

Role of micro-RNAs in Atrial Fibrillation

Amir Shaikh, MD; David D McManus, MD,ScM

Assistant Professor,

Department of Medicine

University of Massachusetts Medical School, Worcester, MA, USA

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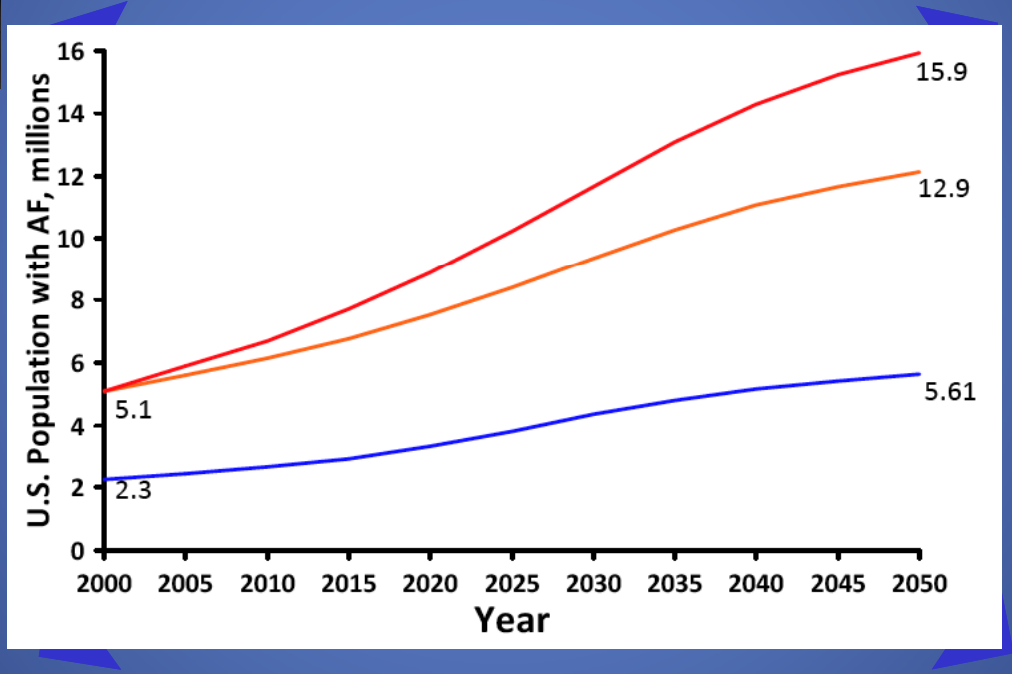






Atrial Fibrillation: A Complex Disease with Far-Reaching Impact





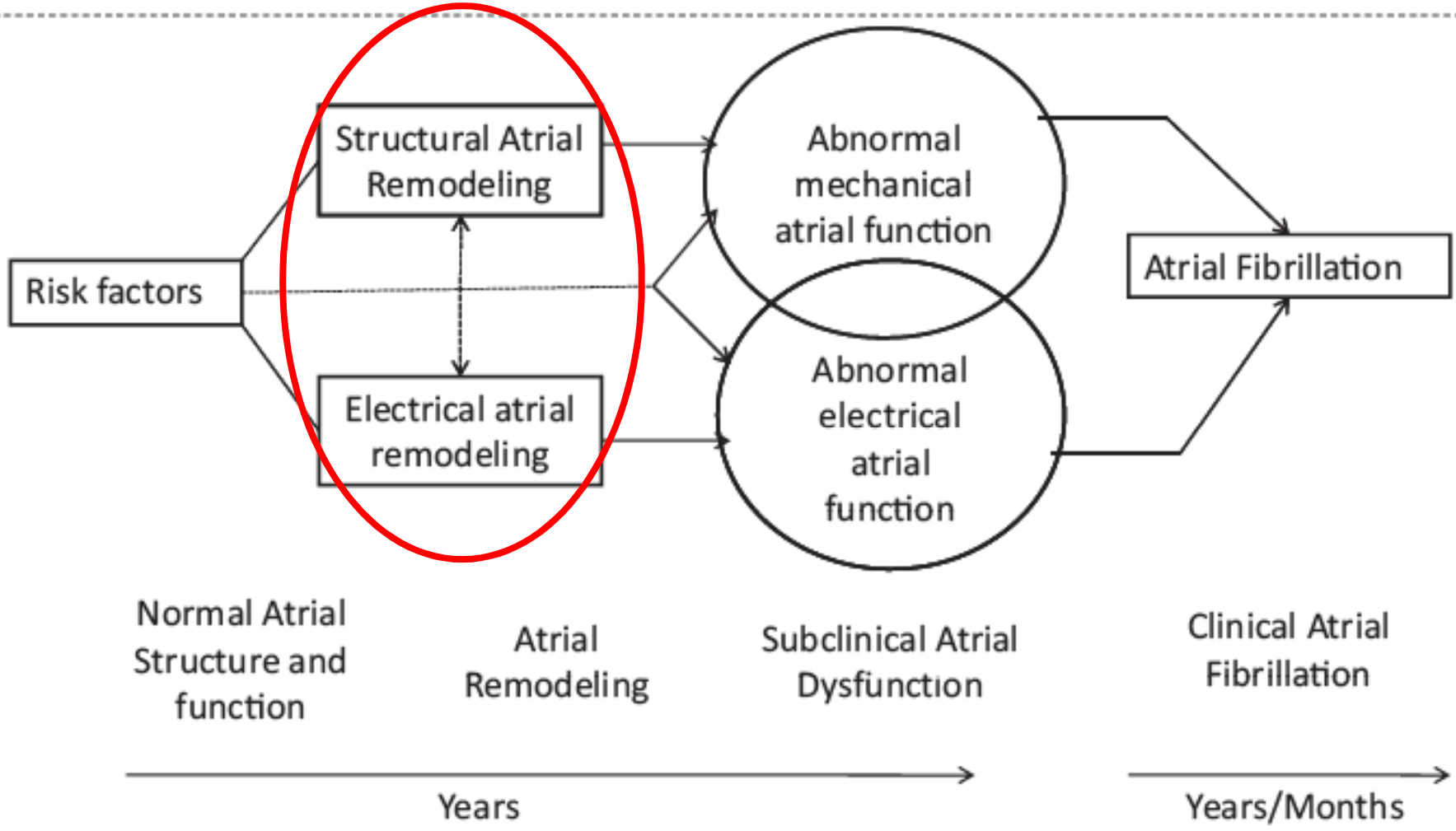






Miyasaka Circulation 2006;11:119; **Go *JAMA* 2001;285:2370**
American Heart Association

Model of Atrial Fibrillation Progression



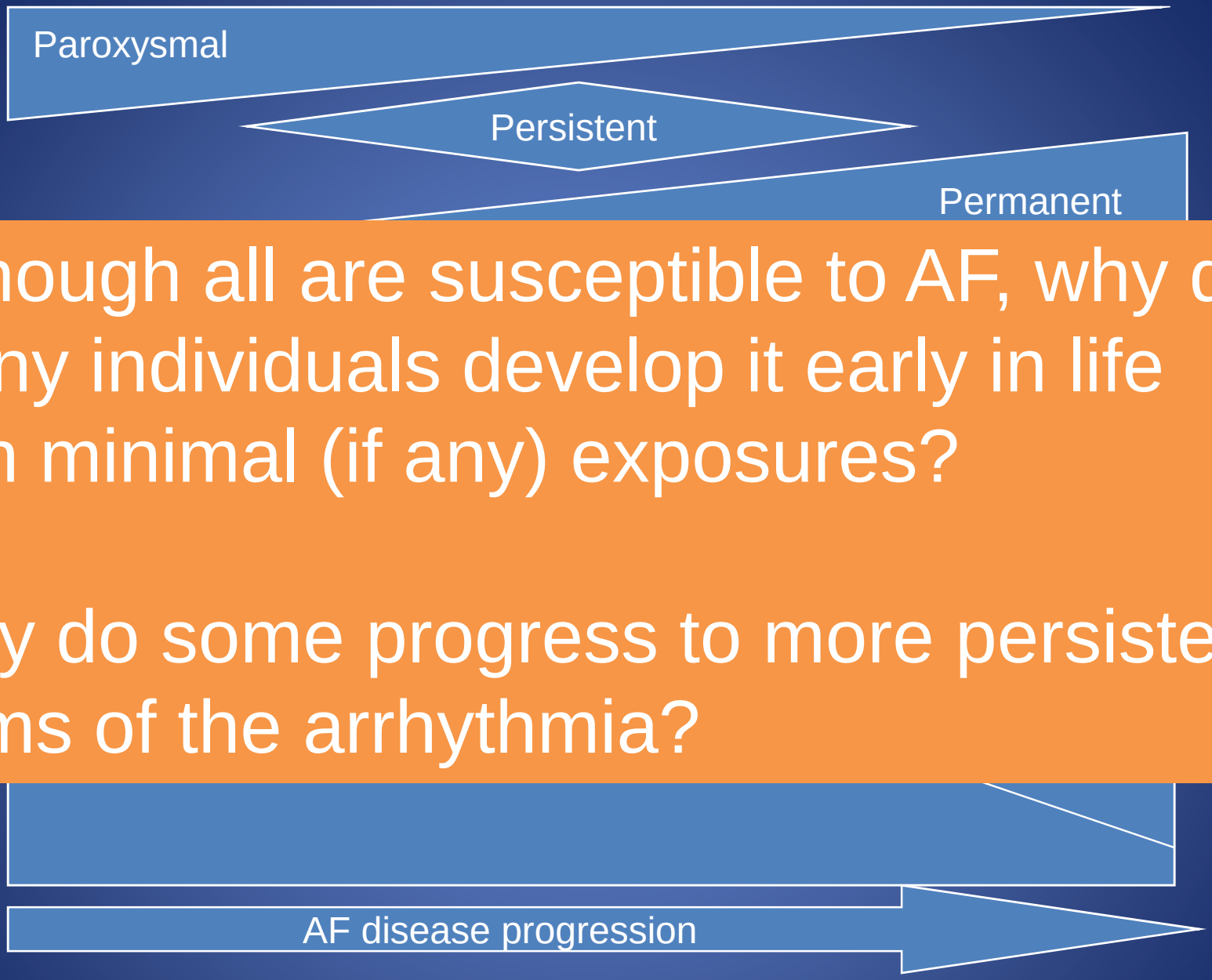
DD McManus, A Shaikh, F Abdhiskek, RS Vasan. *Crit Path Cardiol*. 2011

Focal Triggers Initiate AF and Reentry Perpetuates It

AF requires both a trigger and a vulnerable substrate

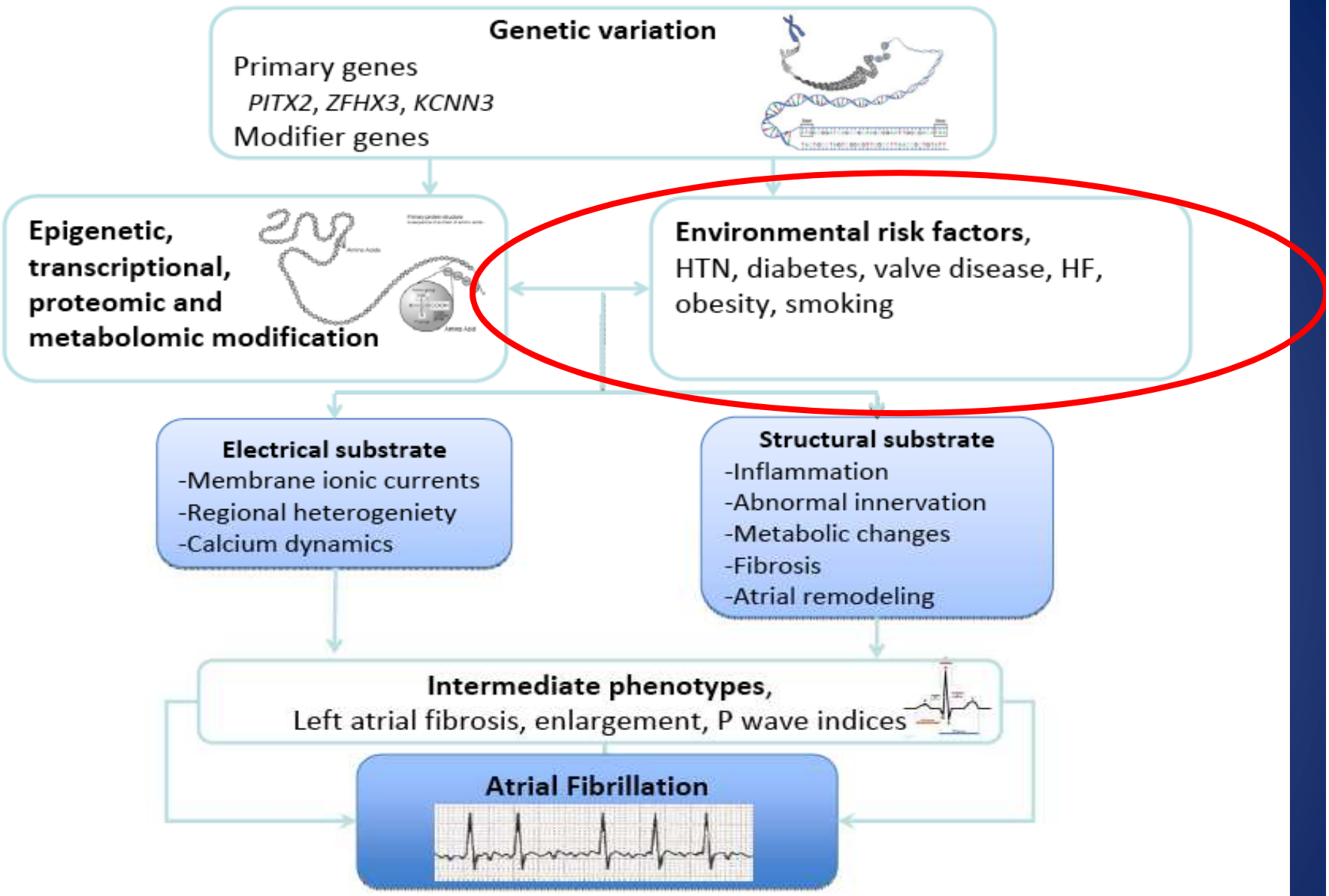
Interplay between intrinsic susceptibility and exposures largely unknown





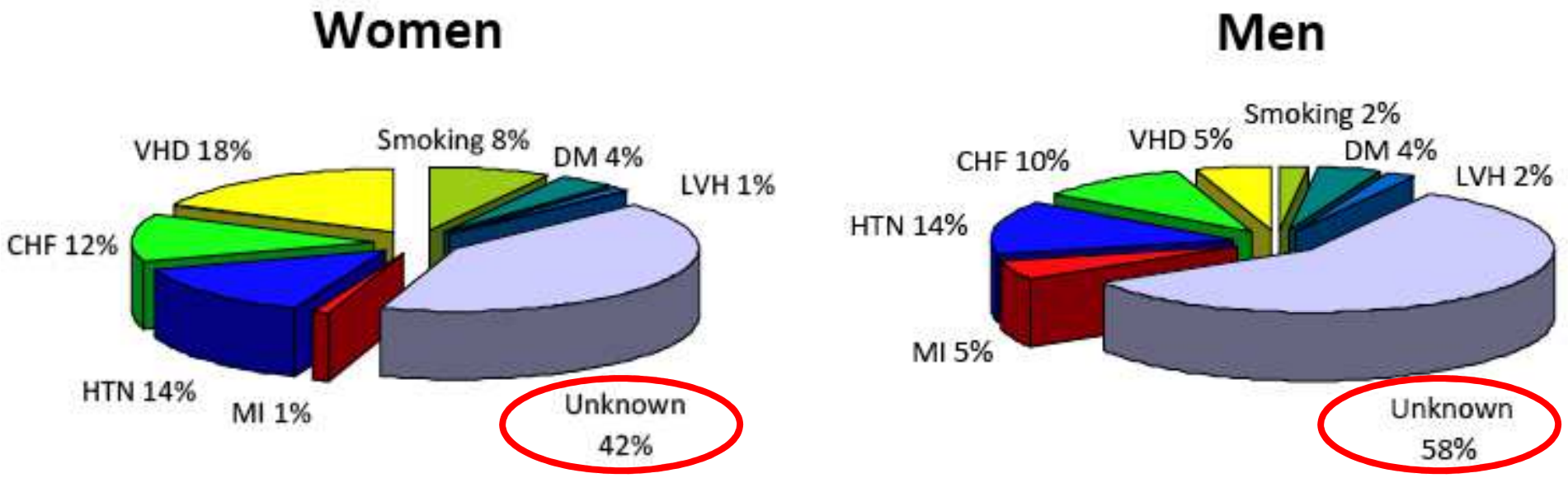
Although all are susceptible to AF, why do many individuals develop it early in life with minimal (if any) exposures?

Why do some progress to more persistent forms of the arrhythmia?

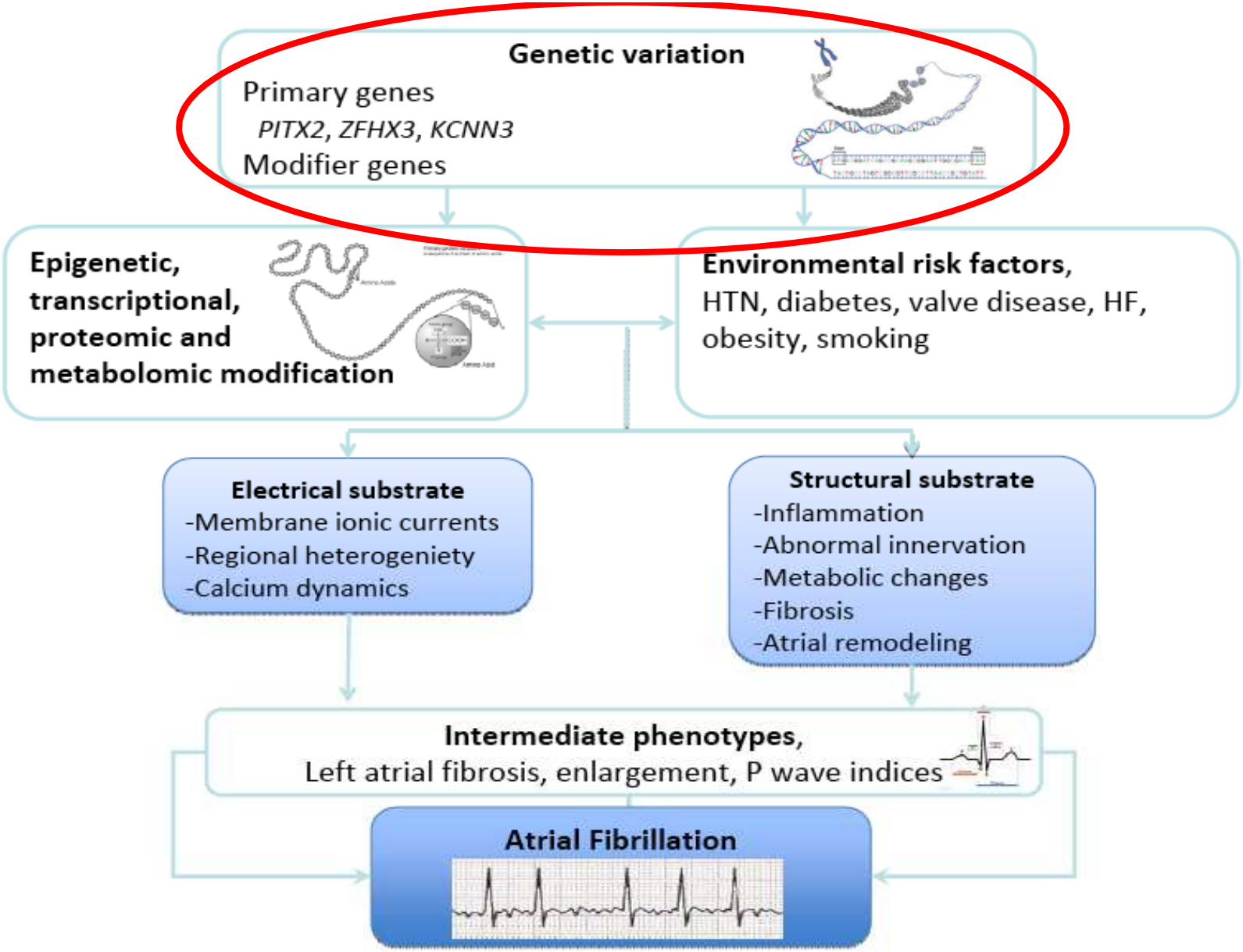


Magnani…McManus…Benjamin. **Atrial fibrillation: current knowledge and future directions in epidemiology and genomics.** *Circulation* 2011.

Population Attributable Risks of Atrial Fibrillation



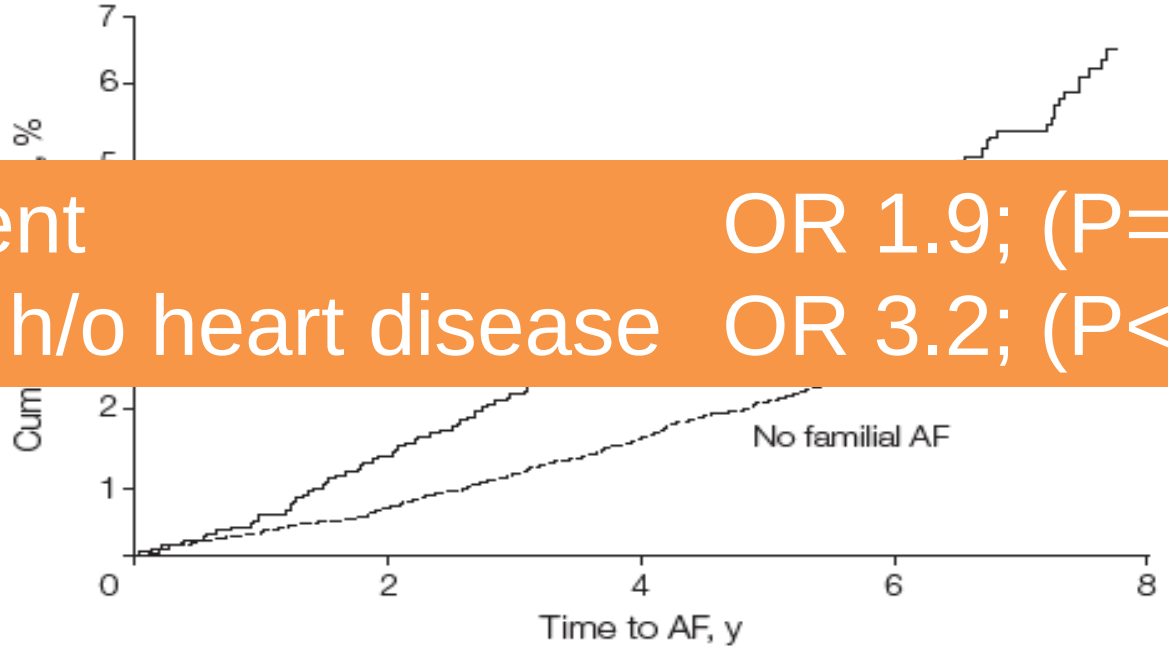
Benjamin JAMA 1994;271:840; Lake Austr NZ J Med 1989;19:321; Psaty Circulation 1997;96:2455; Sawin NEJM 1994;331:1249; Tsang JACC 40:36, 2002



Magnani…McManus…Benjamin. **Atrial fibrillation: current knowledge and future directions in epidemiology and genomics.** *Circulation* 2011.

Family History of AF Associated with Increased AF Risk

Figure 1. Cumulative AF Incidence by Presence or Absence of Antecedent AF in a First-Degree Relative Accounting for Competing Risk of Death



Person-examinations at risk

Familial AF	2393	2334	2256	2063
No familial AF	9578	9383	9121	8498

AF indicates atrial fibrillation. Person-examinations at risk at 8 years are not shown because 8-year follow-up windows were pooled and the majority of censored person-examinations at 8 years contributed to a subsequent risk pool at 0 years.

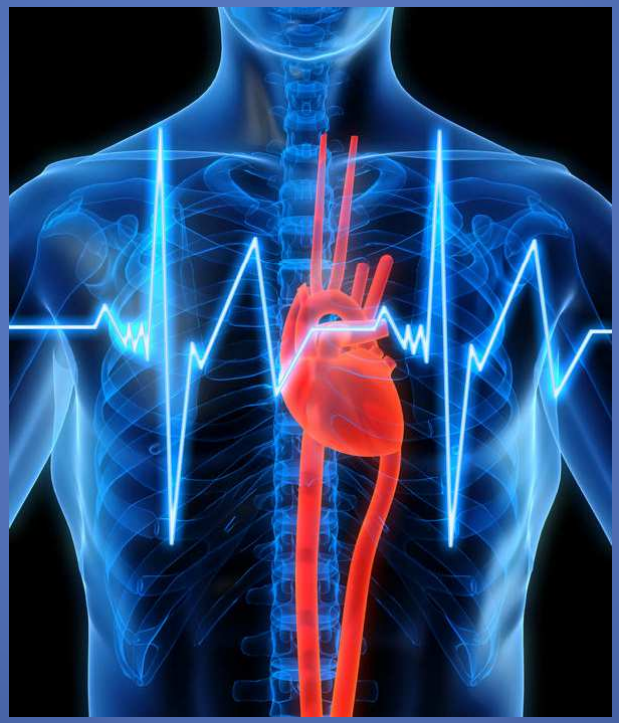
AF ≥ 1 parent OR 1.9; (P=0.02)

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

Lubitz, *JAMA* 2010.

Fox…Benjamin *JAMA* 2004;291:2851

Genetics, Genomics and AF

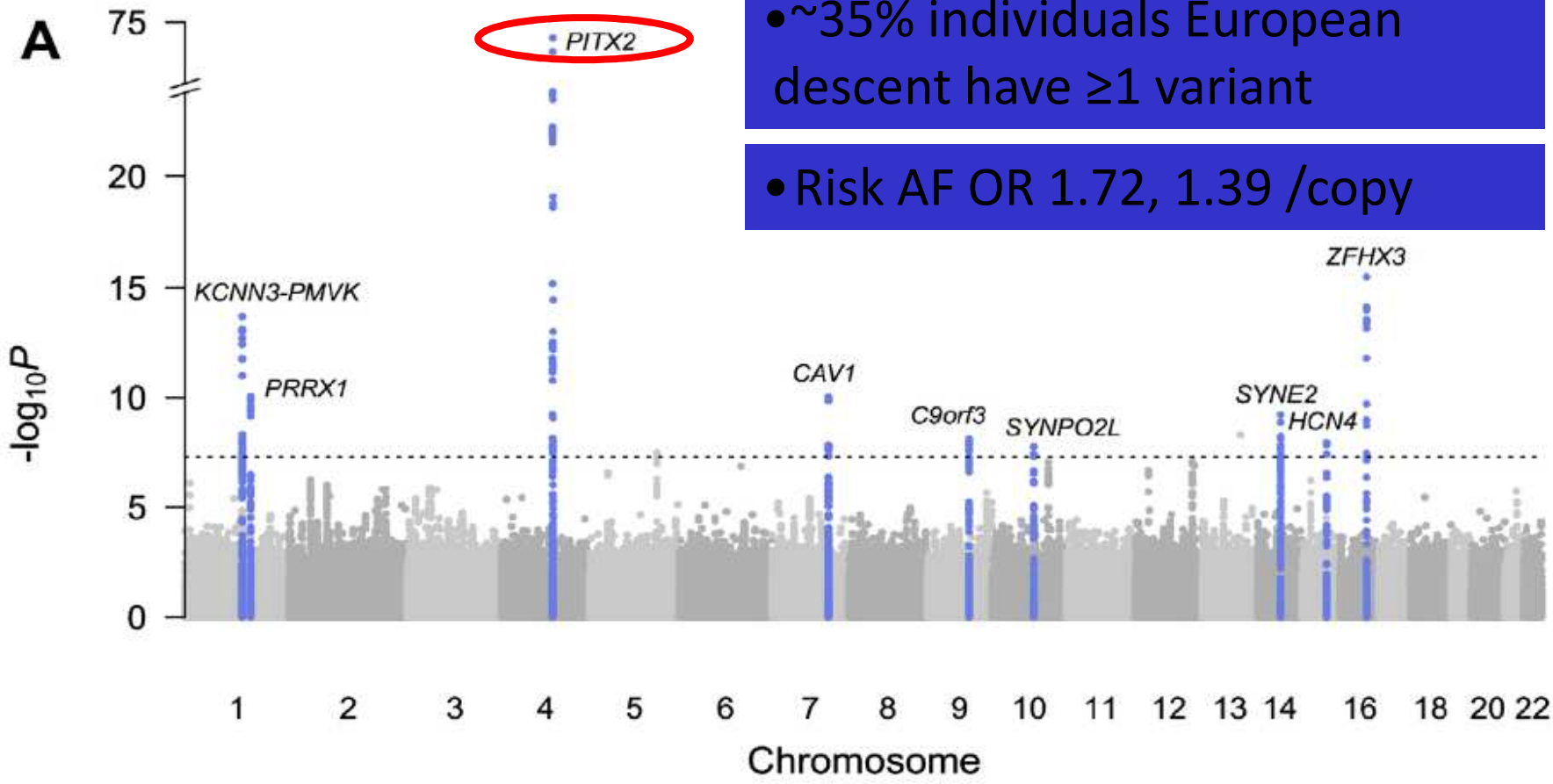


Candidate Genes Associated with AF

Gene	Variant	Cases	Controls	OR	P value	Replicated?
		Candidate Gene Studies				
Connexin 40	-44A, +71G	173	232	1.5	< 0.006	No
Angiotensinogen	M235T	250	250	2.5	<0.001	No
Angiotensinogen	G-6A	250	250	3.3	0.005	No
Angiotensinogen	G-217A	250	250	2.0	0.002	No
Mink	38G	108	108	1.8	0.024	No
GNB3	C825T	291	292	0.46	0.02	No
KCNE5	97T	158	96	0.52	0.007	No
Interleukin 6	-174 G/C	26	84	3.25	0.006	No
CETP	Taq1B	97	97	0.35	0.05	No
KCNE4	E145D	142	238	1.66	0.044	No
ACE	D/D	51	289	1.5	0.16	No
ENOS	894T/T	51	289	3.2	0.001	No
SCN5A	H558R	157	314	1.6	0.002	No
HERG/KCNH2	**K897T**	**1207**	**2475**	**1.25**	**0.0003**	**Yes**

Ellinor *Med Clin N Am* 2008;92:41

Associations between Genetic Variants and AF

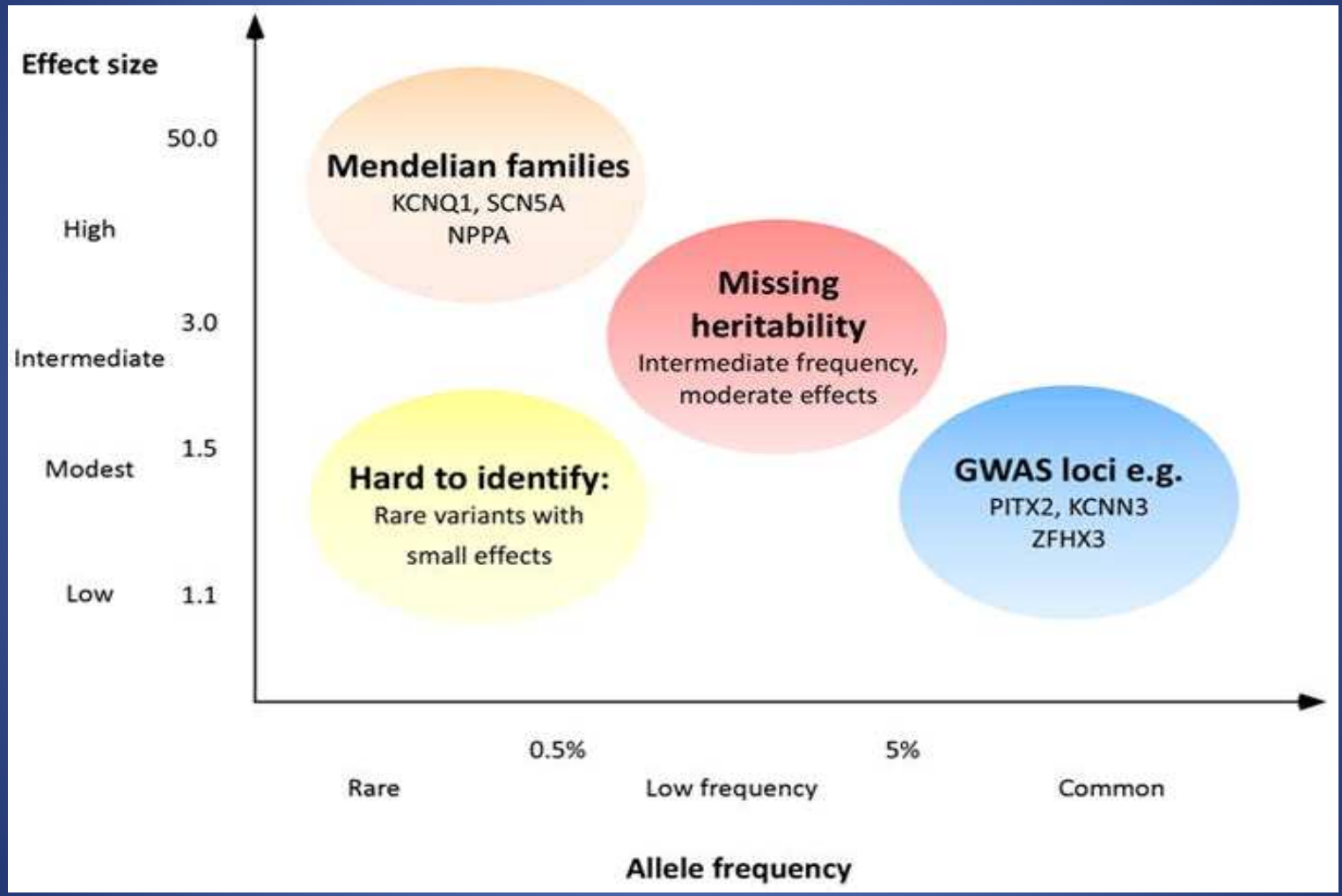


- ~35% individuals European descent have ≥1 variant
- Risk AF OR 1.72, 1.39 /copy

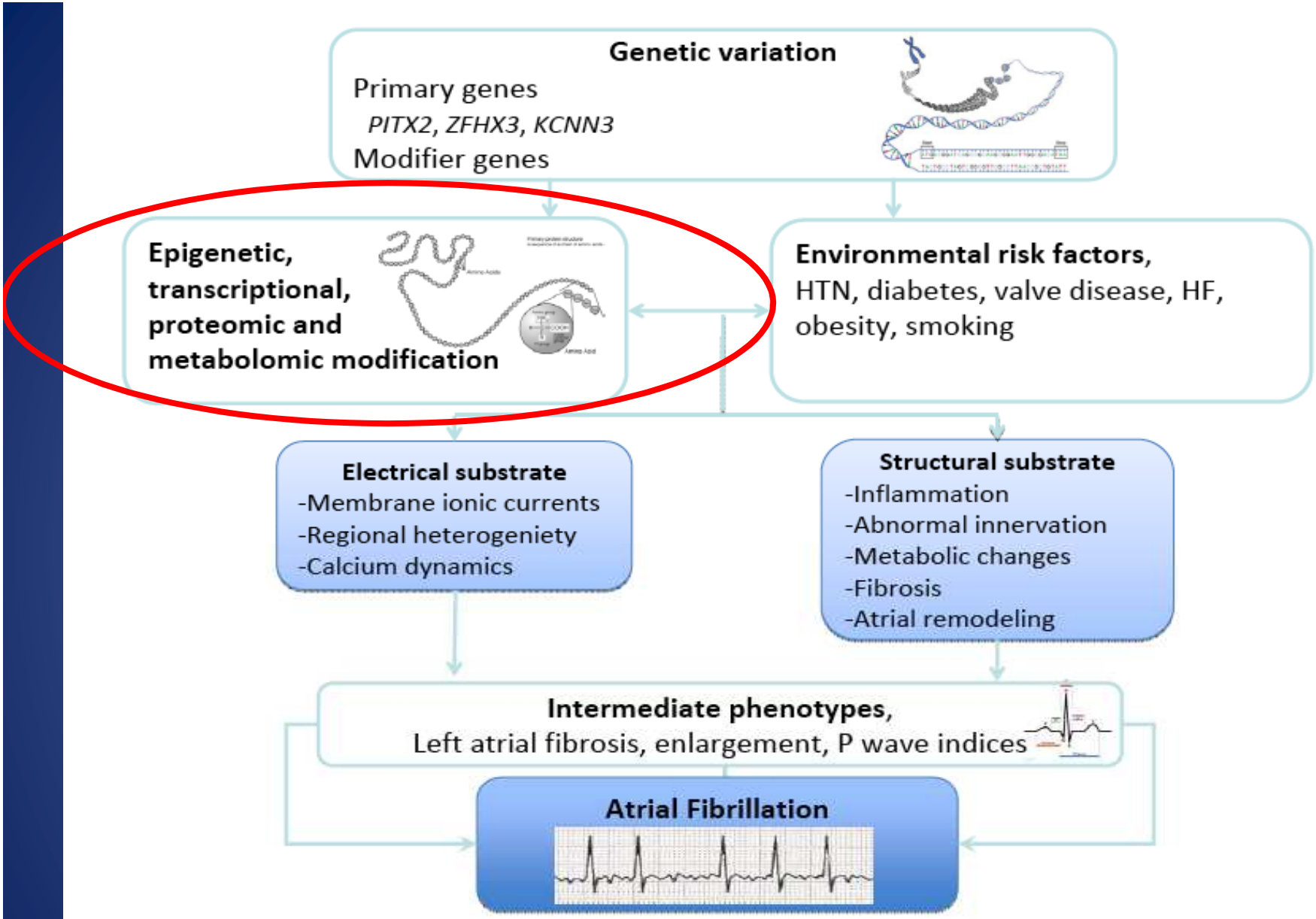
Gudbjartsson *Nature* 2007;448:353

Lubitz…McManus…Ellinor. JACC 2014

IDENTIFIED GENETIC ASSOCIATIONS OF AF AND FUTURE AREAS OF GENOMIC STUDY



Magnani…McManus…Benjamin. **Atrial fibrillation: current knowledge and future directions in epidemiology and genomics.** *Circulation* 2011.



Magnani…McManus…Benjamin. **Atrial fibrillation: current knowledge and future directions in epidemiology and genomics.** *Circulation* 2011.

Heritability Gap in AF – Moving beyond GWAS



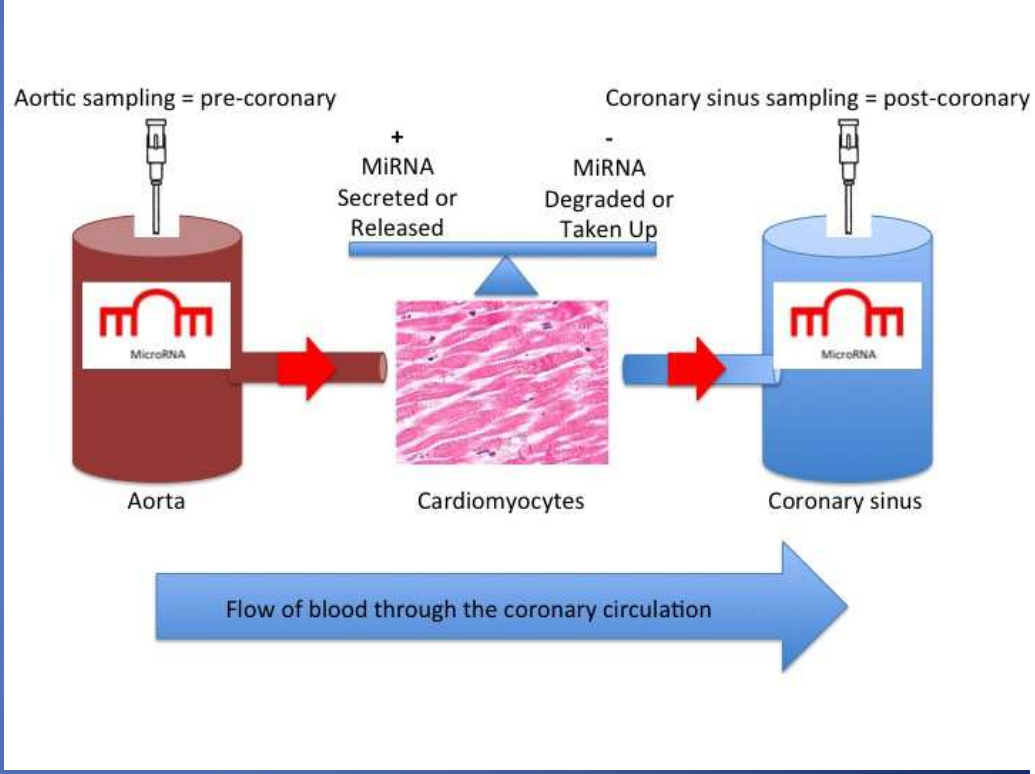
Known unknowns in AF:

- 40% AF risk unexplained by clinical CV risk f
- 2-fol family history of AF
- 90+% of AF heritability unexplained by known SNPs and candidate gene studies
- AF triggers contribute to altered atrial gene expression

Could variable gene expression in stress states explain heritability gap?

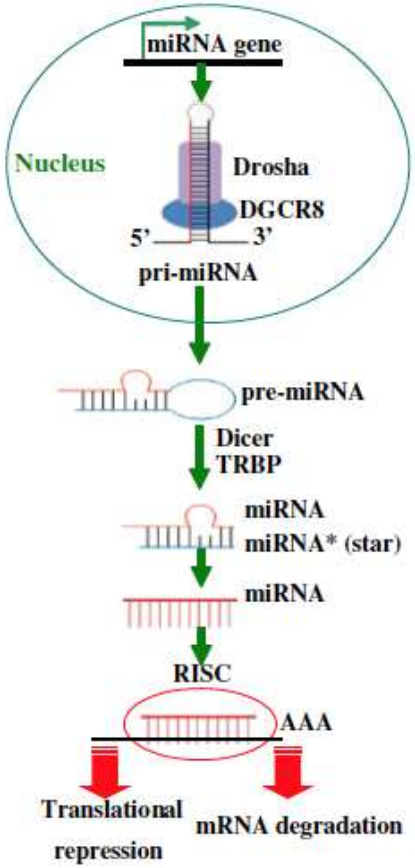
MicroRNA in CVD

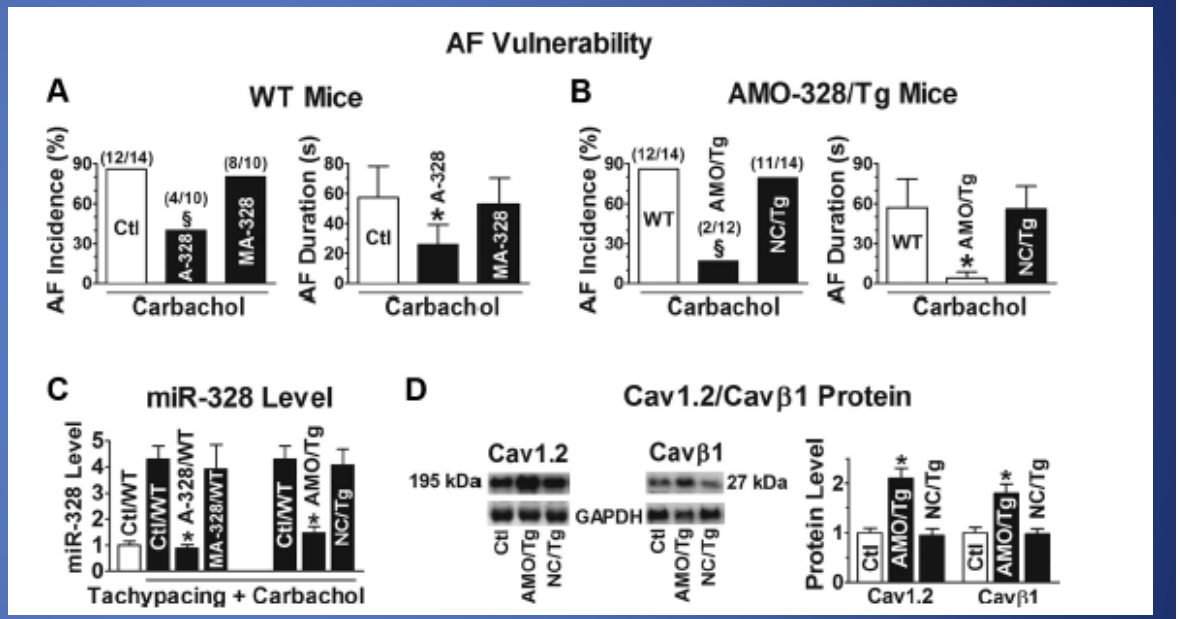
- MicroRNAs (miRNAs) are endogenous, non-coding RNAs
- miRNAs are regulators of gene expression
- miRNAs are released by the heart in the setting of an acute MI, heart failure
- miRNAs are present in the circulation and provide insights into *in vivo* gene expression.



McManus, Ambros. *Circulation* 2011

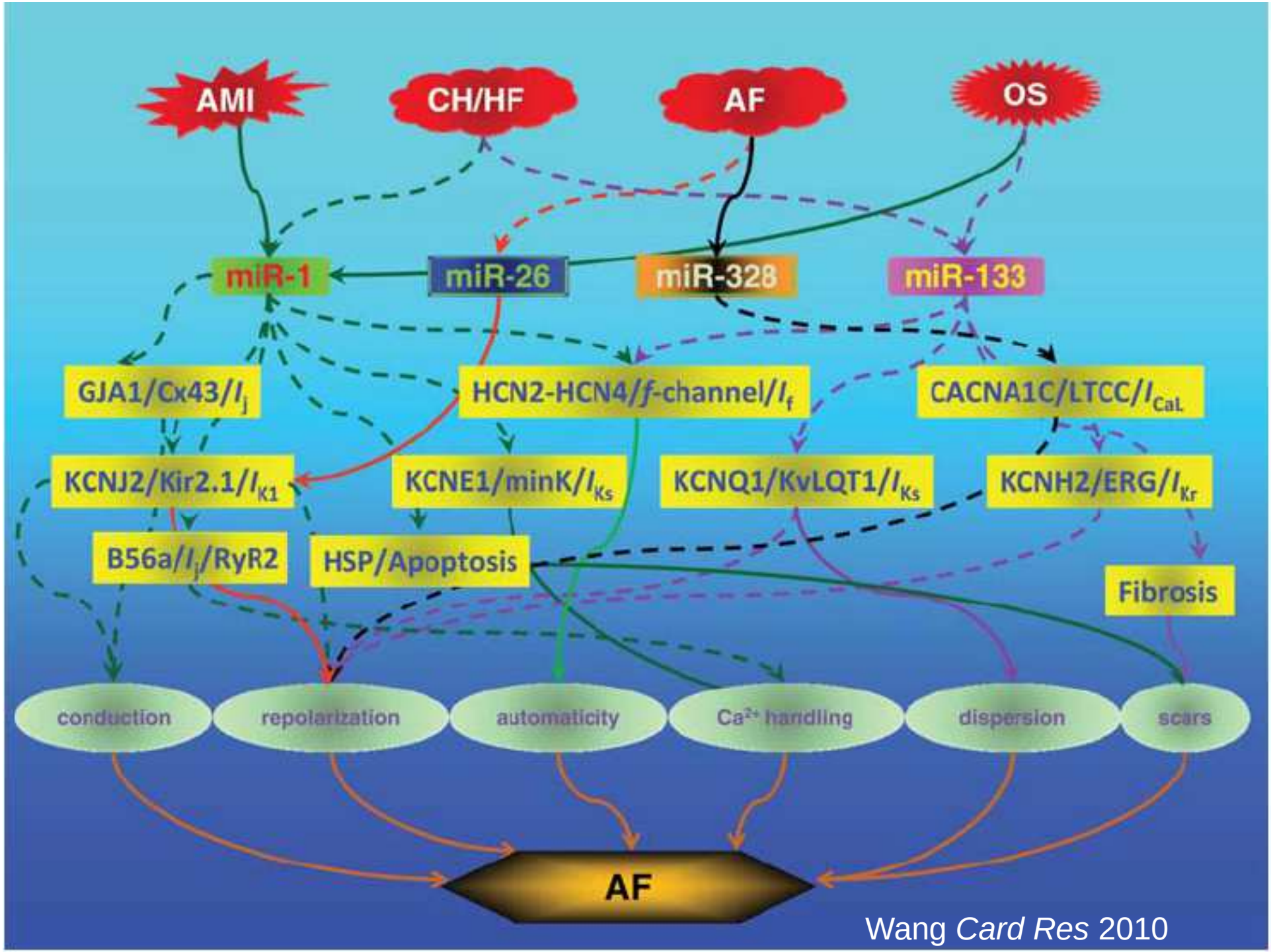
Animal Models suggests Tissue Levels of Mirnas are associated with AF Susceptibility



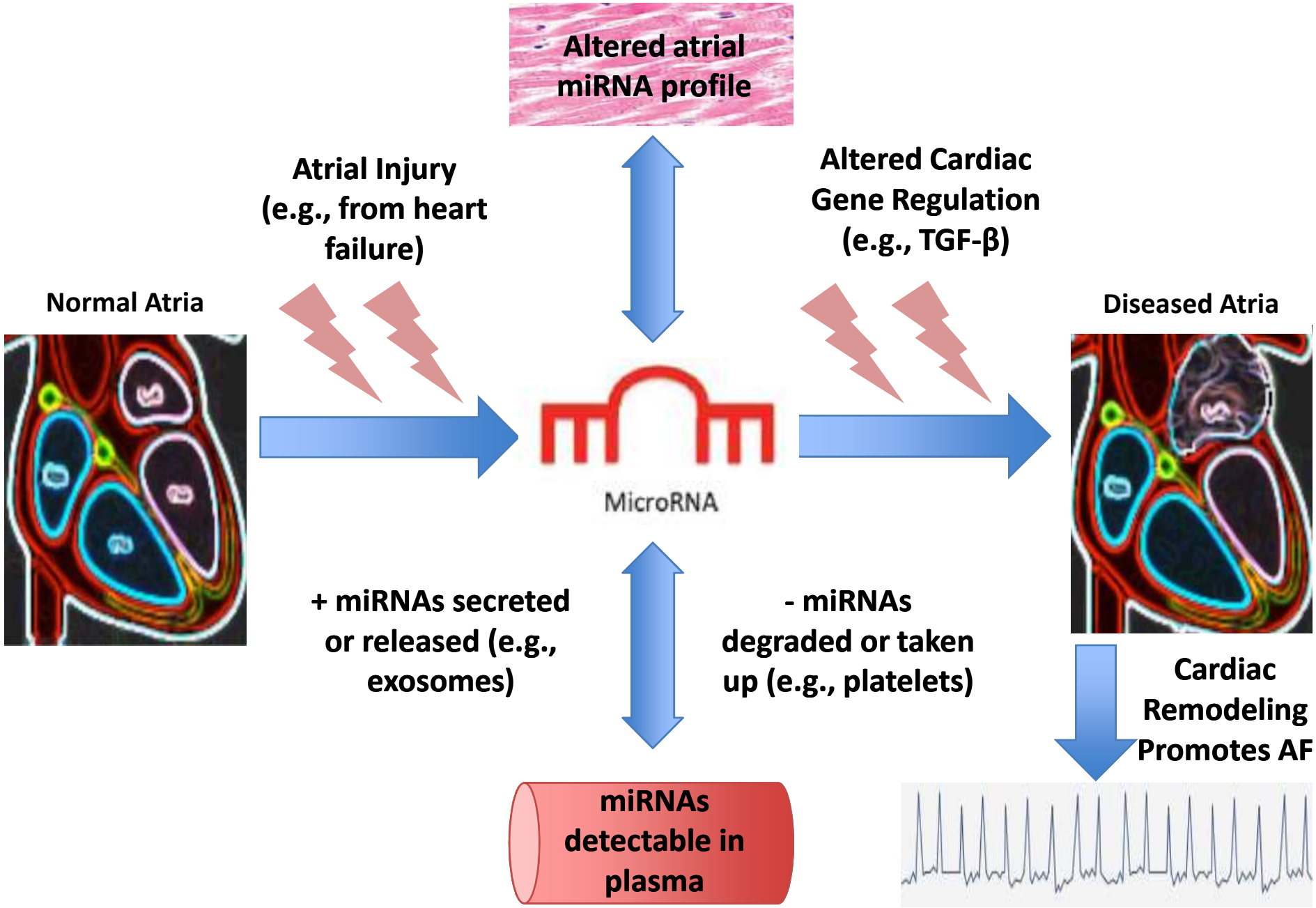


Lu et al Control of Atrial Fibrillation by miR-328

K.-H. Shi et al. / Cellular Signalling 25 (2013) 2079–2084



Wang *Card Res* 2010


Altered atrial miRNA profile
Atrial Injury (e.g., from heart failure)
Altered Cardiac Gene Regulation (e.g., TGF-β)
Normal Atria
Diseased Atria
MicroRNA
+ miRNAs secreted or released (e.g., exosomes)
- miRNAs degraded or taken up (e.g., platelets)
Cardiac Remodeling Promotes AF
miRNAs detectable in plasma

High Throughput Technology exists to assess miRNA expression

- High-throughput miRNA expression profiling systems allow rapid profiling of miRNAs from numerous samples
- Use real-time PCR, or microarray
- Primers correspond to over 1,000 miRNAs
- Accurate, specific and sensitive





Courtesy, Jane Freedman, MD Kahraman Tanriverdi, PhD

miR-328

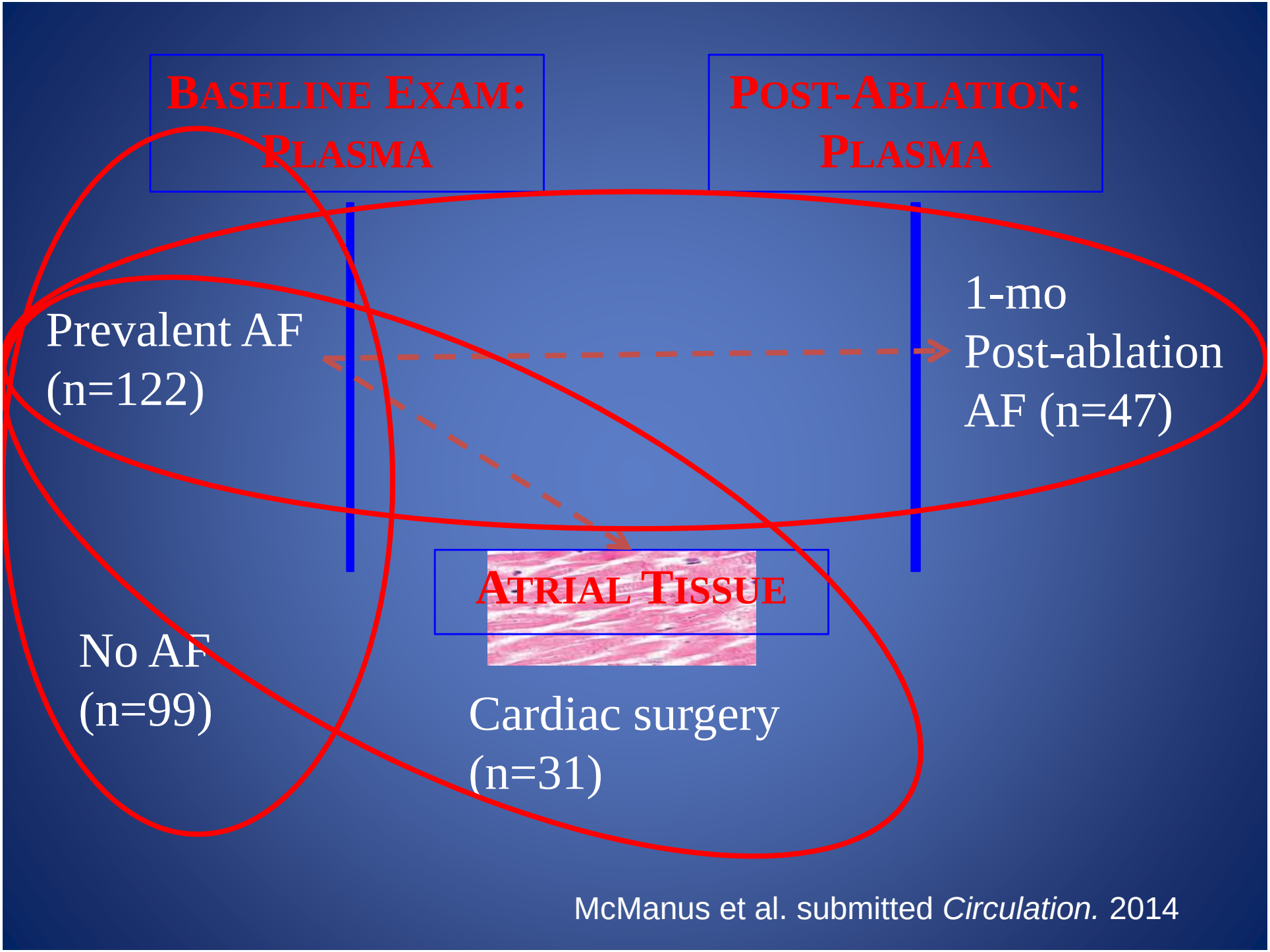


miR-328 contributes to the adverse

- miR-328 is up-regulated in the atria of human subjects with AF
- miR-328 regulates L-type Ca2+ channel density, shortens the atrial effective refractory period
- miR-328 enhances AF vulnerability in animal models

CD44	0.52	0.36
BACE1	0.66	0.07
ABCG2	0.15	1.00
H2AFX	0.73	0.86

McManus et al. *Heart Rhythm* 2014



McManus et al. submitted *Circulation.* 2014

21 Plasma mirnas associated with AF

- 21 miRNAs, including several known to regulate genes associated with cardiovascular disease, were associated with prevalent AF

	N		Average Expression (delta CT)			Multivariable Adjusted***		
		AF	Prevalent AF	No AF	Fold	Odds		
miR-125a-5p	202	106	-2.53	0.85	3.38	0.47	0.38-0.58	4.09×10^{-12}
miR-146a-5p	202	106	-2.19	0.54	2.73	0.38	0.29-0.51	7.8×10^{-12}
miR-148b-3p	198	105	-1.27	0.83	2.10	0.47	0.37-0.59	3.9×10^{-10}
miR-21-5p	209	110	-5.82	-3.76	2.06	0.51	0.41-0.63	9.2×10^{-10}
miR-221-3p	208	109	-3.30	-1.20	2.09	0.50	0.40-0.61	2.6×10^{-10}
miR-223-3p	209	110	-5.88	-3.62	2.27	0.49	0.39-0.60	5.9×10^{-11}

AF=atrial fibrillation; OR=odds ratio; miRNA=; CI= Confidence Interval; P=significance value; Sig=0.05/86 miRNAs = 0.0006

Fold-change is the difference in miRNA expression between individuals with AF compared to no AF

Multivariable adjusted models included age, sex, current smoking, diabetes, prevalent heart failure, and MI

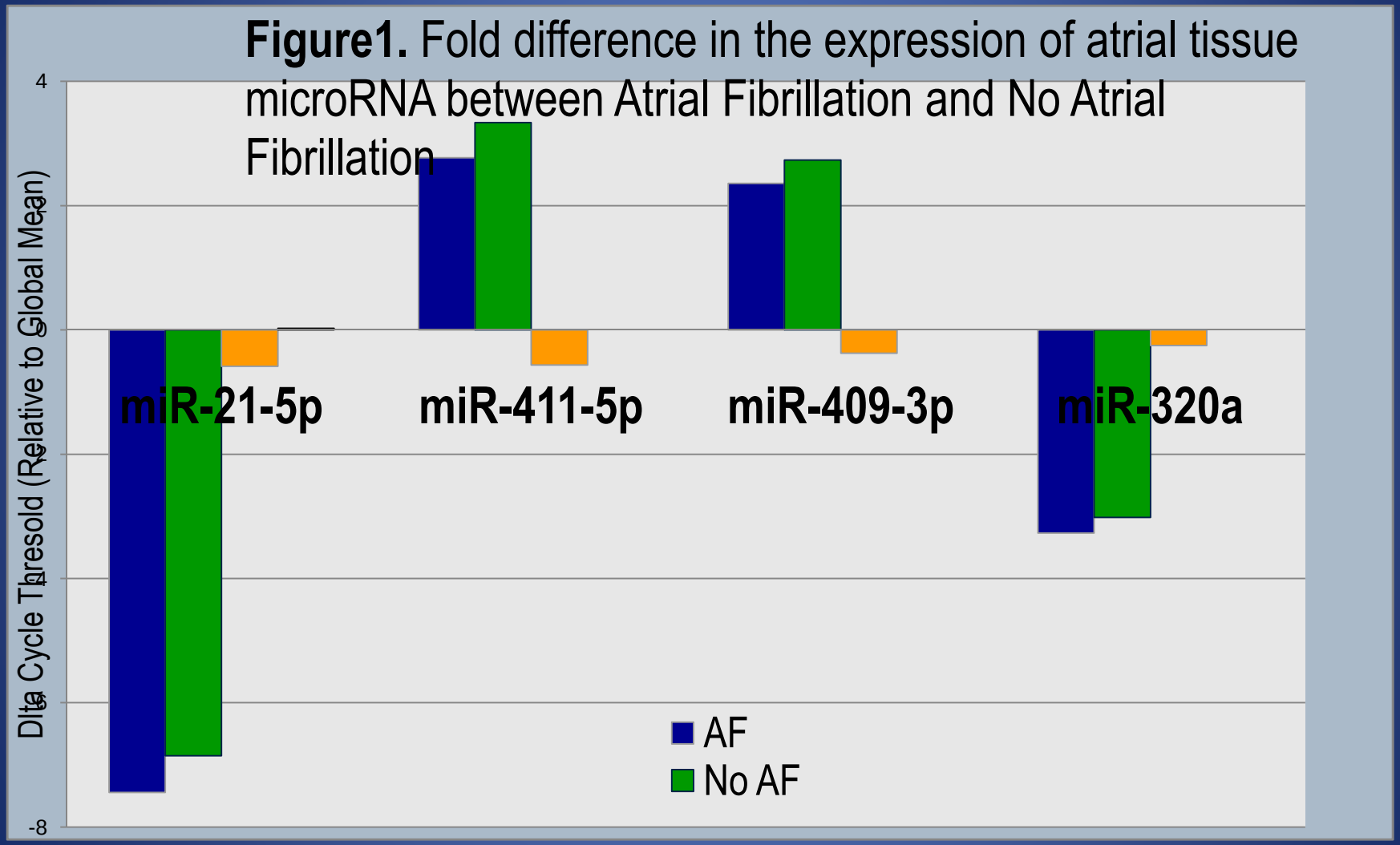
33 Plasma Mirs change pre- to post-ablation

	N		Average Expression (delta CT)			Multivariable Adjusted***		
miRNA	Baseline	Post-Ablation	Baseline	Post-Ablation	Fold Change	Odds Ratio	95% CI	*P* value*
miR-223-3p	47	46	-6.32	-2.64	3.68	3.12	1.98 - 4.93	$1x10^{-6}$
let-7b-5p	47	47	-6.23	-2.67	3.56	3.43	2.08 - 5.66	$1.5x10^{-6}$
miR-30c-5p	47	38	-1.08	1.63	2.71	3.54	2.11 - 5.92	$1.5x10^{-6}$
miR-342-3p	47	47	-2.07	0.54	2.61	4.53	2.41 - 8.51	$2.7x10^{-6}$
let-7c-5p	47	47	-4.66	-0.95	3.71	3.92	2.21 - 6.97	$3.1x10^{-6}$
miR-148b-3p	46	35	-1.49	1.18	2.67	2.94	1.85 - 4.67	$4.9x10^{-6}$
miR-146a-5p	47	36	-2.24	0.91	3.15	3.2	1.93 - 5.33	$7.2x10^{-6}$
miR-125b-5p	47	38	-2.92	1.48	4.40	3.68	2.05 - 6.61	$1.3x10^{-5}$
miR-126-3p	47	44	-5.58	-1.39	4.19	3.81	2.08 - 6.96	$1.4x10^{-5}$
miR-100-5p	47	33	-2.07	1.29	3.36	3.95	2.09 - 7.47	$2.2x10^{-5}$
miR-125a-5p	47	36	-3.22	1.48	4.71	4.86	2.12 - 11.16	$1.9x10^{-4}$

- 33 miRNAs changed from pre- to post-ablation
- 14 miRNAs were also associated with AF

AF=atrial fibrillation; OR = odds ratio; miR = miRNA; CI = Confidence Interval; Bonferroni *p* value cutoff = 0.05/86 miRNAs = 0.0006
Fold-change is the difference in miRNA expression between individuals with AF compared to no AF
Multivariable adjusted models included age, sex

AF vs. No AF in Atrial Tissue

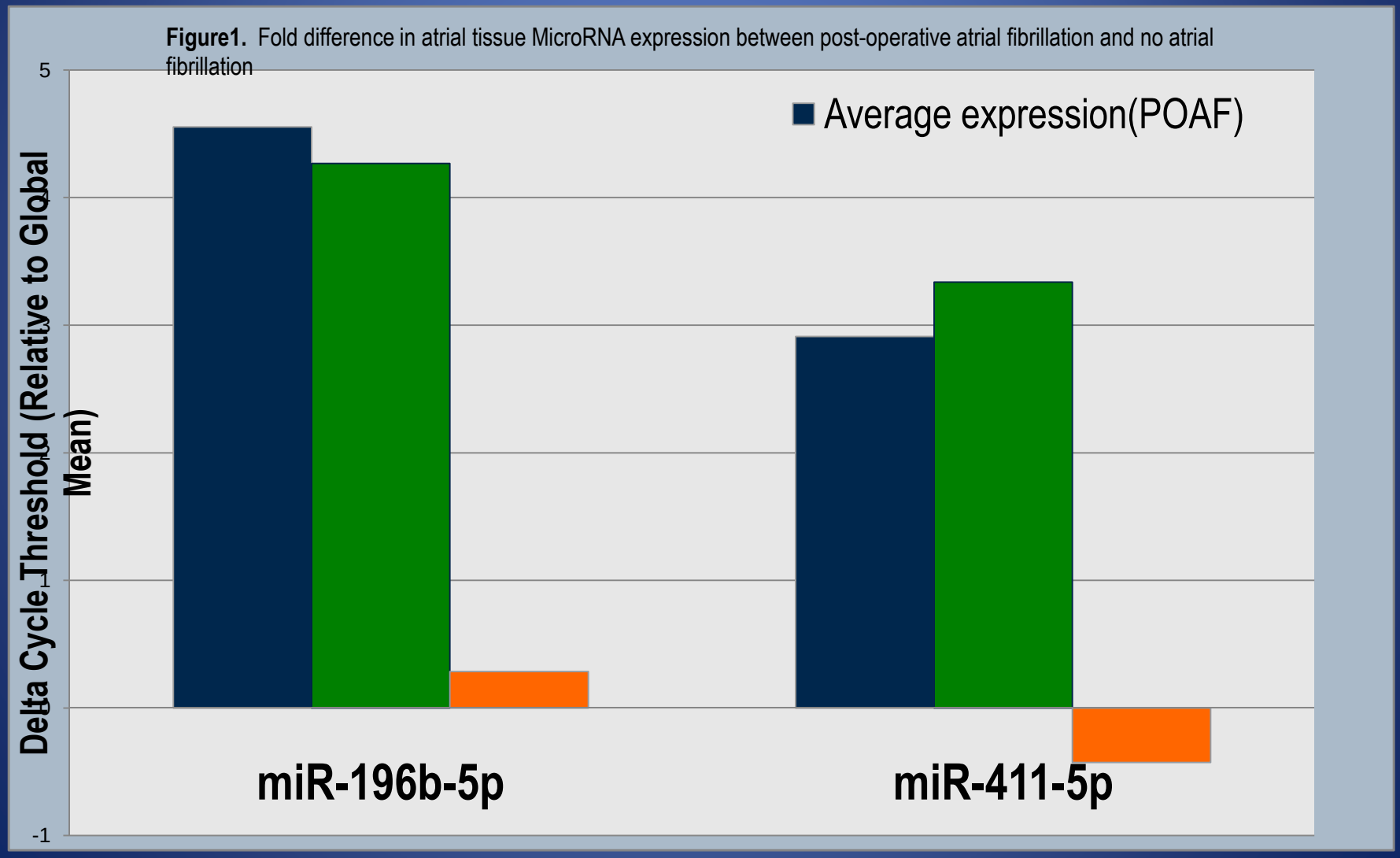


AF vs. No AF in Atrial Tissue

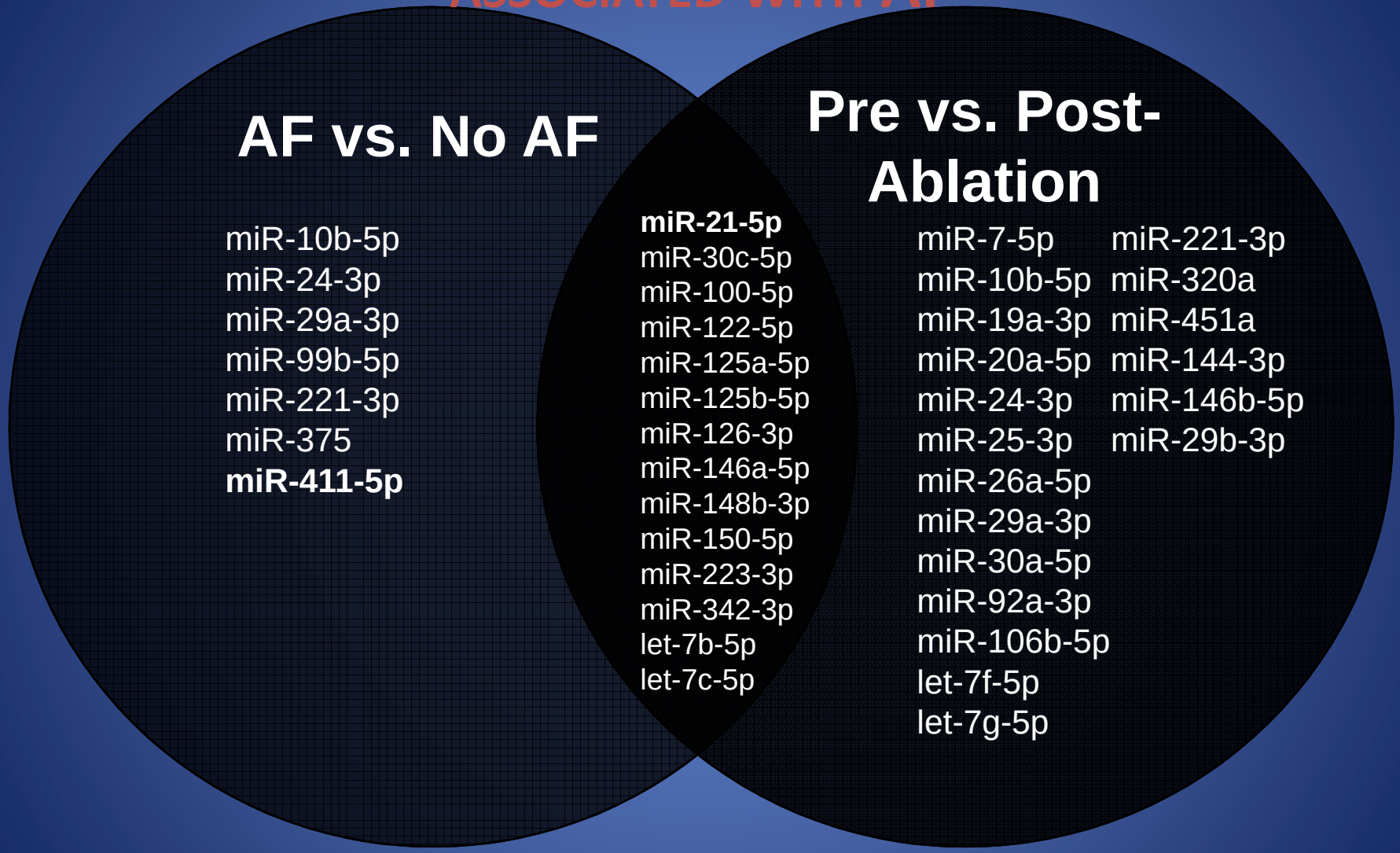
	N		Average Expression (delta CT)			
	Total	AF Cases	AF (n=19)	Control (n=12)	Fold Difference	*P*-value
miRNA 411-5p	31	19	2.770	3.337	- 0.567	0.0170
miRNA 21-5p	31	19	-7.441	-6.853	- 0.588	0.0243
miRNA 409-3p	31	19	2.357	2.732	- 0.375	0.039
miRNA 320a	31	19	-3.268	-3.018	- 0.427	0.0477

Post-Operative AF

Figure1. Fold difference in atrial tissue MicroRNA expression between post-operative atrial fibrillation and no atrial fibrillation



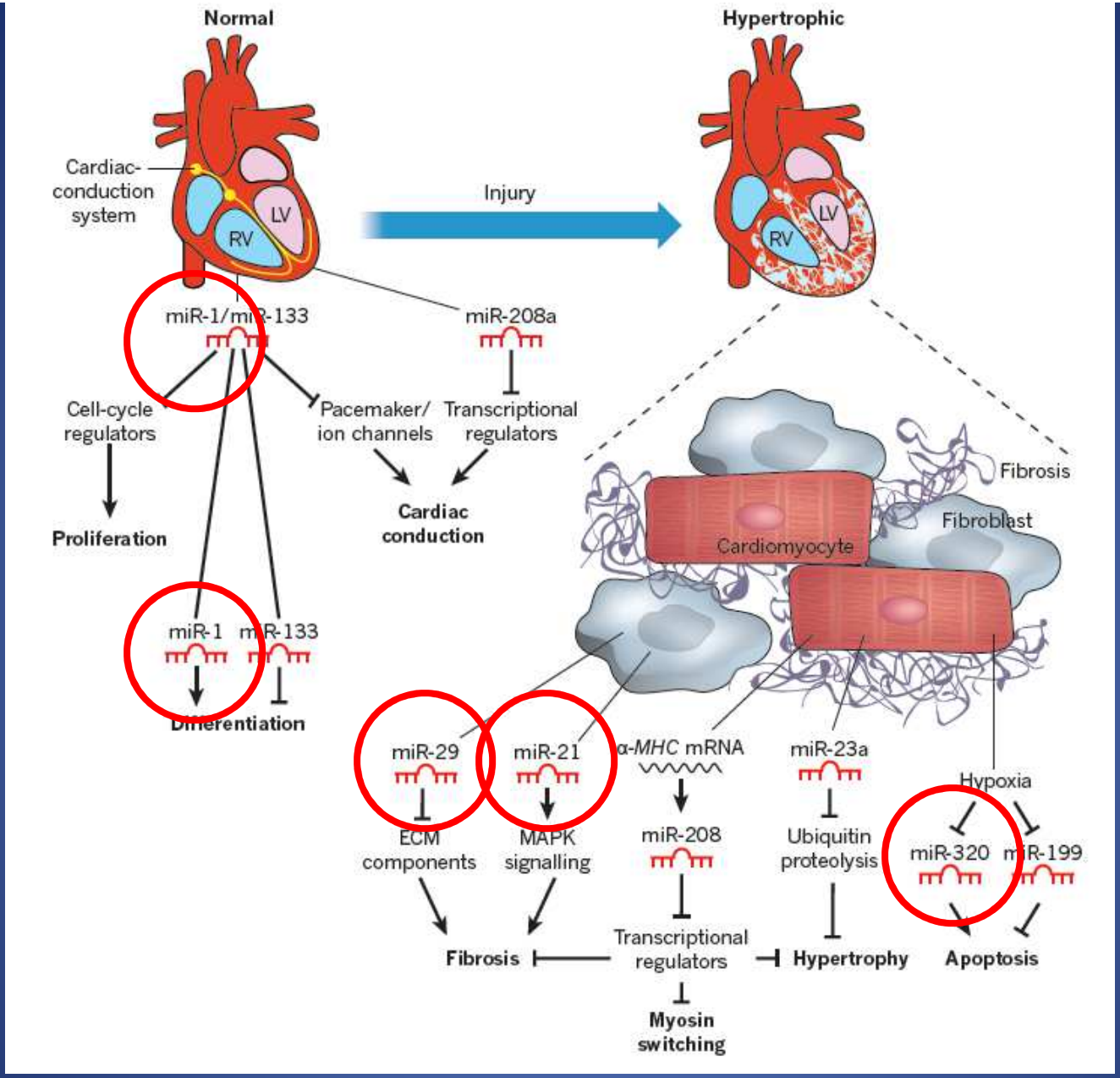
Considerable Overlap in Highly Variant MiRs and those associated with AF



Gene Targets associated with Significant MIRNAs

miRNA	FUNCTION (TARGET GENES)	ASSOCIATED PHENOTYPE
miR-1	Cell cycle regulation; (Ion Channels and gap junction genes, *GJA1*, *KNJ2*)	Cardiac arrhythmia, cardiac development, downregulation in AF
miR-21	Upregulation of the protein sprouty (*ERK-MAPK*), *PDCD4*	Anti-apoptotic factor, cardiac stress response
miR-29	Inhibition of collagen and extracellular matrix proteins (*ELN, FBN1, COL1A1*), Pro-apoptosis (*Mcl-2*)	Regulates deposition of intracellular collagen
miR-92a	Inhibition of neorevascularization (integrin subunit α5 and *eNOS*)	Reduction in cellular apoptosis and improved cardiac function
miR-122	fatty acid beta-oxidation	Contributes to endothelial dysfunction
miR-150	(*c-Myb*), H2O2-induced cardiac cell death	Atherosclerosis, cardiac hypertrophy, heart failure, myocardial infarction, and myocardial ischemia/reperfusion injury
miR-320	Pro-apoptosis (*HSP20* levels); Increases expression of insulin-like growth factor-1	Down-regulated after ischemia reperfusion injury; down-regulated in AF
miR-92a	Inhibition of neorevascularization (integrin subunit α5 and *eNOS*)	Reduction in cellular apoptosis and improved cardiac function

McManus et al. submitted *Circulation.* 2014



Olson, *Nature* 2010

MiRhythm Findings

- We observed associations between AF and plasma miRNAs linked to gene regulatory pathways responsible for cardiac remodeling
- Overlap was observed between plasma miRNAs associated with AF and those changing after ablation
- Studies are needed to explore gene regulatory pathways implicated in susceptibility to AF and to examine the role of miRNAs as circulating biomarkers of diagnostic or prognostic importance in AF

McManus et al. submitted *Circulation.* 2014

Future Directions

- Exploring functional significance of miRNA dysregulation in animal models of AF
- Complete echocardiographic phenotyping of LA structure in FHS and look at genomic and transcriptomic profiles of LA-EF, LAVI
- Leverage AF Registry and Biobank











BU/FHS

-Vasan Ramachandran M

-Emelia Benjamin MD, Sc

-Jared Magnani, MD, MP

-Shuxia Fan

-Susan Cheng, MD MS

-Honghuan Lin, MD

MGH

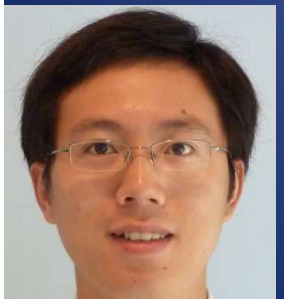
-Patrick Ellinor MD, PhD

-Steven Lubitz, MD

A special thank you to the 650+ AF patients who have entrusted their care to us and participated in the Umass AF Registry, AF Biobank, and InRhythm!















Thank you for your attention!

