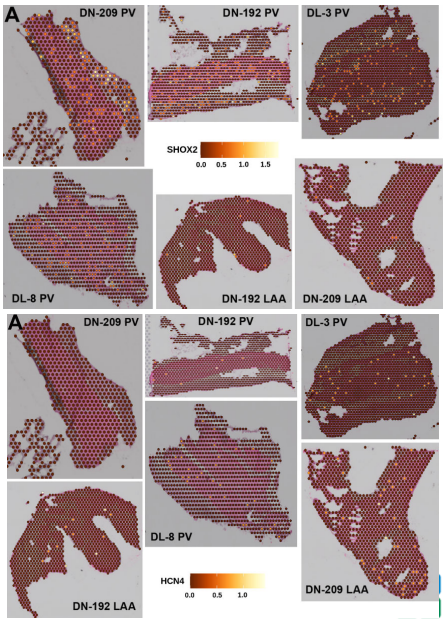
- *PITX2* in PV and LAA CMs
- *SHOX2* in PV CM and endocardium regions in CMs and VSMCs
- *HCN4* in sporadic PV and LAA CMs

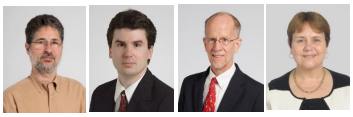


SHOX2

PITX2

HCN4



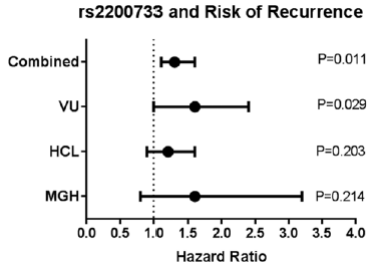


Sun H, Han J, Moravec CS, Okamoto T, McRurry KR, Gillinov AM, Chung MK, Van Wagoner DR, Barnard J, Smith JD

24

Towards Personalized Gene-Based *Interventional Therapies:*
AF genetic risk variants, ablation outcomes, and PV, LA morphology

- Can personalized gene-based stratification guide AF ablation or patient selection for AF therapies?
- Top loci at 4q25, 16q22, 1q21 have been associated with higher risk of:
  - AF recurrence p CV
  - Symptomatic recurrences on AADs
  - Differential responsiveness to AADs
- "Ablatogenomics"
  - Conflicting results, but some risk variants have assd with higher risk of recurrence p AF ablation

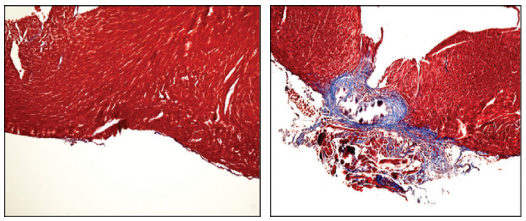


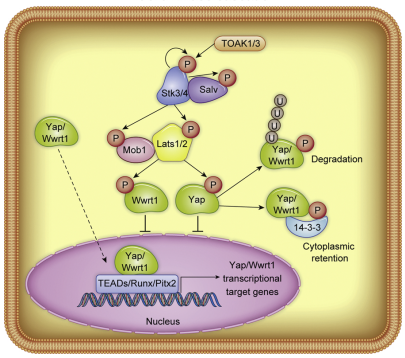
Shoemaker MB et al Circ AE 2015;8:296-302

25

Potential role of *PITX2* in cardiac regeneration

- Role in cardiac regeneration[1]
  - Neonatal mice can regenerate heart tissue
  - *Pitx2*-deficient neonatal mouse hearts fail to repair after apex resection
  - Adult mouse CMs with *Pitx2* gain-of-function regenerate after MI
- Hippo-Yap signaling pathway[2]
  - Regulates neonatal and adult CM proliferation
  - Activation of Hippo kinases inhibits transcriptional co-activators Yap and Taz via phosphorylation and exclusion from the nucleus or degradation
  - Yap activity is required for CM proliferation during development
  - Suppressing Hippo cascade or overdriving Yap induces post-mitotic CM proliferation in mice
  - PITX2 interacts with Yap[1]





Could this explain reconnections post PVI?

[1]Tao G…Martin JF et al. Nature 2016; 534:119-123
[2]Flinn MA, Link BA, O'Meara CC Seminars in Cell & Devel Biol 2020 100:11-19

26

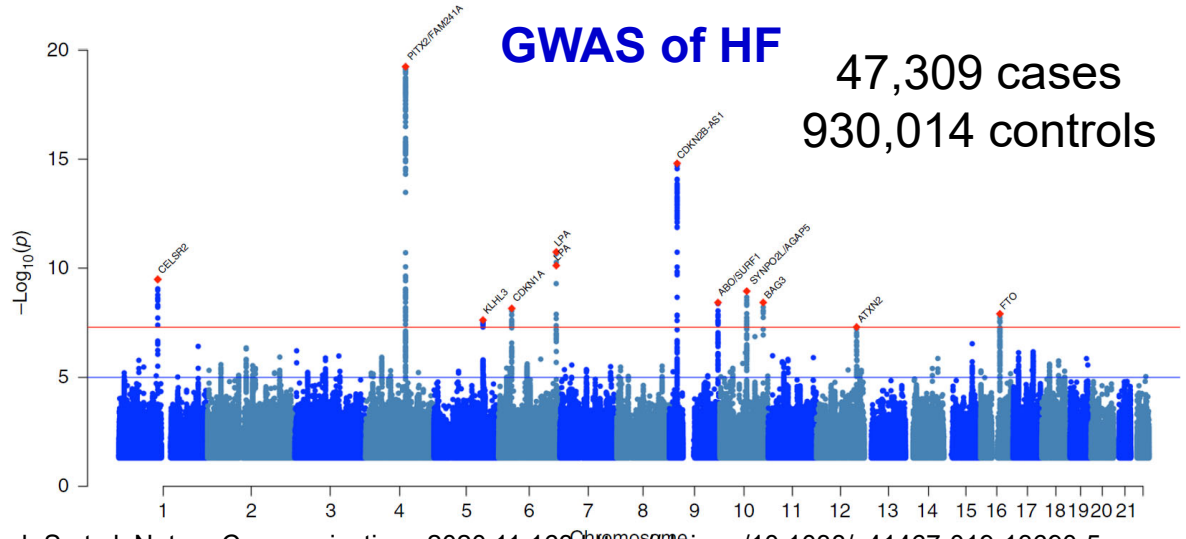
AF and Structural Heart Disease

27

Commonality between AF and Structural Heart Disease
Overlapping genetics of AF and HF

GWAS of HF

47,309 cases
930,014 controls



Shah S et al. Nature Communications 2020 11:163 https://doi.org/10.1038/s41467-019-13690-5

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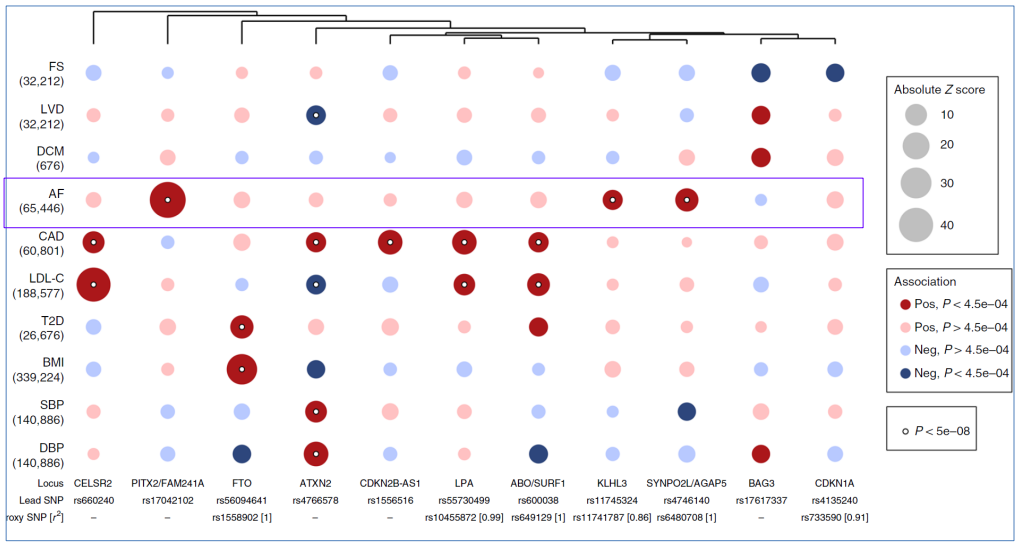
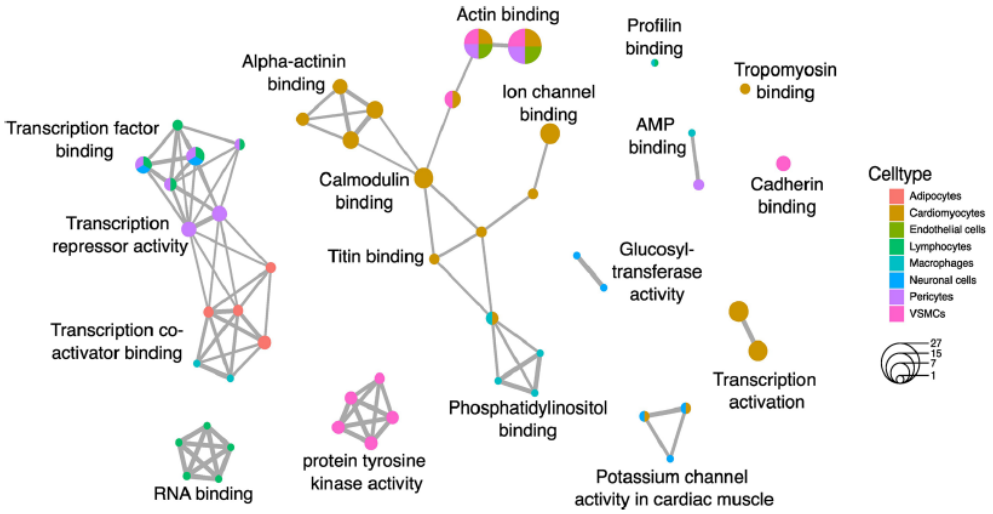
Overlapping genetics of AF and HF



Shah S et al. Nature Communications 2020 11:163 https://doi.org/10.1038/s41467-019-13690-5

29

Cluster analysis of prioritized genes based on cell type specific expression and Gene Ontology (GO)

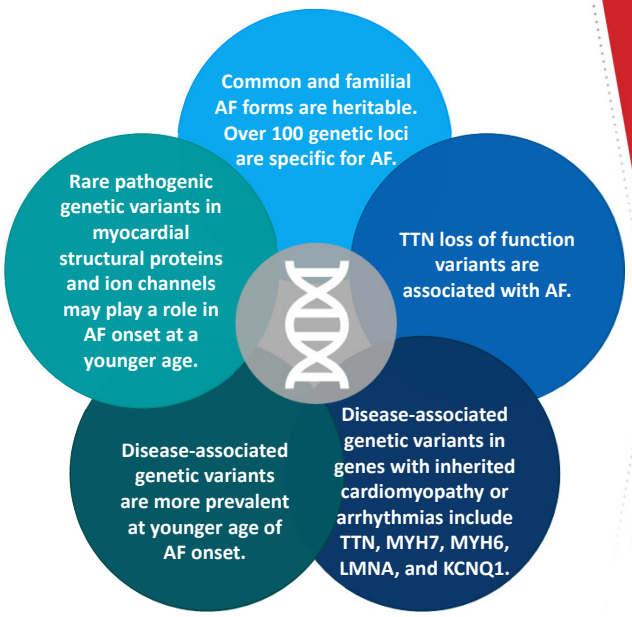


Many of the AF prioritized genes are associated with structural genes in cardiomyocytes

30

Early Onset AF and Genetic Screening

- TOPMed: 1293 pts <66 yo with AF, 10.1% had a disease associated genetic variant in genes associated with cardiomyopathy or channelopathy (e.g. *TTN, MYH7, MYH6, LMNA, KCNQ1*). 16.8% if <30yo
- 23 patients with onset of AF <45yo and no identifiable cause of AF, 24% had a pathogenic/likely pathogenic variant, most in CM genes



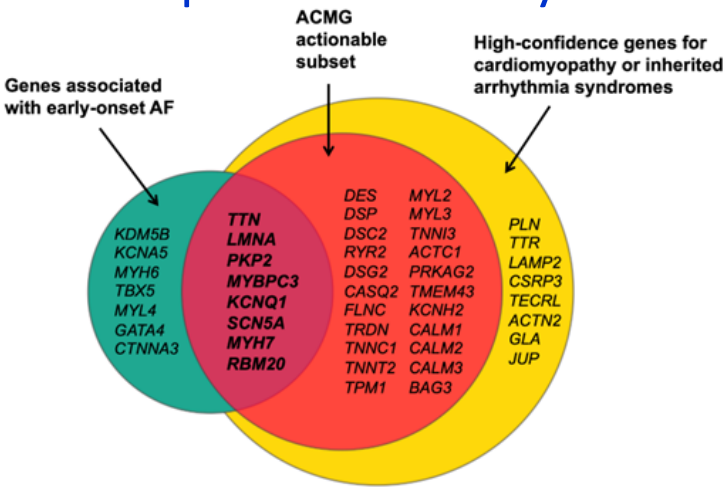
American Heart Association.

Joglar, J. A. et al., 2023 ACC/AHA/ACCP/HRS Guideline for the Diagnosis and Management of Atrial Fibrillation. *Circulation*.

31

31

Overview of genes where rare coding variant have been implicated in early-onset AF



Kany et al. Genetic testing in early onset atrial fibrillation. EHJ 2024 Jul 19:ehae298. doi: 10.1093/eurheartj/ehae298

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Recommendations for Early Onset AF, including Genetic Testing

Recommendations for Management of Early Onset AF, Including Genetic Testing
Referenced studies that support the recommendations are summarized in the Online Data Supplement.

COR	LOE	RECOMMENDATIONS
2b	B-NR	1. In patients with an onset of unexplained AF before 30 years of age, electrophysiological study to evaluate and treat reentrant supraventricular tachyarrhythmias with a targeted ablation may be reasonable because of the high prevalence of reentrant arrhythmias in this group.[1-3]
2b	B-NR	2. In patents with an onset of AF before 45 years of age without obvious risk factors for AF, referral for genetic counseling, genetic testing for rare pathogenic variants, and surveillance for cardiomyopathy or arrhythmia syndromes may be reasonable.[4,5]

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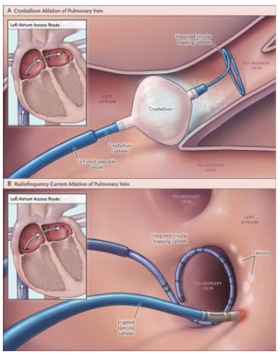
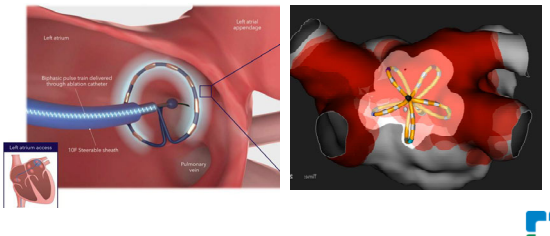
Perspectives from AF genetics/genomics

- Top AF locus: near a gene implicated in formation of PVs during development
  - Pulmonary vein ostia are the target of AF ablation
  - Top risk loci appear to predict tendency for AF recurrence after ablation
- There is overlap in genetics of AF and structural heart genes





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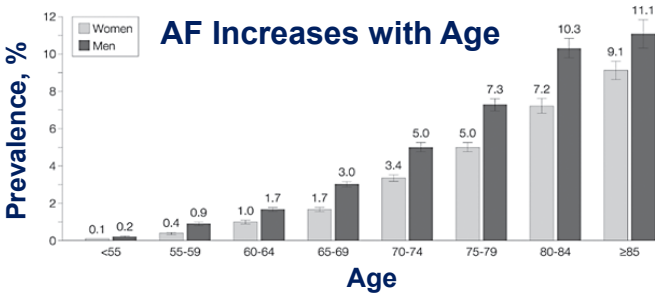
The Age Paradox

- **Several of the top genetic loci are active early in cardiac development**
- ***However, AF generally does not develop until 5-7 decades later***



Go AS, et al. JAMA 2001;285:2370-5

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From Genomics to AF Mechanisms

Genes to Function

DNA
↓
mRNA
↓
Protein
↓
Function

Genomics⇒multi-omics

- Identification of functional links and mechanisms for GWAS-identified variants
- Identification of new potential upstream therapies:
  - Developmental genes: tough targets
  - What can we target in adulthood?

How is gene expression affected by AF?

↓

AF Susceptibility and Persistence

How do AF-risk SNPs affect local gene expression?

↓

What are the causal SNPs and which genes do they affect?

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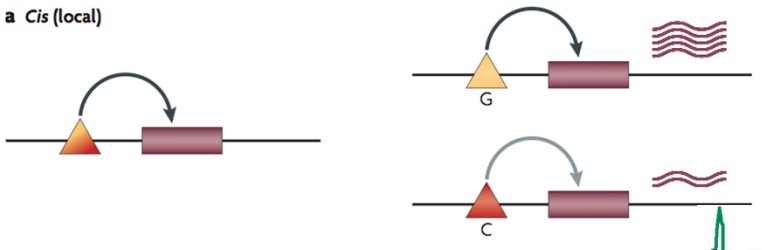
What are the genes affected by AF GWAS SNPs?

Objective: Use transcriptomics to identify functional SNPs that affect gene expression

Do AF Associated SNPs Influence RNA Expression of Nearby Genes in Human Left Atria?

- mRNA gene expression is analyzed as a quantitative trait
- SNPs that influence local (cis) gene expression ⇒ *cis*-eQTLs

Resource: >1000 human atrial tissue bank



Cheung VG, Spielman RS (2009) Genetics of human gene expression: mapping DNA variants that influence gene expression. Nat Rev

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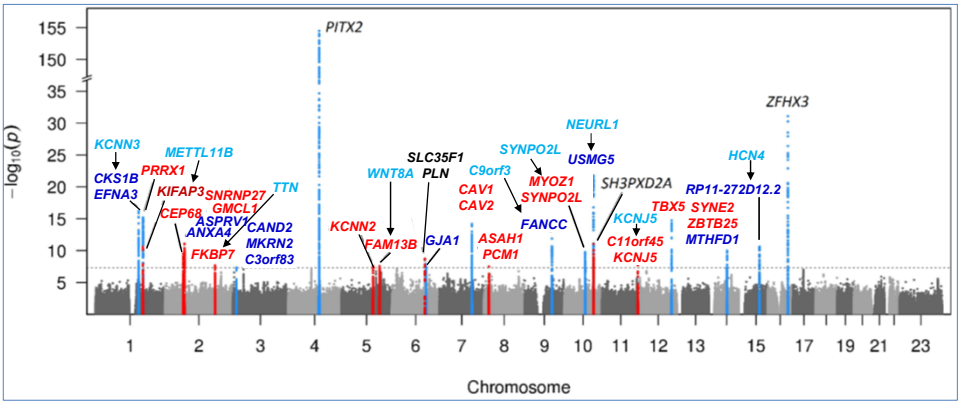
2017 – 24 AF GWAS loci

RNA-Seq: Significant eQTLs found for 12



GWAS
18,398 AF
91,536 referents

ExWAS and RVAS
22,806 Cases
132,612 referents

Significant eQTLs
RNASeq p<0.05 (6)
RNASeq q<0.05 (12)
RNA microarray p<0.05 (1)

eQTL gene was not necessarily the closest gene: candidate genes were re-annotated at 8 loci

http://afeqtls.lerner.ccf.org

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Christophersen IE, et al, Nature Genetics 2017 Jun;49(6):946-952
Hsu J, et al., Circ Genomics Precision Med 2018

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cis-eQTLs were not found for *PITX2* at the top GWAS locus on chr. 4q25

- TIMING?

- LOCATION, LOCATION, LOCATION?

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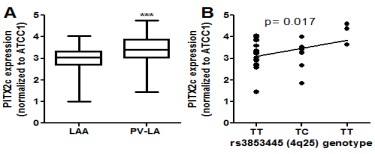
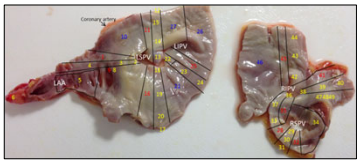
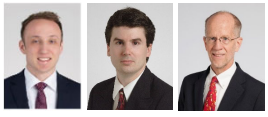
PITX2c in the PV-LA junction

- Regional studies
  - Unadj eQTL in PV-LA
    - Rs3853445 – Minor allele protective for AF (OR 0.59); *PITX2c* expression is higher in homozygotes for the minor allele
  - *PITX2c* expression in PV-LA

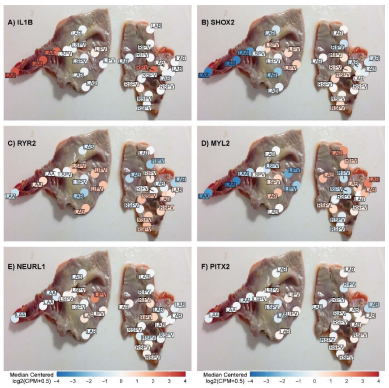
SNP	p value
rs2200733	0.83
rs3853445	0.02
rs1448818	0.81
rs10033464	0.59

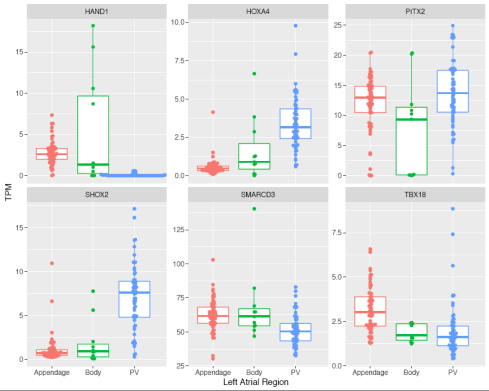






Harwood S, McCurry K, Okamoto T, Castel L, Smith JD, Van Wagoner DR, Barnard J, Chung, MK





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If predisposition to AF is determined by genes active during development, why does AF not manifest until decades later?

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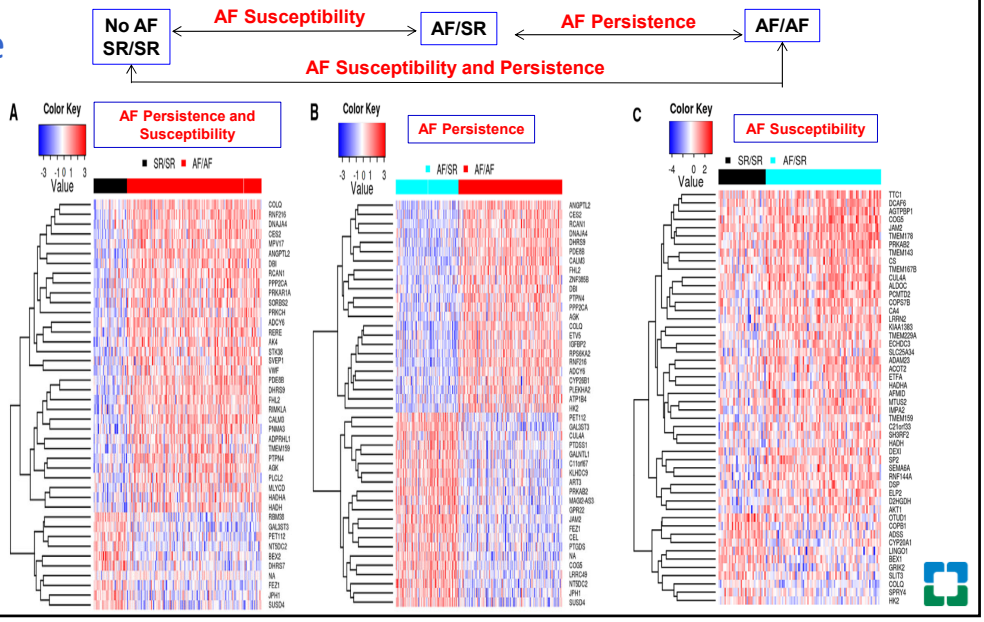
What happens to gene expression with AF?

Differential gene expression by Rhythm:

in 239 human adult LAA tissues



Deshmukh A, Barnard J, Sun H, Newton D, Castel L, Pettersson G, Johnston D, Roselli E, Gillinov AM, McCurry K, Moravec C, Smith JD, Van Wagoner DR, Chung MK. Left atrial transcriptional changes associated with atrial fibrillation susceptibility and persistence. Circ Arrhythm Electrophysiol 2015;8:32-41.

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Gene Set Testing

AF Susceptibility (AF/SR vs No AF)	
CREB/ATF Oxidoreductase Activity	Oxidative stress
HSF1 targets ATF6 targets	Heat shock, unfolded protein response
P53 targets	Stress response, tumor suppression, apoptosis
SRF targets	Cell cycle, growth, differentiation
E2F1 targets	Cell stress response - Cell cycle, tumor suppression
MEF2A targets MYOD targets	Myocyte differentiation
Electron transport activity	Mitochondrial function

AF Persistence (AF/AF vs AF/SR)	
HSF2 targets	Heat shock response
Ion channel activity	Ion channels
MEF2 targets MYOD targets	Myocyte differentiation

Susceptibility

- **↓transcriptional responses to cell stress, unfolded proteins**
- **↑myocyte differentiation gene expression**

Persistence

- **changes in ion channel expression**
- **↓transcriptional responses to cell stress**

AF Susceptibility and Persistence

↓ transcriptional responses to cell stress

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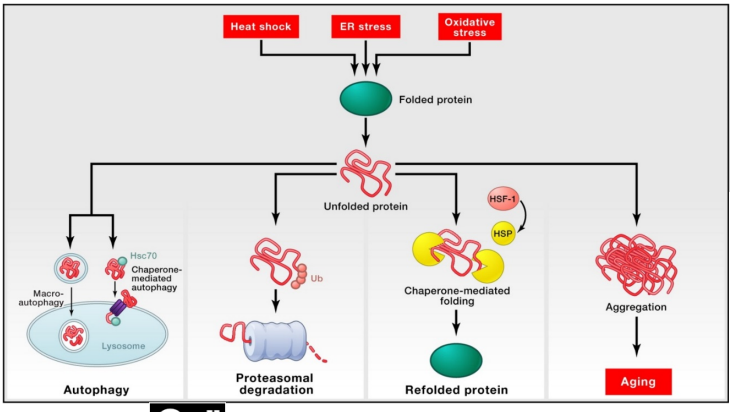
Heat Shock Response

- System designed to protect cells from stress
- Ensures proteins are folded appropriately
- Heat shock factor 1
  - Master regulator of the HSR in mammals



Cell PRESS *Lopez-Otin, et al. Cell* 2013 153, 1194-1217

Hallmarks of Aging

- Amyloid - Insoluble protein aggregates from misfolded protein components
- Atrial amyloid reported in >90% of persons >90 yo
- Atrial amyloid is derived from deposits of atrial natriuretic peptide
- Amyloid can be proteotoxic, may insert into cell membrane and form peptide channels, causing Ca++ dysregulation, membrane depolarization, mitochondrial dysfcn, direct cytotoxicity

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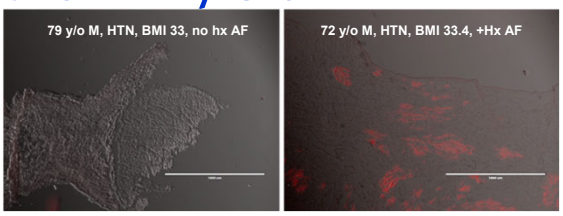
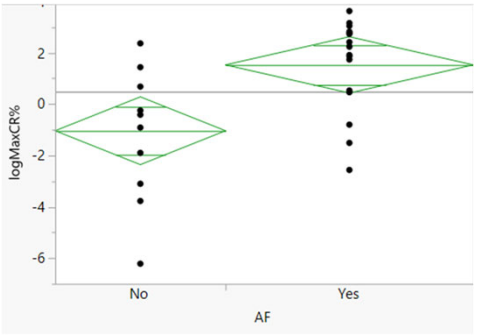
Left Atrial Amyloid

28 LAA samples
- 16 with history of AF
- Matched for sex, age, BMI



Mean amyloid content (% scanned tissue area)
- AF 10.8±2.6%
- No AF 1.29±0.7%

P<0.005



LA amyloid content not associated with
- Age
- Sex
- HTN
- BMI

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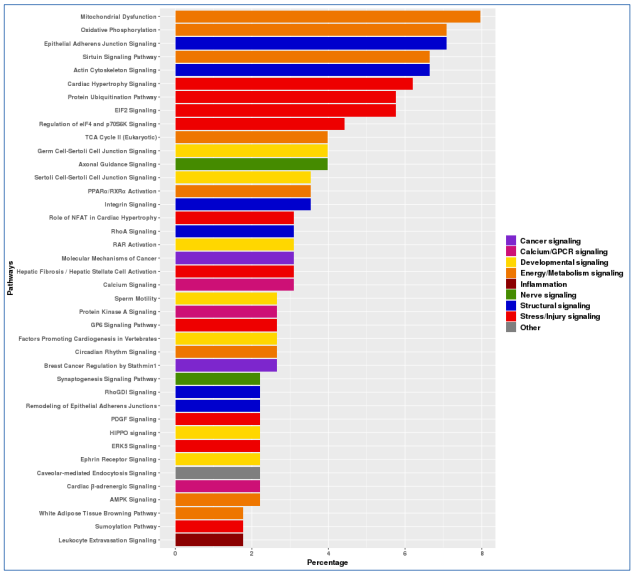
Gene Coexpression Analyses of Top AF GWAS Implicated Genes



Top Canonical Pathways by Ingenuity Pathway Analyses (IPA®)

Metabolic Pathways

- *Oxidative Phosphorylation*
- *Mitochondrial Dysfunction*
- *Structural signaling*
- *EIF2 Signaling*
- *Regulation of eIF4 and p70S6K Signaling*

Stress Response

Left atrial RNAseq co-expression analysis of genes associated with AF risk via GWAS
Wass, et al., Heart Rhythm 2023;20:1219-1226

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Gene Coexpression Analyses of Top AF GWAS Implicated Genes

Top Canonical Pathways by Ingenuity Pathway Analyses (IPA®)

- *Oxidative Phosphorylation*
- *Mitochondrial Dysfunction*

→ **Metabolic Pathways**

- *EIF2 Signaling*
- *Regulation of eIF4 and p70S6K Signaling*

→ **Stress Response**

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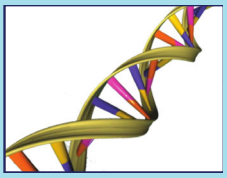
Dual Risk Model
The Perfect Storm for AF

Genetically Susceptible Patients, Inadequate Transcriptional Responses To Stress Predispose To AF In Later Life

Genetic Susceptibility

Pulmonary Vein Triggers

Genes active in development



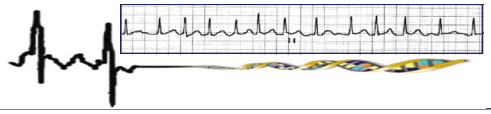
Aging **Obesity**

Overwhelmed or ↓Transcriptional Response to Cell Stress

Stress

Metabolic Stress
Mitochondrial Dysfunction
Oxidative Stress
Abnormal Proteostasis

Atrial Fibrillation



Potential for novel upstream therapies

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Potential Novel Upstream Targets

Candidate pathways

- Metabolic stress
- Oxidative stress
- Mitochondrial dysfunction
- Proteostasis

Candidate genes

SYNPO2L/MYOZ1	SYNE2
FAM13B	CAV1
TBX5	PRRX1
CEP68	KIFAP3
KCNN2	KCNJ5
ZBTB25/MTHFD1	

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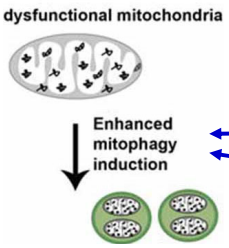
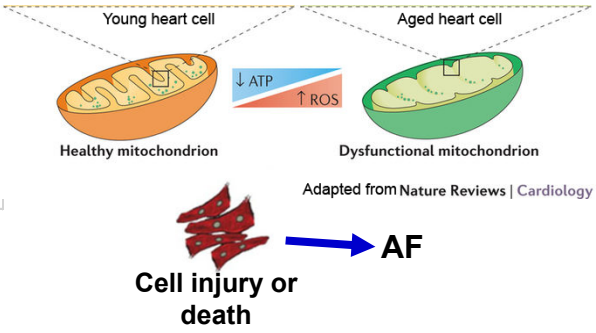
FAM13B Association with AF

- Knock down *FAM13B* expression in heart cells grown from adult stem cells ➡ altered the expression of >400 genes
- Pathway in common with the aging heart: the accumulation of dysfunctional mitochondria



***FAM13B* knockdown**

Gene symbol	% change
FAM13B	-78%
PARK2	-49%
ATPIF1	-35%



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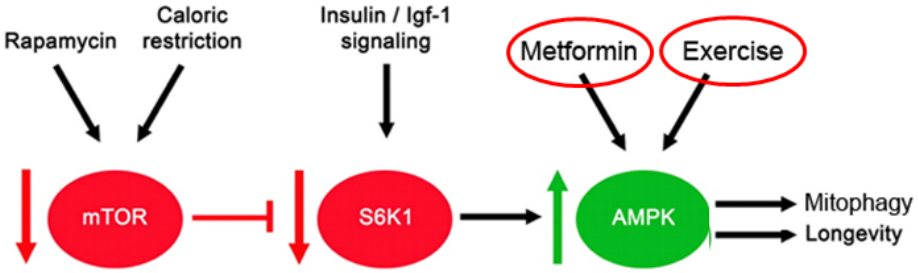
FAM13B Hypothesis

Aging and ↓ *FAM13B* expression:

- **↓ mitophagy**
- **↓ ATP production,**
- **altered ion conductance**
- **↑ reactive oxygen species, cell death, and fibrosis**

Can reduced mitophagy be rescued in aging or genetically predisposed hearts? Central role of AMP Activated Protein Kinase (AMPK)



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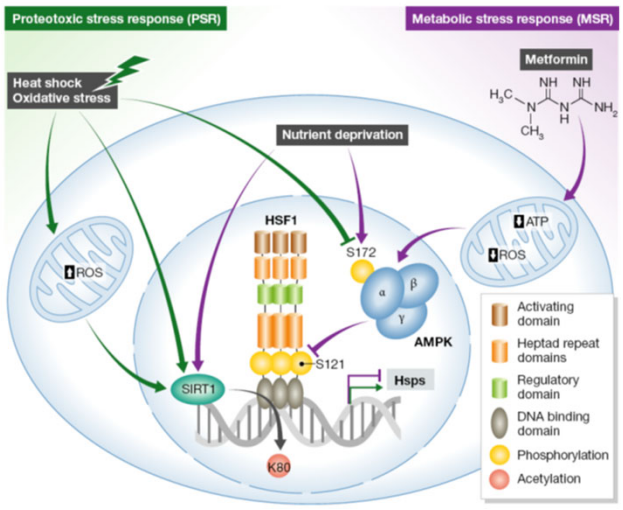
Cellular stress response cross talk maintains protein and energy homeostasis

HSF1 – master regulator of protein stress responses

AMPK – master regulator of metabolic stress responses

- Activated in response to ↑AMP:ATP
- Acts to ↑ATP generation and ↓ATP expenditure



Swan and and Sistonen. EMBO J 2015; 34:2 267-9

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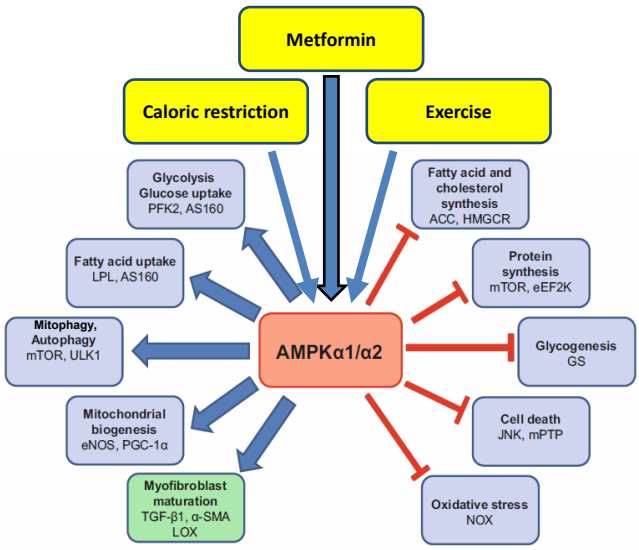

Modified from: Daskalopoulos EP, et al. AMPK in Cardiovascular Diseases. *EXS*. 2016;107:179-201

AMPK has diverse and at times contradictory roles in cell death and survival that is dependent on:

- Type of stress
- Duration of stress
- Degree of stress/induction
- Cell type

Each situation must be studied individually

Villanueva-Paz, et al. AMPK Regulation of Cell Growth, Apoptosis, Autophagy, and Bioenergetics. EXS. 2016;107:45-71

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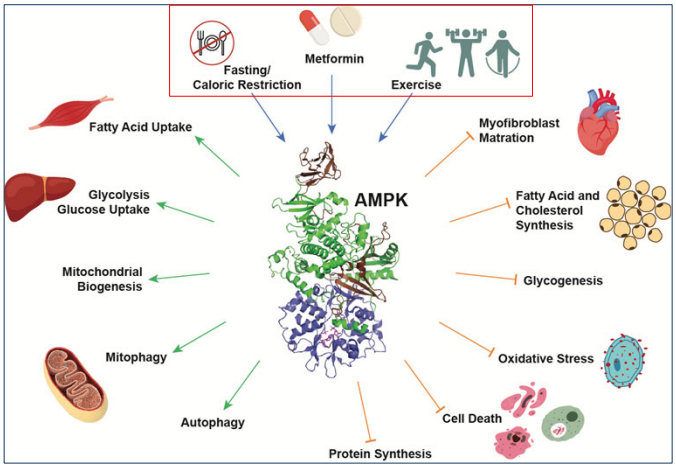
Rationale for testing Metformin and LRFM

- AF associated with ↓ transcriptional responses to stress
- Coexpression analyses of AF genetic risk loci implicate metabolic and other stress pathways and other targetable genes, including PRKAA2 (subunit of AMPK)



- AMP kinase is the master regulator of metabolic stress in cells.
- *Metformin, caloric restriction and exercise have been reported to have beneficial effects on AMP Kinase.*

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From Basic Lab to the Bedside

Testing a novel upstream therapy for AF

Metformin

- 1st line for diabetes and pre-diabetes
- ↑AMPK, a master regulator of metabolic stress responses
  - Works in the mitochondria with downstream activation of AMPK to regulate glucose metabolism and energy utilization
  - Lowers ROS production and ↑s cardiomyocyte ATP production in an AMPK dependent mechanism
- Promotes mitophagy to clear dysfunctional mitochondria
- Promotes weight loss
- Being studied for its potential anti-aging effects
- Has been used off-label in studies without hypoglycemia
- *Observational study in Taiwan: use ass'd with 20% less AF*

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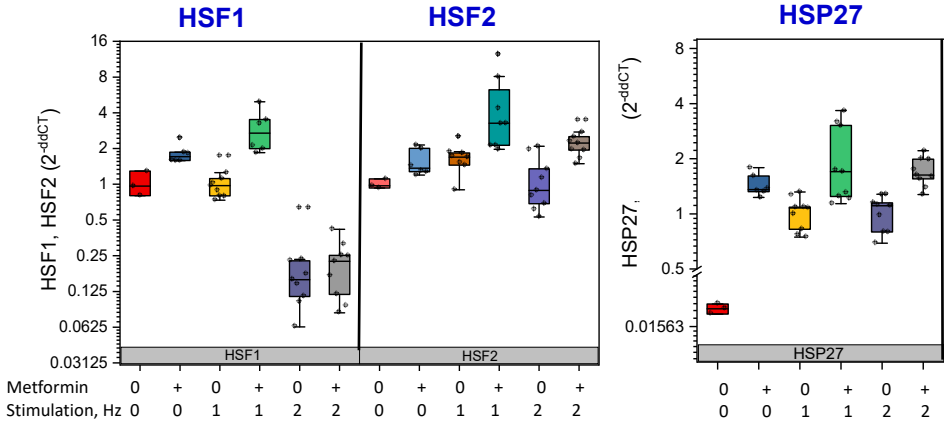
Metformin

A potential drug targeting mitochondrial dysfunction, metabolic and proteotoxic stress

HSP response to pacing stress in iCMs with/without metformin



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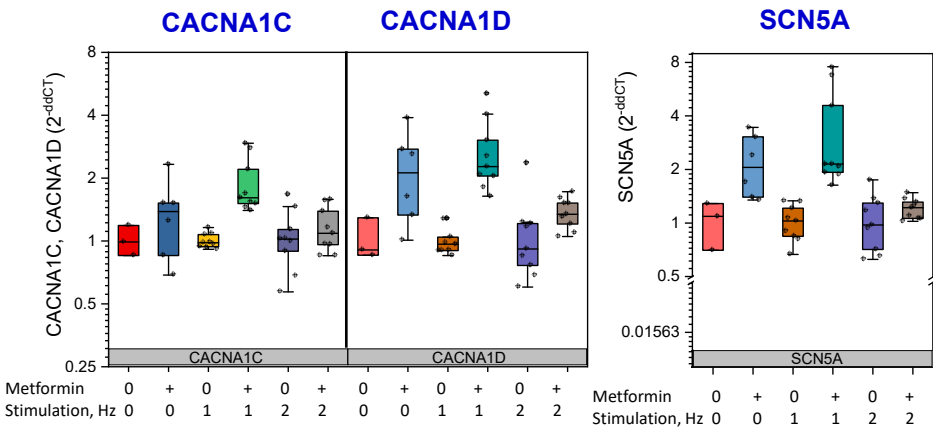
Ion channel gene expression response to pacing stress in iCMs with and without metformin



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Upstream Targeting for the Prevention of AF:
Targeting Risk Interventions and Metformin for Atrial Fibrillation (TRIM-AF)

TRIM AF — Upstream Targeting for the Prevention of Atrial Fibrillation: Targeting Risk Interventions and Metformin for Atrial Fibrillation

TRIM-AF Steering Committee Chair:	**Mina K. Chung MD**
TRIM-AF PI:	**Niraj Varma MD**
Biomarker Substudy PI:	**Ayman Hussein MD**
Center Fellow:	**So Jin Youn MD**
Clinical Trials Design and Analyses:	**John Barnard PhD, Steven Nissen MD**
Preventive Cardiology:	**Leslie Cho MD, Haitham Ahmed MD, Gordon Blackburn PhD**
Cardiac Electrophysiology:	**Khaldoun Tarakji MD, Walid Saliba MD, Oussama Wazni MD**
Endocrinology:	**Sangeeta Kashyap MD**
Sleep Medicine:	**Reena Mehra MD**
Cardiovascular Medicine Fellow:	**Kevin Trulock MD**
Consultants:	**Prash Sanders MBBS, Stephen Nicholls MBBS PhD**

CLEVELAND AF

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TRIM-AF Study Design

- Prospective, randomized, open-label, blinded endpoint 2x2 study in patients with implanted PMs or ICDs with atrial leads

Patients with AF (N=220)	
Control	Metformin
LRFM	LRFM + Metformin

- Metformin Extended Release: *up to 750mg BID*
- Lifestyle and risk factor modification (LFRM): *Preventive Cardiology*
  - Exercise assessment with tailored exercise program
  - Nutrition/diet assessment and counsel
  - RF reduction
- Outcomes: Daily AF burden, activity, events

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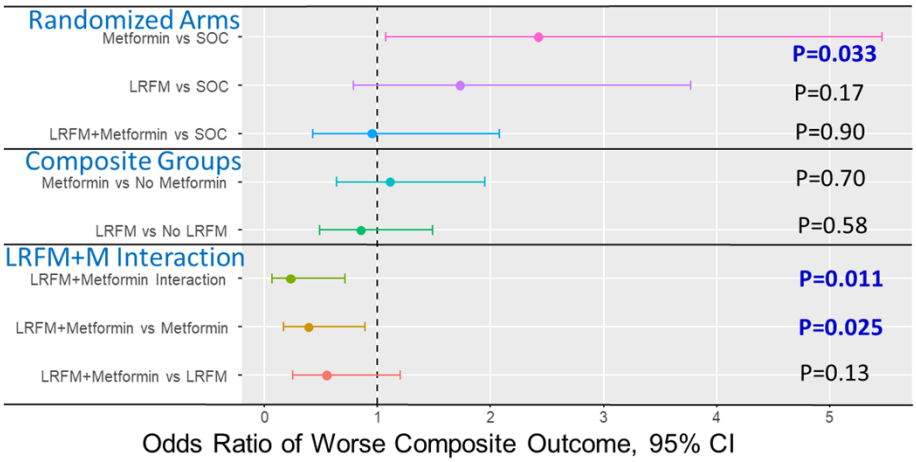
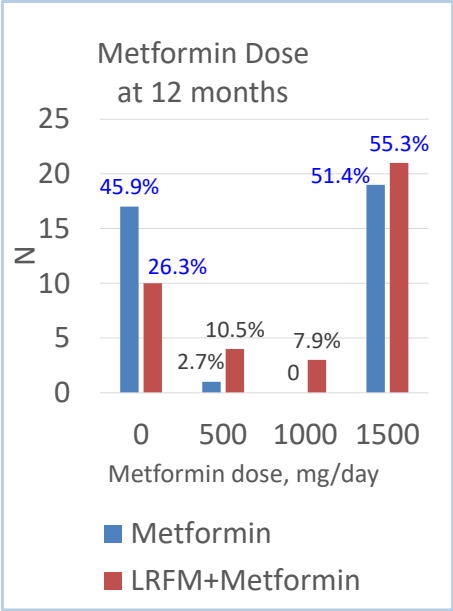
Composite primary endpoint - ITT

ITT Composite AF Burden % Change 3-12 months and survival at 1 year



- Metformin trended worse than SOC
- No significant differences in the LRFM or LRFM+Metformin group compared to SOC or in composite groups
- LRFM+Metformin showed significant interaction

60



Limitations

- Inability to tolerate metformin
- COVID-19 limited recruitment, in-person visits, shut-down, and sample size
- Inability to achieve weight and fitness targets
- SOC improved in AF burden, likely had LRFM counseling effects

Next Steps

- 2-year data
- On-treatment analyses
- In vitro testing of iCMs
- Testing of structural gene targets and candidate drugs from network systems biology methods

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Towards Personalization of AF Treatments

Dissect patient-specific response mechanisms to upstream therapies

Blood samples from Metformin arms of TRIM-AF trial

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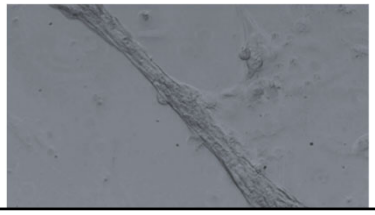
Genomic Prediction of Atrial Subtypes

DNA

Predict AF burden response to Metformin or other candidate drugs

Adult-derived stem cells differentiated to atrial-like muscle cells

Electrical and mechanical stimulation *maturation*

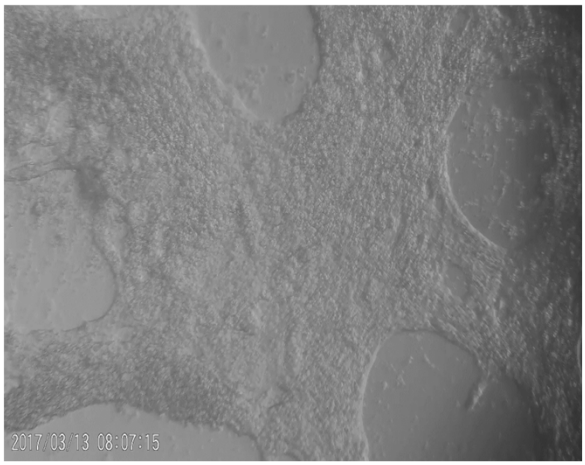


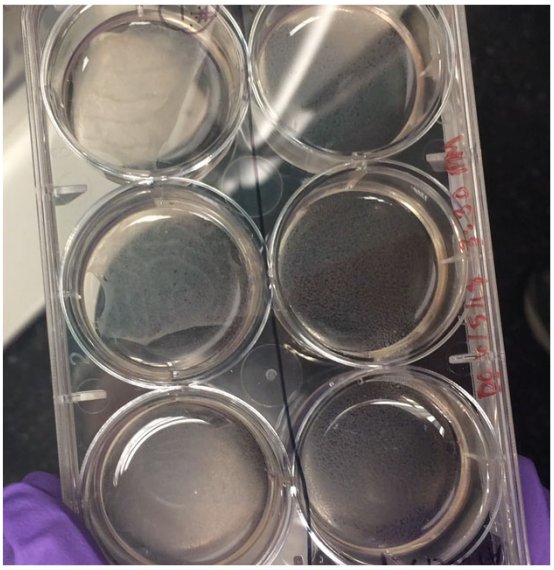
RESPONSE TO STRESS
± drug intervention
- Electrophysiology
- Contractility
- Mito/metabolic activity
- Gene expression

62

iPSCs->Cardiomyocytes

iCells undergoing differentiation to cardiomyocytes – day 10





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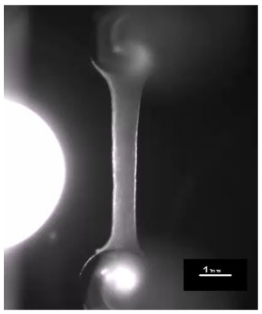
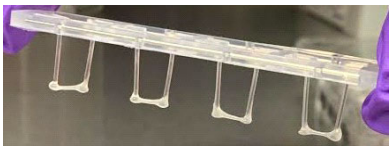
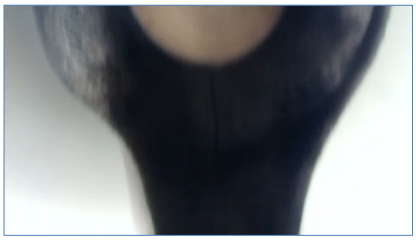
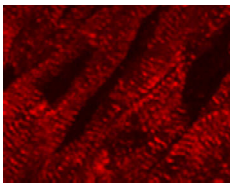
Engineered atrial-like heart tissues

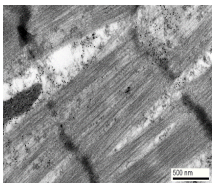


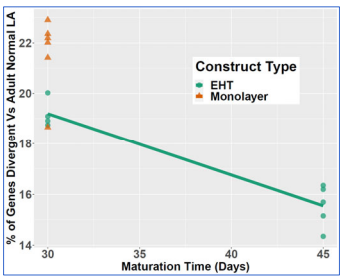
Lemme M, et al. *Stem Cell Reports* ***11, 1378-1390.***











- Aligned myocyte connections, improved structure
- More adult-like action potential characteristics, more negative resting potential
- Transcriptomic profile closer to adult LAA
- Should facilitate patient to patient comparisons and drug testing

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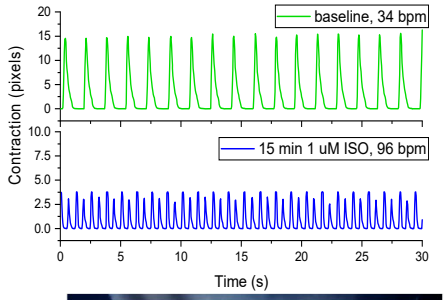
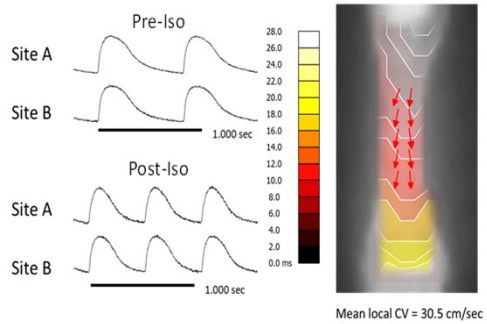
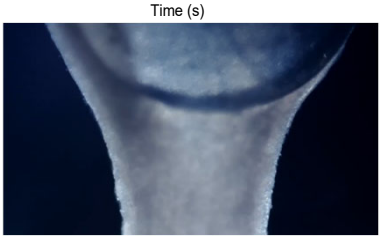
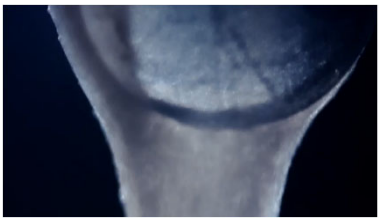
Using EHTs to evaluate the impact of metabolic stressors on atrial muscle







45 day EHT, baseline



45 day EHT, 1 µmol/L isoproterenol (15 min)

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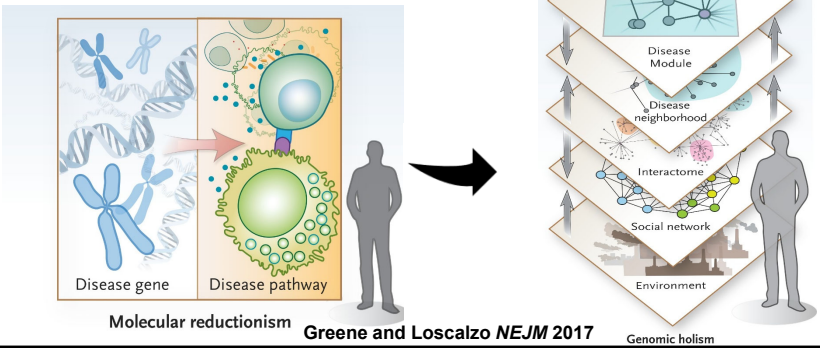
From Genes to Drugs

Network Systems Biology approaches to drug repurposing and discovery

- Genetically supported targets: ~double clinical development success rates
- Network medicine
  - incorporate layers of data related to biological systems and environmental factors
  - understand complex diseases, identify disease networks
  - Identify potential drug targets



Greene and Loscalzo *NEJM* 2017

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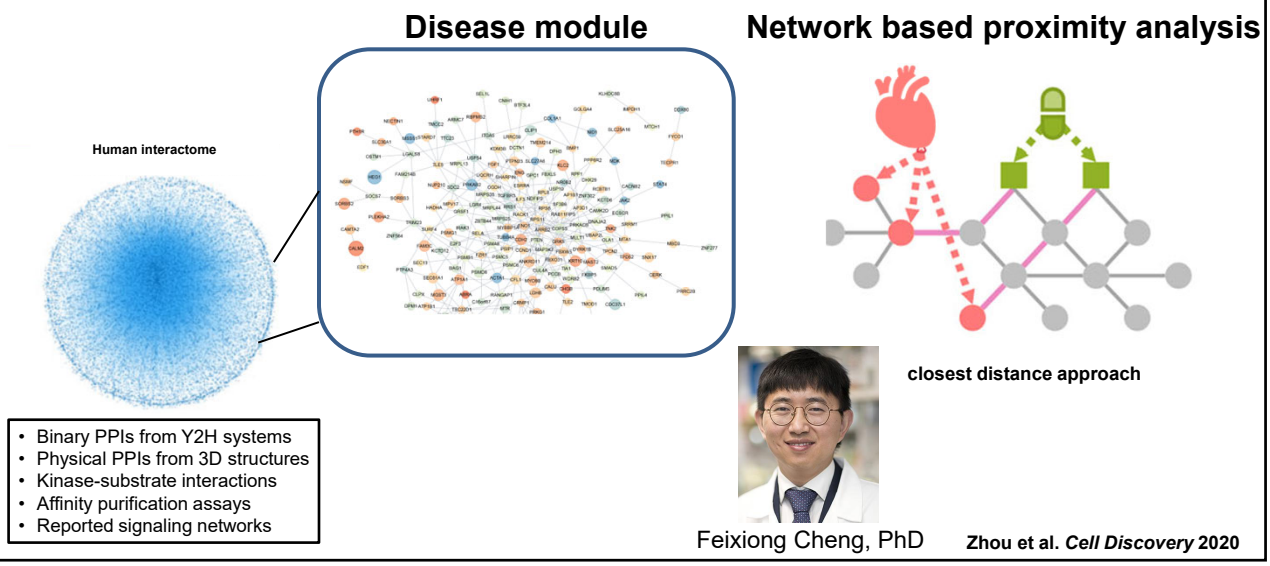
Network Medicine uses the Human Interactome to map diseases and novel drug target



Feixiong Cheng, PhD

Zhou et al. *Cell Discovery* 2020

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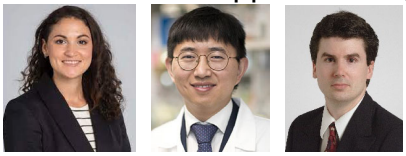
Network Systems Biology approaches to drug repurposing for prevention of AF and AF progression

- Proteins that associate with and functionally govern the AF phenotype are localized in an AF disease module within the comprehensive protein-protein interactome (PPI) network
- Proteins that serve as drug targets for a specific disease may be suitable drug targets for another disease owing to common protein-protein interactions and functional pathways elucidated by the PPI
- Multi-omic approach – Genetics, RNASeq, proteomics, metabolomics, snRNASeq
- Validation performed in large patient/insurance databases
- Focus on FDA-approved drugs shortens the time to testing for AF



Jessica Castrillon Lal; Feixiong Cheng, PhD; John Barnard, PhD, Mina Chung MD

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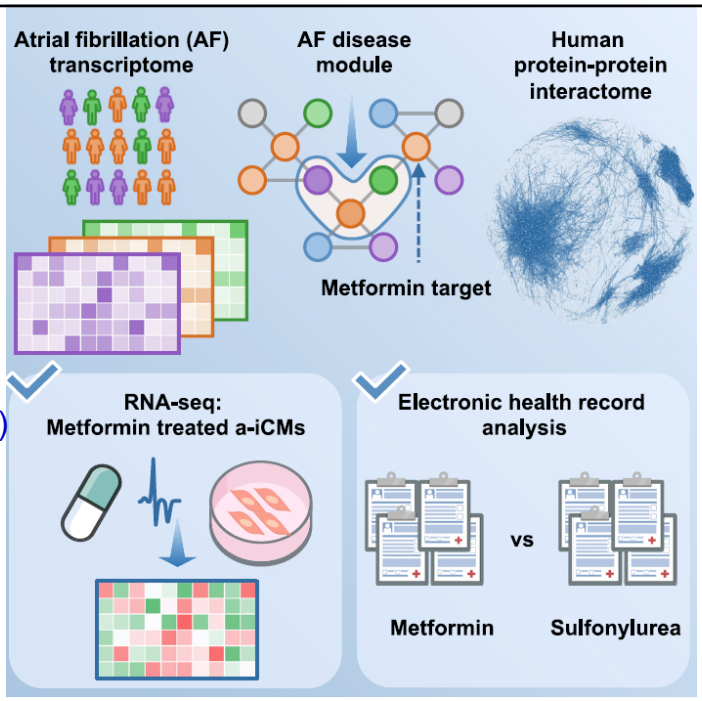
Cell Reports Medicine
CellPress
OPEN ACCESS

Transcriptomics-based network medicine approach identifies metformin as a repurposable drug for atrial fibrillation

Jessica C. Lal,[1,2,12] Chengsheng Mao,[3,12] Yadi Zhou,[1] Shamone R. Gore-Panter,[4,5] Julie H. Rennison,[4] Beth S. Lovano,[4] Laurie Castel,[4] Jiyoung Shin,[3] A. Marc Gillinov,[6] Jonathan D. Smith,[2,4] John Barnard,[7] David R. Van Wagoner,[4] Yuan Luo,[3,*] Feixiong Cheng,[1,2,8,10,*] and Mina K. Chung[4,9,11,13,*]

- Integrated transcriptomics (RNASeq)
- In vitro studies of metformin-treated a-iCMs
- Validation performed in a large patient/insurance database



Cell Rep Med. 2022 Oct 18;3(10):100749. doi: 10.1016/j.xcrm.2022.100749. Epub 2022 Oct 11

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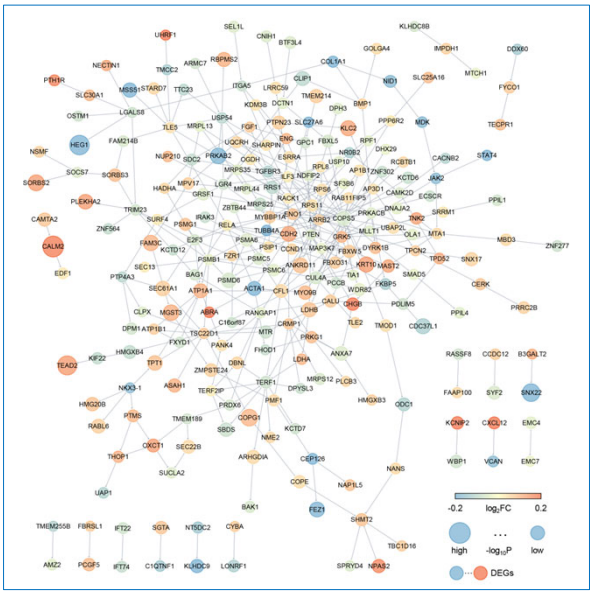
AF disease network highlights 245 AF enriched genes.

- LAA cohort mRNASeq used to generate the AF disease network
- AF GWAS loci found in the network: *CAMK2D, PMVK, ASAH1, HCN4*
- AF hubs related to cardiac integrity and metabolic fitness: *LDHB, CDH2, UQCRH, PDLIM5, COPS5, OXCT1*



Jessica Castrillon Lal, Feixiong Cheng, PhD; John Barnard, PhD, Mina Chung MD
AHA 2021

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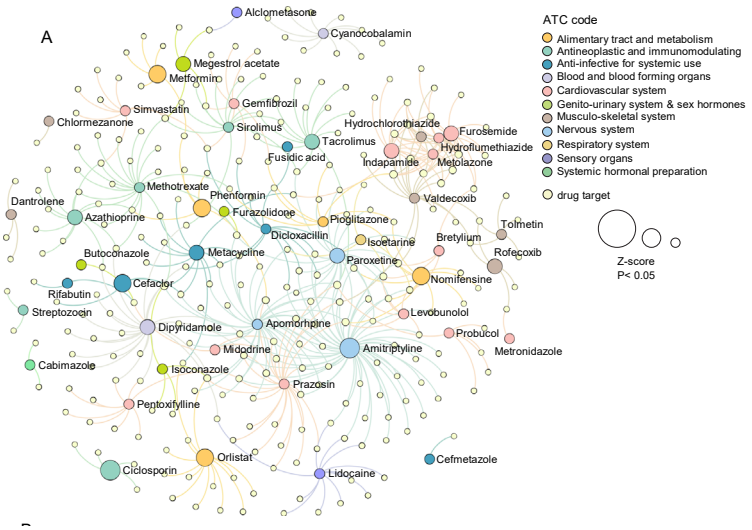
The top 55 drug candidates nominated by Network Proximity Analysis



Drug	from	Z	Proximity_Pvalue
Phenformin	AF	-2.475	0.002
Metformin	AF	-1.97	0.015
Furosemide	AF	-1.912	0.031
Indapamide	AF	-1.691	0.037
Metacycline	AF	-1.674	0.042
Rofecoxib	AF	-1.67	0.004
Midodrine	AF	-1.372	0.052
Furazolidone	AF	-1.33	0.049
Naringenin	AF	-1.325	0.063
Dantrolene	AF	-1.193	0.014
Simvastatin	AF	-1.185	0.117
Alclometasone	AF	-1.094	0
Rifabutin	AF	-1.062	0.066
Streptozocin	AF	-1.048	0

Using a Z-score cutoff value Z < -1, we focused on 55 drug candidates from total 2,891 drugs

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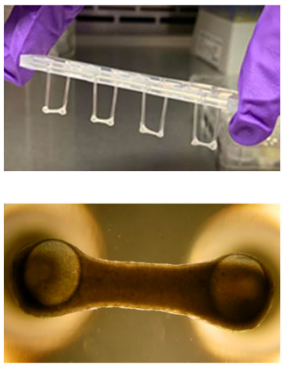
Dantrolene

- Dantrolene stabilizes RyR2 and limits spontaneous RyR2 openings
- Used as a muscle relaxant

Engineered heart tissues from iPSC-derived iCMs

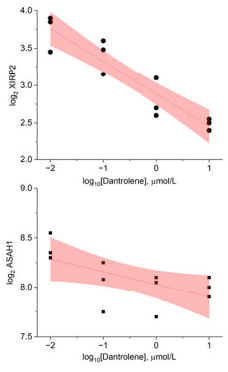


Top differentially expressed genes in dantrolene-treated a-EHTs compared to differential expression in atrial samples collected from patients in AF vs SR.

Differentially Expressed Genes		
Gene	AF	Dantrolene
XIRP2		
ASAH1		
UNC5B-AS1		
KATNIP		
TNNI3		
MYH7B		

-0.5 0.5
Log Fold Change

Dantrolene downregulates *XIRP2* and *ASAH1* expression.



- XIRP2 is a gene associated with pathologic angiotensin-II driven signaling in the heart
- Lower XIRP2 expression is associated with less hypertrophy and fibrosis, pathways contributing to an AF substrate
- ASAH1 is a known AF risk gene
- We have reported that phospholamban (PLN), a regulator of intracellular Ca^{2+} uptake, was the 2nd most highly co-expressed gene with ASAH1 [2].
- Atrial natriuretic peptide (NPPA), a marker of atrial wall stress, was the 7th most highly co-expressed gene with ASAH1 [2].
- While not significant after FDR correction, DAN reduced mRNA expression of both PLN and ANP.

- Side effects: dizziness, muscle weakness, hepatotoxicity

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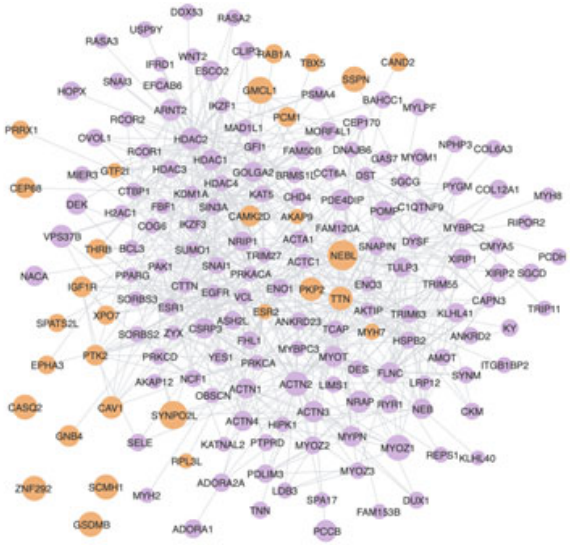
New AF disease module identified by a PPI-based deep learning approach



Drug	Z	Description
Aminophylline	-3.848	PDE inhibitor; adenosine A2B receptor blocker
Sucrose	-3.616	Sugar
Sapropterin	-3.427	Cofactor in synthesis of nitric oxide
Dronedarone	-3.410	Class III AAD, similar to amiodarone
Oxtriphylline	-3.300	PDE inhibitor
Theophylline	-3.253	PDE3, 4 inhibitor; adenosine A2B receptor blocker
Nisoldipine	-3.224	Calcium channel blocker
Malathion	-2.974	Parasympathomimetic organophosphate
Imiquimod	-2.937	TLR7 agonist
Cefixime	-2.909	Cephalosporin
Sildenafil	-2.860	PDE5 inhibitor

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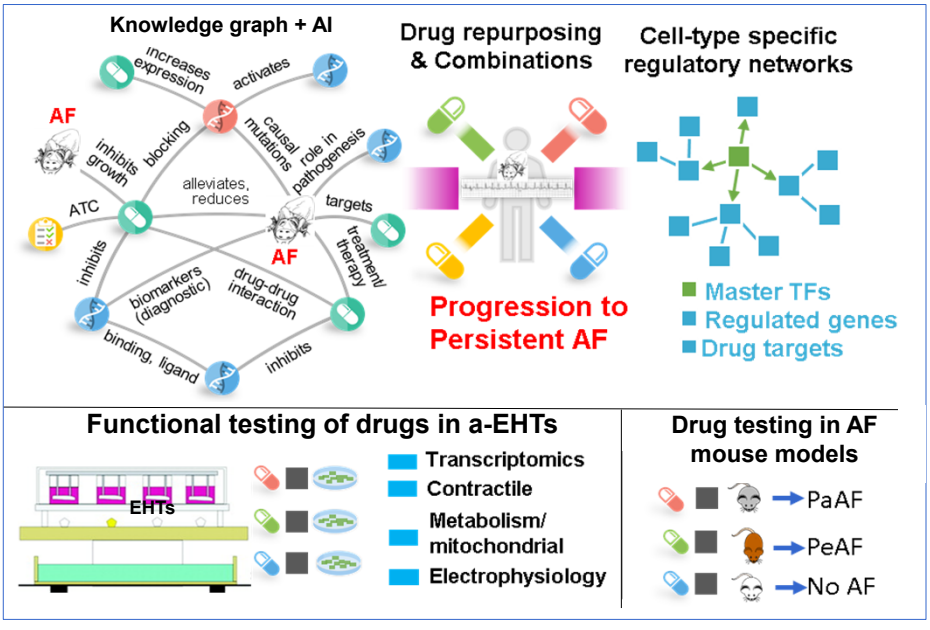
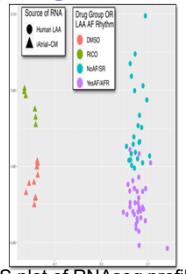
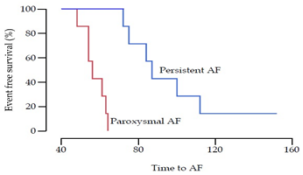
Identification and functional testing of drugs targeting AF progression





MDS plot of RNAseq profiles from ricolinostat treated a-iCMs and LAA. Ricolinostat shifted the a-iCM profile towards SR.



CREM heterozygote mice and progression to high burden ("persistent") AF. (N=7, 2 females, 5 males). 4-minute ECGs were recorded 3x/week. Persistent AF was defined as continuous AF present in at least 4 consecutive measures (≥1 week).

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AF Genomics: From Bedside to Bench to Bedside

- Large GWAS for AF implicate over 100 AF risk loci.
- The top locus is near a gene implicated in PV formation
- Transcriptomic studies suggest that AF is associated with impaired or overwhelmed responses to cell stress
- A dual risk model proposes that in genetically susceptible individuals, inadequate transcriptional responses to stress predispose to AF in later life

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AF Genomics: From Bedside to Bench to Bedside

- Drugs targeting metabolic, oxidative, or protein handling stress may be novel upstream agents that can be studied for AF
- Multi-omics based preventive therapies for patients at high clinical or genetic risk for AF may aid rational, personalized selection of preventive therapy, but need to be tested
- Patient-specific iPSCs may become useful for functional or drug testing in the future
- Integration of multiomics, imaging, network and AI-based analyses may identify new targets for AF

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Thank You!

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