Clonal hematopoiesis of indeterminate potential and the risk of atrial fibrillation: prevalence and an association with progression

Hyo-Jeong Ahn, MD[a*], Hong Yul An, MD[b*], Jiwoo Lim, MS[b], Song Han, PhD[b], Su-Yeon Choi, MD, PhD[c,d], Heesun Lee, MD, PhD[d], Soonil Kwon, MD[a], So-Ryoung Lee, MD, PhD[a], Gregory Y. H. Lip, MD[e,f], Seil Oh, MD, PhD[a,c], Siddhartha Jaiswal, PhD[g], Youngil Koh, MD, PhD[a,b,h,i] Eue-Keun Choi, MD, PhD[a,c]

[a] Department of Internal Medicine, Seoul National University Hospital, Seoul, Republic of Korea
[b] Genome Opinion Incorporation, 04799, Seoul, Republic of Korea
[c] Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea
[d] Department of Internal Medicine, Seoul National University Hospital Healthcare System Gangnam Center, Seoul, Korea.
[e] Liverpool Centre for Cardiovascular Science at University of Liverpool, Liverpool John Moores University and Liverpool Chest & Heart Hospital, Liverpool, United Kingdom
[f] Danish Center for Clinical Health Services Research, Department of Clinical Medicine, Aalborg University, Aalborg, Denmark
[g] Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA.
[h] Biomedical Research Institute, Seoul National University Hospital, Seoul, Republic of Korea.
[i] Cancer Research Institute, Seoul National University Hospital, Seoul, Republic of Korea.

Korean Heart Rhythm Society

COI Disclosure

This study was partially supported by Genome Opinion Inc.

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- Background
- Clinical research
- (Basic research)
- Expected clinical implications and future research
- Conclusion

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- **Background**
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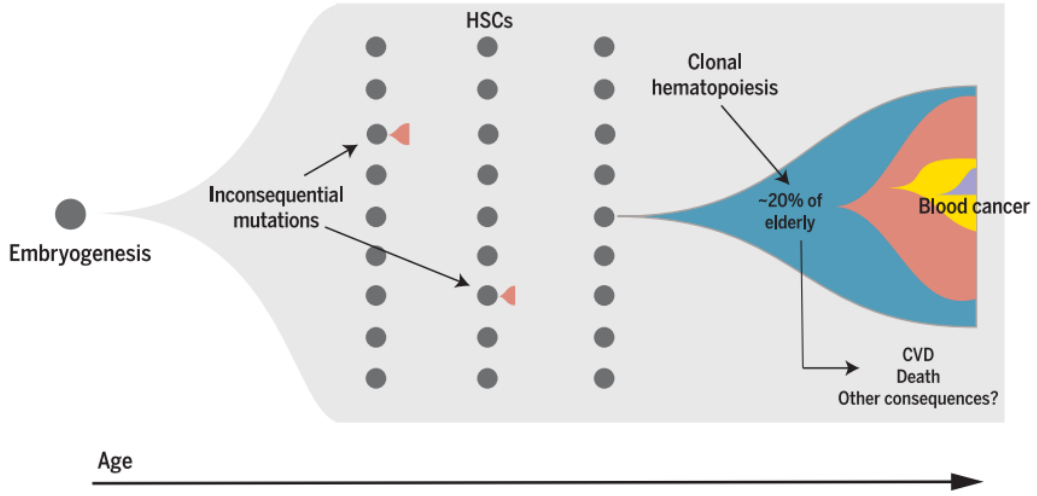
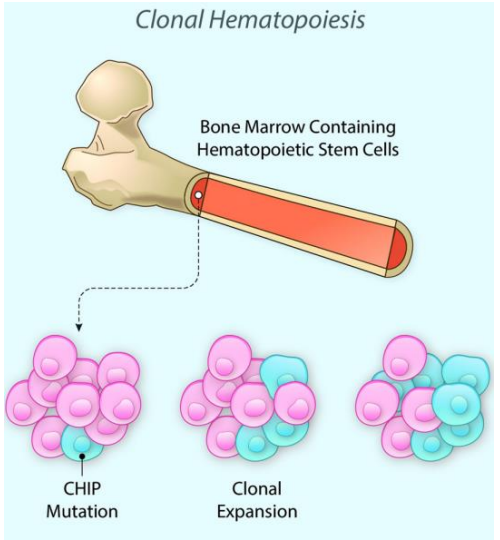
Clonal Hematopoiesis: selective growth advantage of a HSC

- Clonal hematopoiesis of indeterminate potential (**CHIP**) is the clonal expansion of hematopoietic stem cells bearing acquired somatic mutations, especially ***DNMT3A, TET2,*** and ***ASXL1*** ** Involved in epigenetic regulation*

- Most individuals with CHIP will never develop a hematologic malignant condition, hence the term *indeterminate potential*
  ** Medical uncertainty*

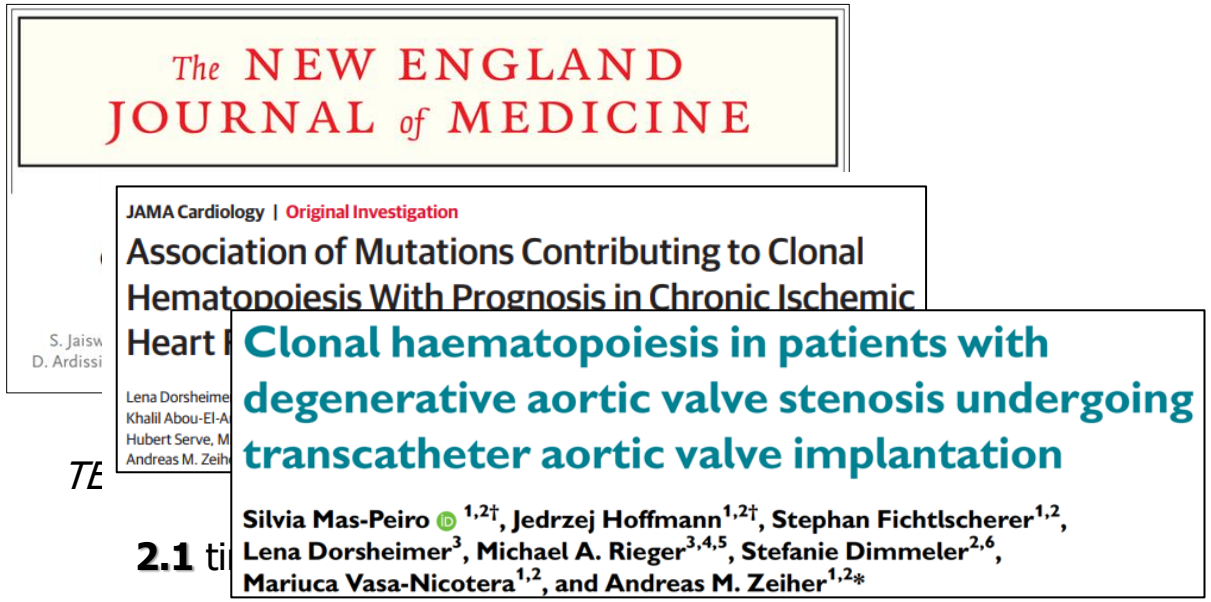




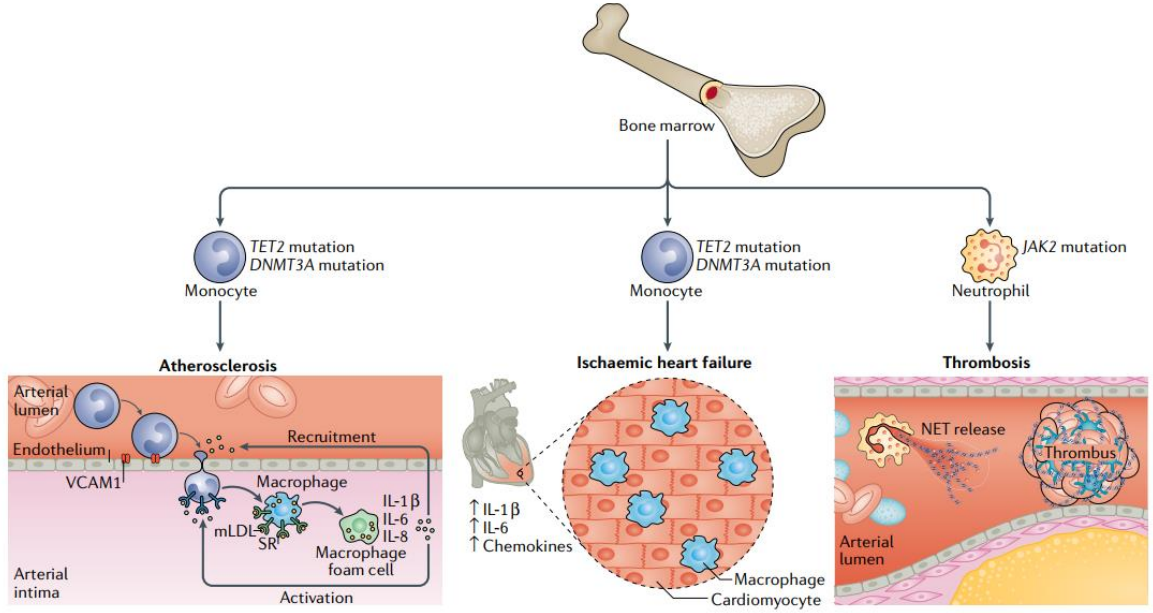
Jaiswal S et al. Science. 2019; Jaiswal S, et al. Nat Rev Cardiol. 2020; Sidlow R, et al. JAMA Cardiol. 2020

Clonal Hematopoiesis, a newly recognized CV risk factor

- **Age-related** condition: rare in the young, but 10-20% of those > 70 years
- CHIP associates with a striking **elevation in cardiovascular risk**
- Mouse experiments: causality of **CHIP** with **accelerated atherosclerosis and HF**



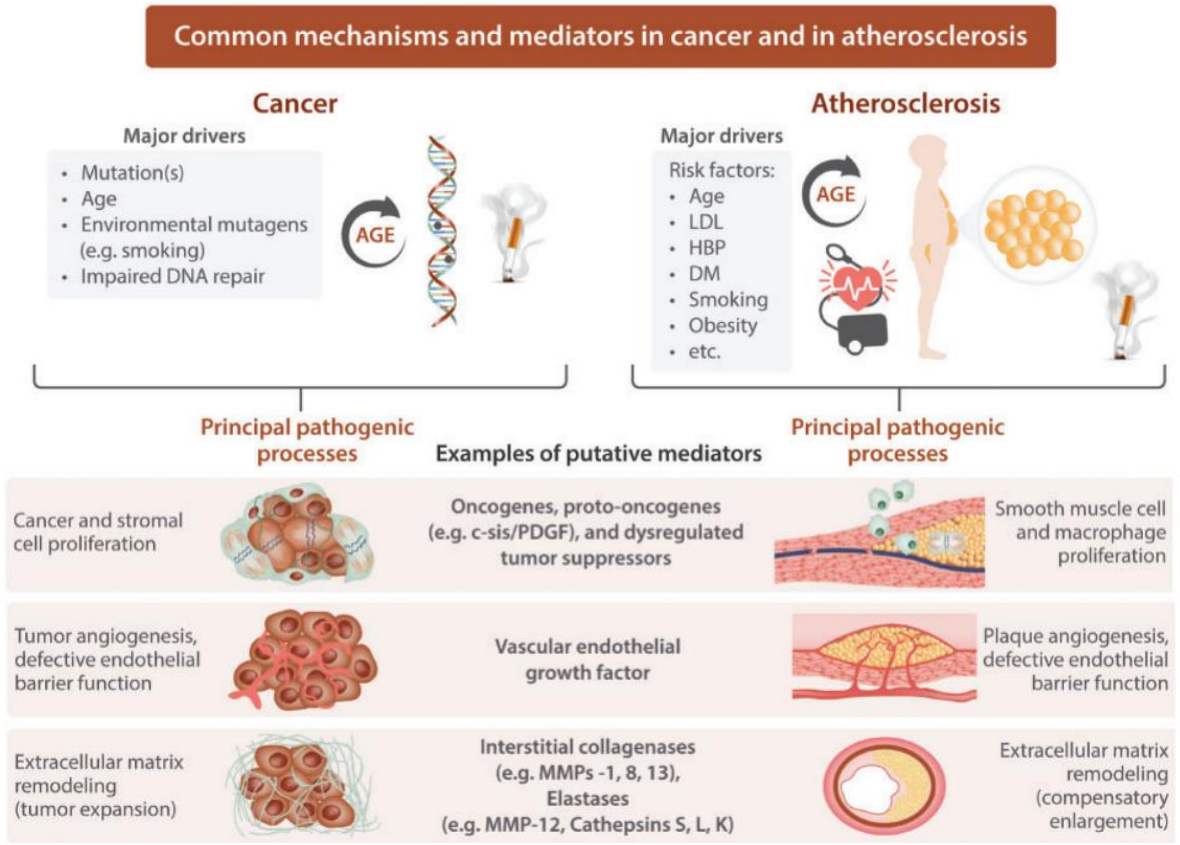
DNMT3A or TET2 mutations had **an increased mid-term all-cause mortality**



Jaiswal S, et al. N Engl J Med. 2017; Dorsheimer L, et al. JAMA Cardiol. 2019; Mas-Peiro S, et al. Eur Heart J. 2020

Inflammation:
a common contributor to cancer, aging, and CVD

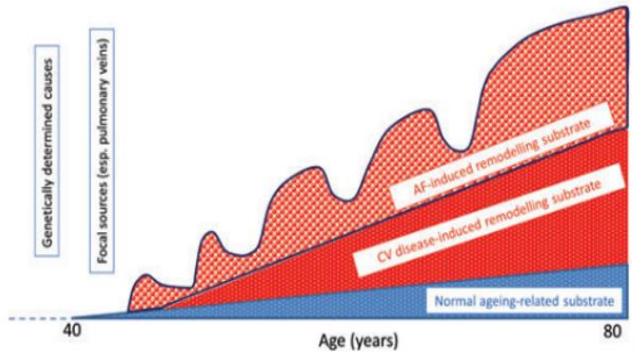
- Key pathogenesis: **Inflammatory reactions of PBCs**

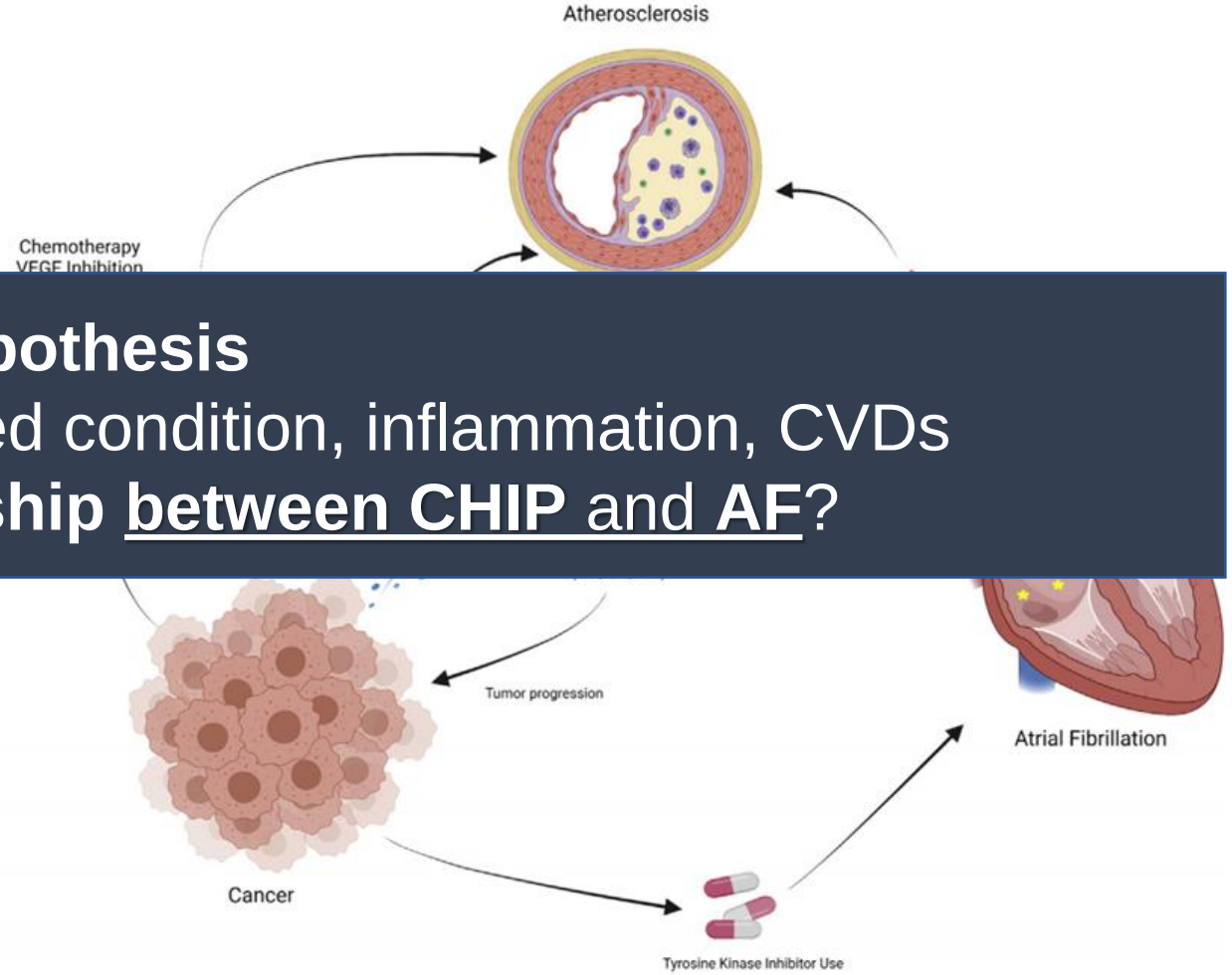


Peter Libby, et al. Cardiovasc Res. 2019

CHIP and atrial fibrillation (AF)?

- AF is a disease of aging
- Inflammation as a pathophysiology
- Shares common risk factors with atherosclerotic CVDs





1st Hypothesis

Both CHIP and AF – age related condition, inflammation, CVDs

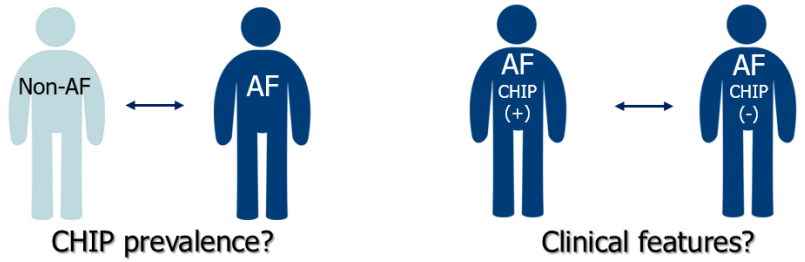
Will there be **any relationship <u>between CHIP</u>** and **<u>AF</u>**?

Leiva O, et al. JACC CardioOncol. 2021

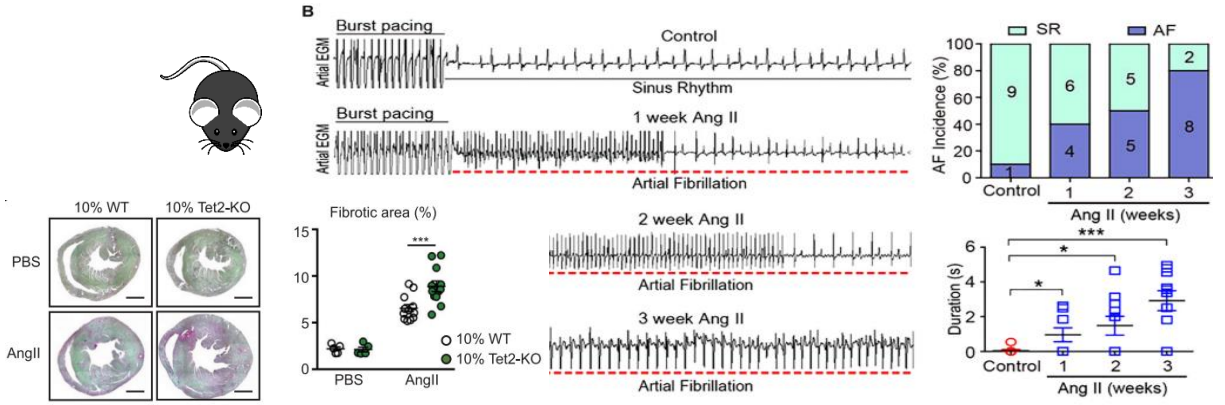
Aim of the study

- To evaluate the association between CHIP and AF from bench to bedside

- **Part I**. Clinical association between CHIP and AF
  - To compare the prevalence of CHIP mutations in Non-AF vs. AF
  - To find clinical features stratified by the CHIP mutations within AF patients



- **Part II**. Mechanism of CHIP on AF
  - Validation of pathophysiology in mice model
  - Role of CHIP in angiotensin II-induced atrial fibrosis in mice



Leiva O, et al. JACC CardioOncol. 2021; Li J, et al. Hypertension. 2018; Wu YX, et al. Front Physiol. 2019

Contents

- Background
- **Clinical research :**

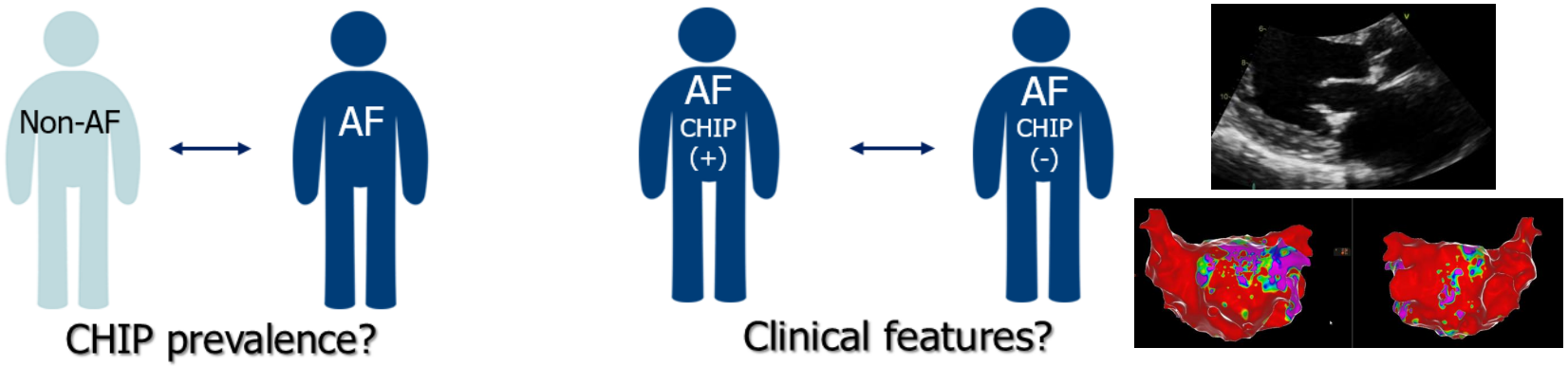
  CHIP and AF: the prevalence and associated clinical features
- (Basic research)
- Expected clinical implications and future research
- Conclusion

CHIP and atrial fibrillation (AF)?

- **Analysis**

  1) Non-AF vs. AF: The prevalence of CHIP mutations

  2) In AF, **CHIP(+) vs. CHIP (-)**:
     Distinctive AF-related features (AF type, duration, LA remodeling parameters)



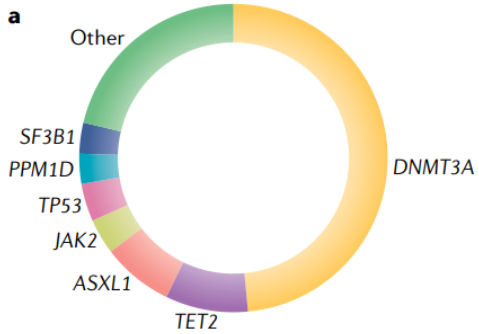
Methods

- **Study population**
  - *AF (n=1,004)*
    - 50-79 years, no hx. of cancer/VHD/cardiac surgery in 2021/4 – 2022/12
  - *Control (n=3,341)*
    - Non-AF healthy adults, health examination in 2011/5 – 2017/1

- **A deep targeted sequencing of 24 CHIP driver mutations** from peripheral blood-derived mononuclear cells
  - **CHIP (+)** if a variant allele fraction (VAF) reach 2.0%

ASXL1	*ATM*	*BCOR*	*CBL*	*CHEK2*	*CREBBP*	*DNMT3A*	*EP300*	*GNAS*	*IDH2*
JAK2	*KMT2D*	*KRAS*	*NOTCH1*	*NOTCH2*	*PPM1D*	*SETD2*	*SF3B1*	*SRSF2*	*STAG2*
STAT3	*TET2*	*TP53*	*U2AF1*						

* VAF (variant allele fraction): the percentage of reads that support a mutant allele out of the total number of reads.



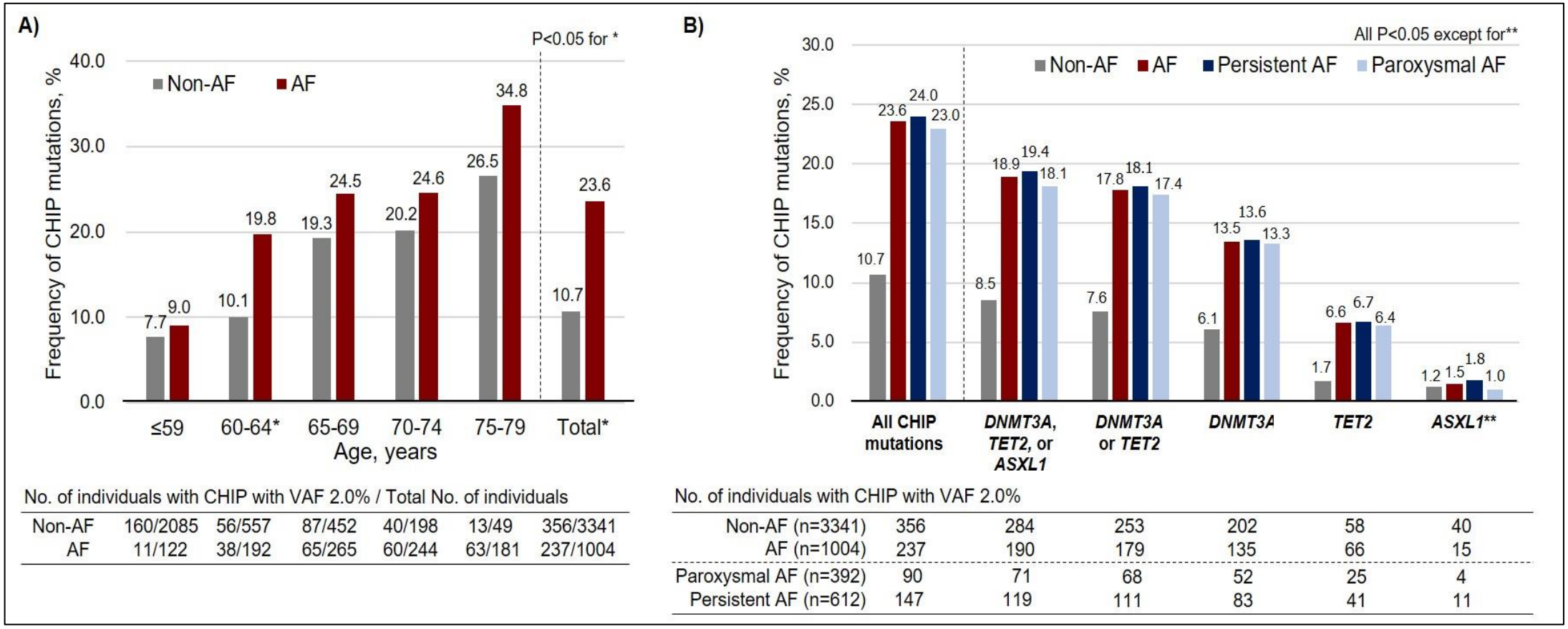
Niroula A, et al. Nat Med. 2021; Jaiswal S, et al. Nat Rev Cardiol. 2020

Results – Baseline characteristics

	Non-AF (GENIE cohort)	AF	p-value
	N=3,341	N=1,004	
Age (years)	58.5 ± 6.5	67.6 ± 6.9	<0.001
Sex (Male)	2328 (69.7%)	697 (69.4%)	0.907
Body mass index (kg/m^2)	24.0 ± 2.7	25.3 ± 3.5	<0.001
Comorbidities			
Hypertension	1320 (39.5%)	756 (75.3%)	<0.001
Diabetes	527 (15.8%)	313 (31.2%)	<0.001
Chronic kidney disease	82 (2.5%)	51 (5.1%)	<0.001
Stroke/TIA/TE	14 (0.4%)	119 (11.9%)	<0.001
Vascular disease	112 (3.4%)	119 (11.9%)	<0.001
Smoking			
Never	1831 (54.8%)	606 (60.4%)	<0.001
Ex	1068 (32%)	305 (30.4%)	
Current	442 (13.2%)	93 (9.3%)	
Measurements			
Systolic blood pressure (mmHg)	119.0 ± 13.4	127.2 ± 14.9	<0.001
Diastolc blood pressure (mmHg)	78.5 ± 9.8	74.6 ± 10.3	<0.001
Heart rate (/min)	65.8 ± 9.9	75.0 ± 14.8	<0.001
Hb (g/dL)	14.6 ± 1.3	14.1 ± 1.5	<0.001
Hct (%)	44.0 ± 3.7	42.8 ± 4.0	<0.001
Blood urea nitrogen (mg/dL)	15.0 ± 3.7	16.7 ± 4.8	<0.001
Creatinine (mg/dL)	0.9 ± 0.2	0.9 ± 0.2	<0.001
eGFR (mL/min/1.73m^2)	87.0 ± 14.8	78.6 ± 15.8	<0.001

	AF
N	**N=1,004**
Family history of AF	37 (3.7%)
EHRA	
0	5 (0.5%)
1	605 (60.3%)
2	326 (32.5%)
3	68 (6.8%)
AF duration (days)	1563.0 (596.0-2813.0)
CHA_2DS_2-VASc Score	2.6 ± 1.3
Alcohol	376 (37.5%)
Echocardiography	
LVEF (%)	59.2 ± 5.7
LA diameter (mm)	45.9 ± 7.2
LAVI (mL/m^2)	47.8 ± 19.2
E/E'	10.2 ± 6.6
CIED	69 (6.9%)
Treatment	
Electrical cardioversion	281 (28.0%)
Catheter ablation	
No	605 (60.3%)
Radiofrequency	232 (23.1%)
Cryo	135 (13.4%)
Both	32 (3.2%)

Result – Non-AF vs. AF
CHIP, More frequent in patients with AF



No. of individuals with CHIP with VAF 2.0% / Total No. of individuals

Non-AF	160/2085	56/557	87/452	40/198	13/49	356/3341
AF	11/122	38/192	65/265	60/244	63/181	237/1004

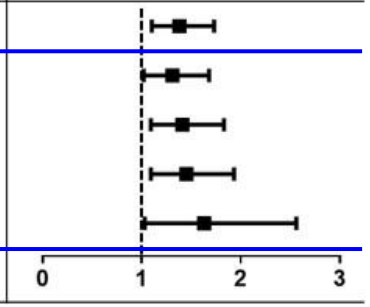
No. of individuals with CHIP with VAF 2.0%

Non-AF (n=3341)	356	284	253	202	58	40
AF (n=1004)	237	190	179	135	66	15
Paroxysmal AF (n=392)	90	71	68	52	25	4
Persistent AF (n=612)	147	119	111	83	41	11

All CHIP mutations are **more frequently observed in AF** patients across the age
DNMT3A, TET2, and ***ASXL1*** are the most common CHIP mutations in AF

Result – Non-AF vs. AF
CHIP, *1.4* - fold more frequent in patients with AF

	Prevalence of CHIP mutations (VAF ≥ 2.0%)		OR (95% CI) of CHIP in AF						OR (95% CI) of Model 3
	Non-AF (n=3,341)	AF (n=1,004)	Model 1	P	Model 2	P	Model 3	P	
All CHIP mutations	356 (10.7%)	237 (23.6%)	1.46 (1.18-1.81)	0.001	1.44 (1.15-1.80)	0.001	1.38 (1.10-1.74)	0.006	
DNMT3A, TET2,* or *ASXL1	284 (8.5%)	190 (18.9%)	1.41 (1.12-1.79)	0.004	1.38 (1.09-1.76)	0.009	1.32 (1.03-1.69)	0.030	
DNMT3A* or *TET2	253 (7.6%)	179 (17.8%)	1.53 (1.20-1.95)	0.001	1.50 (1.17-1.93)	0.001	1.42 (1.10-1.84)	0.008	
DNMT3A	202 (6.1%)	135 (13.5%)	1.54 (1.18-2.02)	0.002	1.53 (1.16-2.02)	0.003	1.45 (1.09-1.93)	0.012	
TET2	58 (1.7%)	66 (6.6%)	1.79 (1.18-2.73)	0.007	1.72 (1.11-2.66)	0.015	1.65 (1.05-2.60)	0.030	



Model 1: adjusted by age, sex; **Model 2**: adjusted by age, sex, smoking, body mass index; **Model 3**: adjusted by age, sex, smoking, body mass index, diabetes, hypertension

CHIP mutations are ***1.4***-fold more prevalent in AF patients than in non-AF
As a single gene, ***TET2*** has the highest OR (***1.7***)

Result – In AF, CHIP (+) vs. CHIP (-):

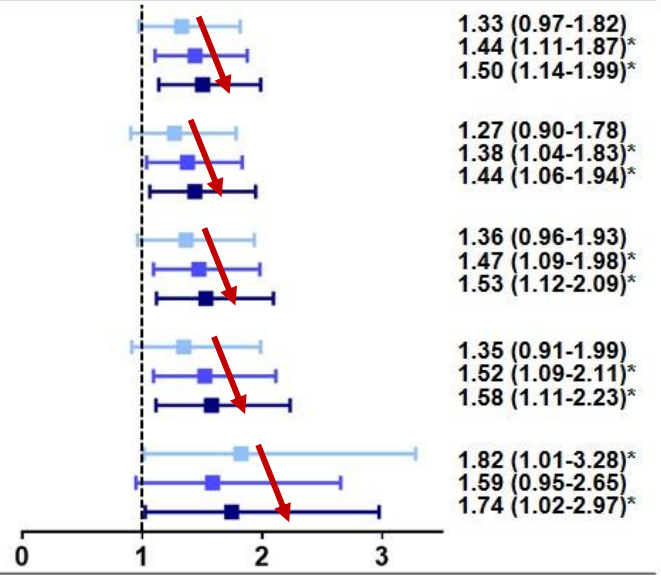
Then, what would be the distinctive features stratified by CHIP presence?

	No DTA (VAF 2%) N=814	DTA (VAF 2%) N=190	p-value
Age	67.2 ± 7.0	69.7 ± 6.0	<0.001
Sex (Male)	565 (69.4%)	132 (69.5%)	0.986
Body mass index (kg/m^2)	25.4 ± 3.7		
Type of AF			
Paroxysmal	321 (39.4%)		
Persistent	493 (60.6%)		
Family history of AF	32 (3.9%)		
EHRA			
0	4 (0.5%)		
1	479 (58.8%)		
2	270 (33.2%)		
3	61 (7.5%)		
AF duration (days)	1465.0 (583.0-2720.0		
Comorbidities			
Hypertension	606 (74.4%)		
Diabetes	236 (29.0%)		
Congestive heart failure	142 (17.4%)		
Chronic kidney disease	37 (4.5%)		
Chronic liver disease	5 (0.6%)		
Stroke/TIA/TE	95 (11.7%)		
Vascular disease	99 (12.2%)		
CHA_2DS_2-VASc Score	2.5 ± 1.3		

	No DTA (VAF 2%) N=814	DTA (VAF 2%) N=190	p-value
Smoking			0.764
Never	487 (59.8%)	119 (62.6%)	
Ex	250 (30.7%)	55 (28.9%)	
Current	77 (9.5%)	16 (8.4%)	
Alcohol	309 (38.0%)	67 (35.3%)	0.489
Echocardiography			
LVEF	59.1 ± 5.8	59.6 ± 5.0	0.308
LA diameter	45.8 ± 7.2	46.5 ± 7.2	0.308
LAVI (mL/m^2)	47.6 ± 19.4	48.7 ± 18.3	0.549
E/E'	9.9 ± 4.0	11.8 ± 13.1	0.002
CIED	52 (6.4%)	17 (8.9%)	0.209
Treatment			
Electrical cardioversion	229 (28.1%)	52 (27.4%)	0.833
Catheter ablation			0.018
No	476 (58.5%)	129 (67.9%)	
Radiofrequency	202 (24.8%)	30 (15.8%)	
Cryo	113 (13.9%)	22 (11.6%)	
Both	23 (2.8%)	9 (4.7%)	

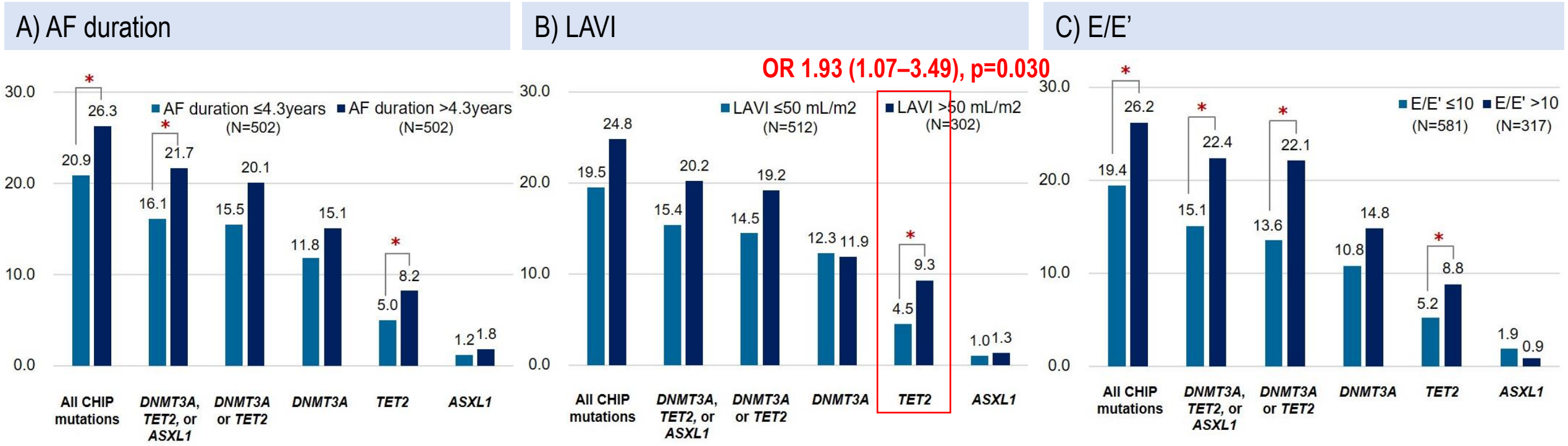
Result – In AF, CHIP (+) vs. CHIP (-): CHIP (*DNMT3A, TET2, ASXL1*) & clinical features in AF

	Prevalence of CHIP mutations (VAF ≥ 2.0%)				OR (95% CI) of Model 3
	Non-AF (n=3,341)	PAF (n=392)	PeAF (n=612)	LsPeAF (n=517)	
All CHIP mutations	356 (10.7%)	90 (23.0%)	147 (24.0%)	129 (25.0%)	1.33 (0.97-1.82) 1.44 (1.11-1.87)* 1.50 (1.14-1.99)*
DNMT3A, TET2,* or *ASXL1	284 (8.5%)	71 (18.1%)	119 (19.4%)	104 (20.1%)	1.27 (0.90-1.78) 1.38 (1.04-1.83)* 1.44 (1.06-1.94)*
DNMT3A* or *TET2	253 (7.6%)	68 (17.4%)	111 (18.1%)	97 (18.8%)	1.36 (0.96-1.93) 1.47 (1.09-1.98)* 1.53 (1.12-2.09)*
DNMT3A	202 (6.1%)	52 (13.3%)	83 (13.6%)	73 (14.1%)	1.35 (0.91-1.99) 1.52 (1.09-2.11)* 1.58 (1.11-2.23)*
TET2	58 (1.7%)	25 (6.4%)	41 (6.7%)	37 (7.2%)	1.82 (1.01-3.28)* 1.59 (0.95-2.65) 1.74 (1.02-2.97)*



Result – In AF, CHIP (+) vs. CHIP (-):

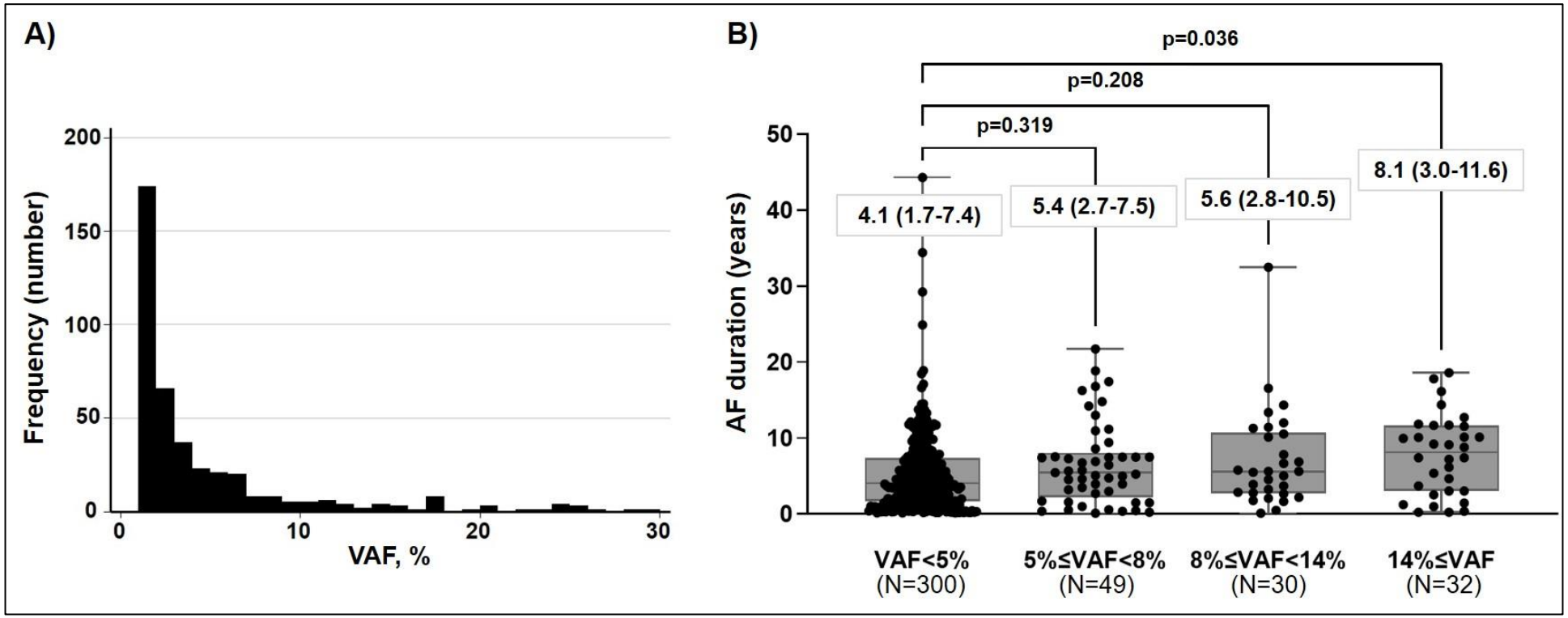
Frequency of CHIP mutations according to AF duration, LA volume index, and E/E



A ***higher*** prevalence of CHIP in AF with:
Longer duration, increased LA volume, and elevated E/E'

Result – In AF:

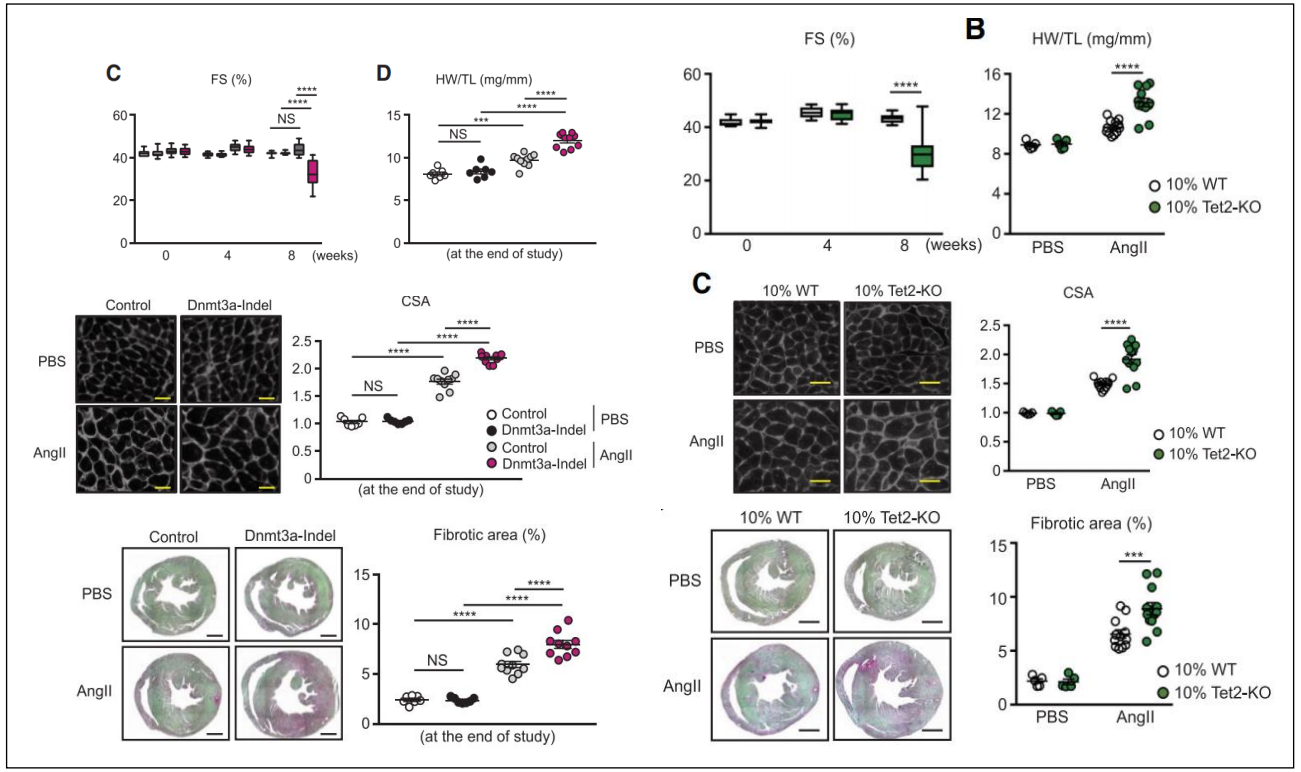
Dose-response relationship with CHIP VAF



A ***longer duration of AF*** according to the ***higher VAF*** in patients with AF with CHIP of VAF > 0%

What would be the mechanism?

- *DNMT3A*, *TET2:* Greater cardiac hypertrophy, decreased cardiac function, higher levels of fibrosis

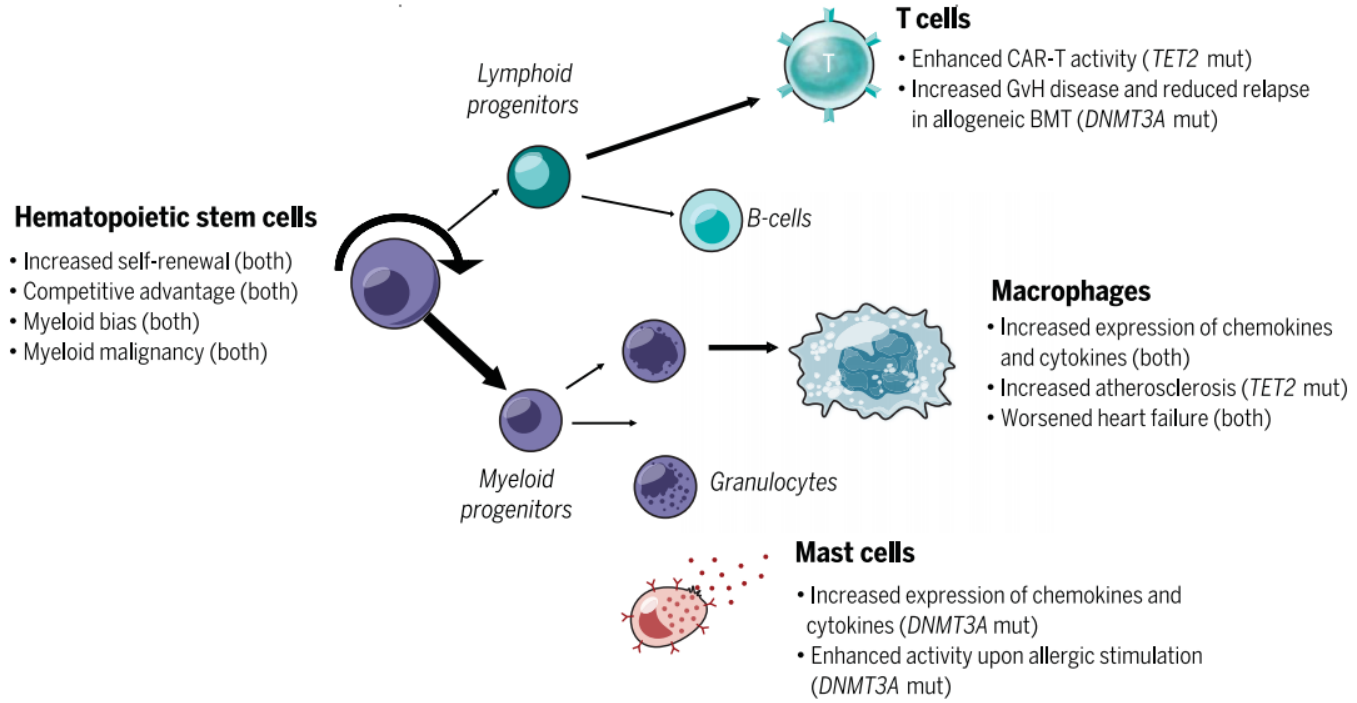


CHIP mutations might contribute to the development/progression of AF
via atrial hypertrophy and ***fibrosis***

Heimlich JB, et al. Circ Res. 2022; Sano S, et al. Circ Res. 2018

What would be the mechanism?

- CHIP mutations: **enhanced activity of immune cells** with increased secretion of chemokines and cytokines

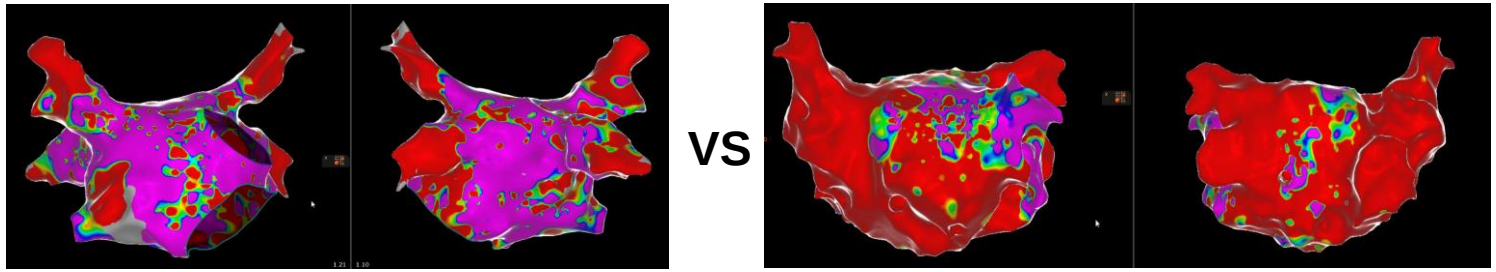


the higher prevalence of CHIP would be possibly due to ***higher inflammatory reactions*** in AF patients.

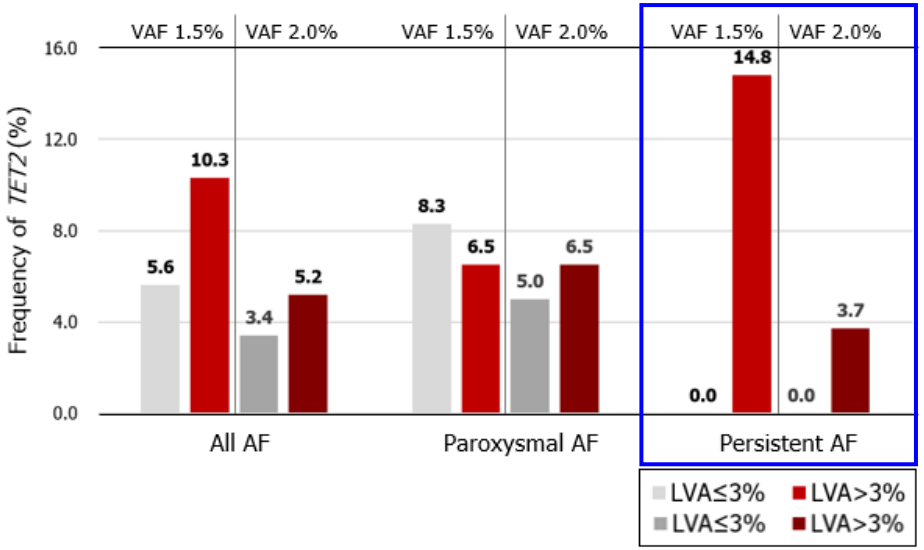
Jaiswal S et al. Science. 2019; Jaiswal S, et al. Nat Rev Cardiol. 2020

Signal in our data

- *Persistent AF, RFCA, LVA : 56 patients*



		LVA≤3%	LVA>3%	p-value
All AF	*TET2* (VAF 1.5%)	5/89 (5.6%)	6/58 (10.3%)	0.29
	TET2 (VAF 2.0%)	3/89 (3.4%)	3/58 (5.2%)	0.59
Paroxysmal AF	*TET2* (VAF 1.5%)	5/60 (8.3%)	2/31 (6.5%)	0.75
	TET2 (VAF 2.0%)	3/60 (5.0%)	2/31 (6.5%)	0.77
Persistent AF	*TET2* (VAF 1.5%)	0/29 (0.0%)	4/27 (14.8%)	0.031
	TET2 (VAF 2.0%)	0/29 (0.0%)	1/27 (3.7%)	0.3



For persistent AF, *TET2* mutation was more frequent in **patients with LVA>3%** than LVA≤3%

Contents

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- Clinical research
- ***(Basic research)***
- Expected clinical implications and future research
- Conclusion

Role of clonal hematopoiesis of indeterminate potential in angiotensin II-induced atrial fibrosis in mice

- **Hypothesis**

  1) CHIP is associated with the development of AF via atrial fibrosis/inflammation
  2) Inflammasome signaling would play a role in CHIP-AF

- **Aim**

  In TET2(-) vs (+) mice,

  1) To compare Ang II-induced atrial fibrosis/inflammtion and AF inducibility
  2) To evaluate whether inflammasome signaling blocking agent suppress Ang II-induced atrial fibrosis and AF inducibility

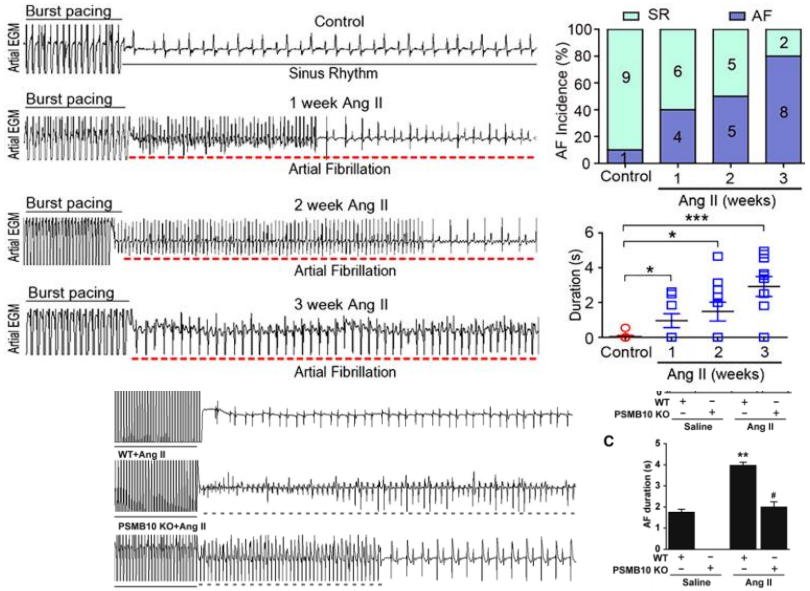
Method – Overall scheme

Production of TET2 KO mice (13 weeks of age), *n*=10 for each group

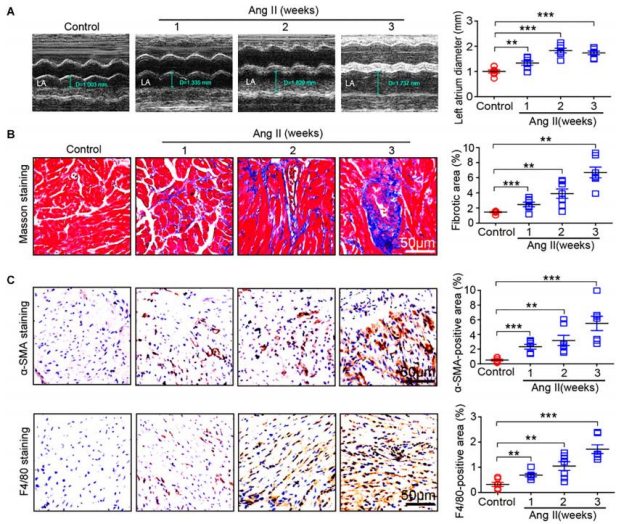
Control (Saline)		Ang II		Ang II + inflammasome signaling blocking agent	
Tet2 -/-	Tet2 +/+	Tet2 -/-	Tet2 +/+	Tet2 -/-	Tet2 +/+

3 wks

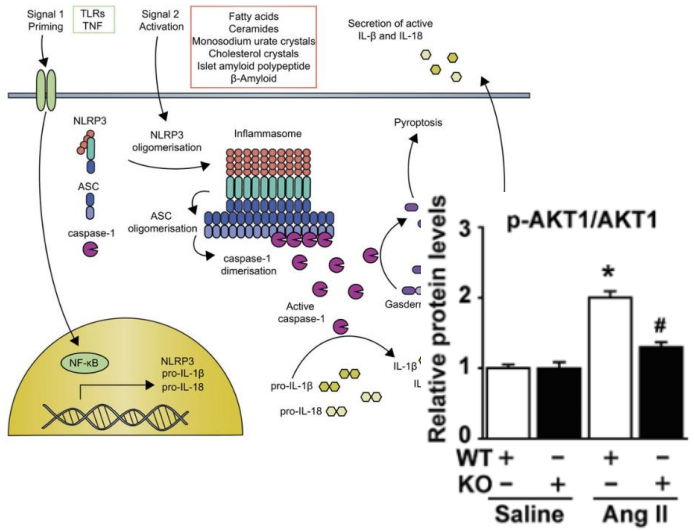
I. Electrophysiological study: Inducibility of AF



II. Analysis of atrial fibrosis



III. Measurements of biomarkers



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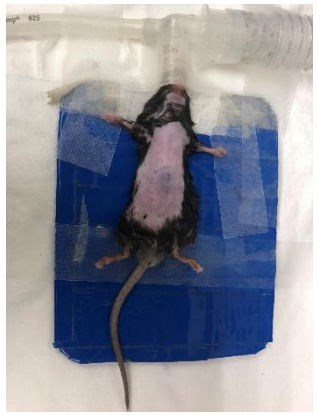
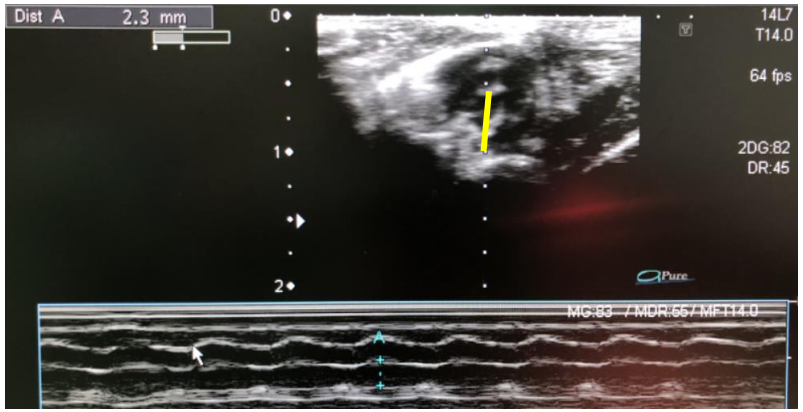
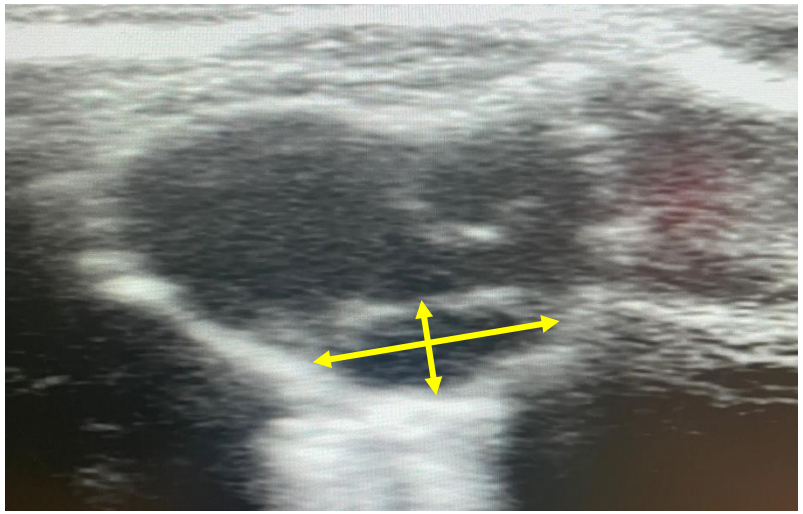
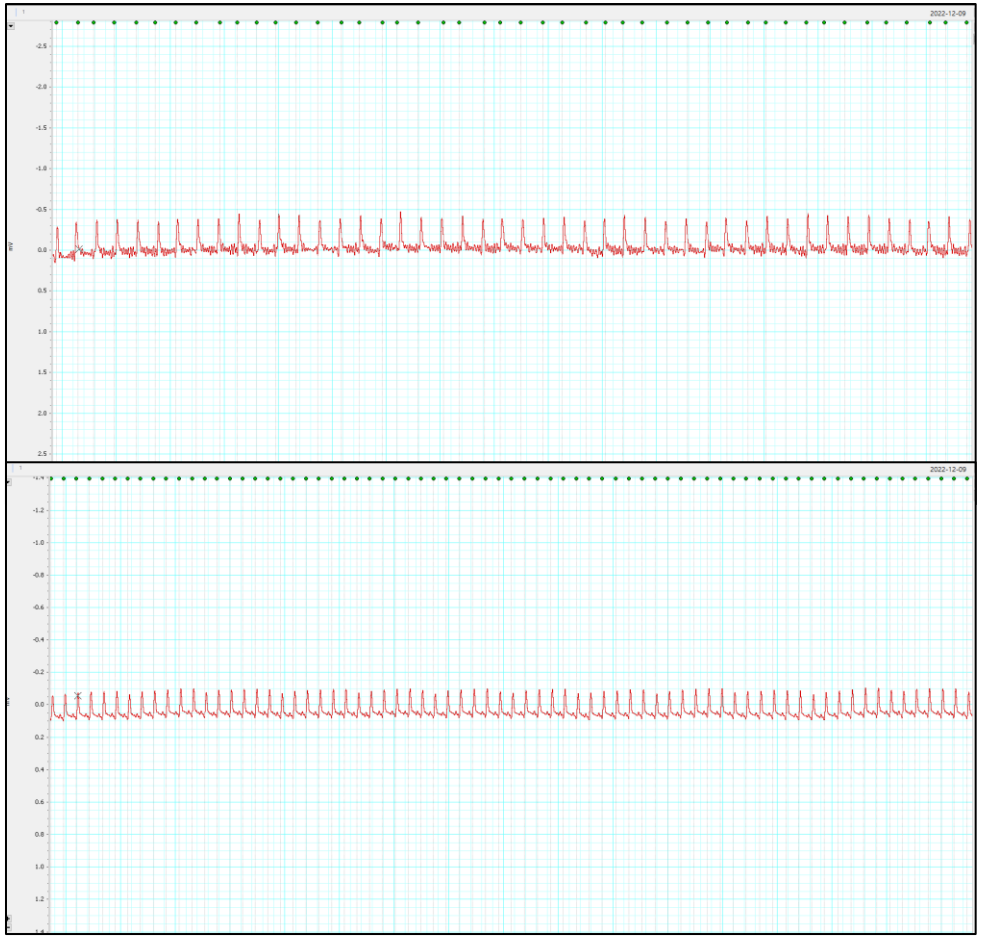
Pilot experiment – Baseline echocardiography and ECG

- Control (n=6) / Ang II (n=6, 1000 ng/kg/min)



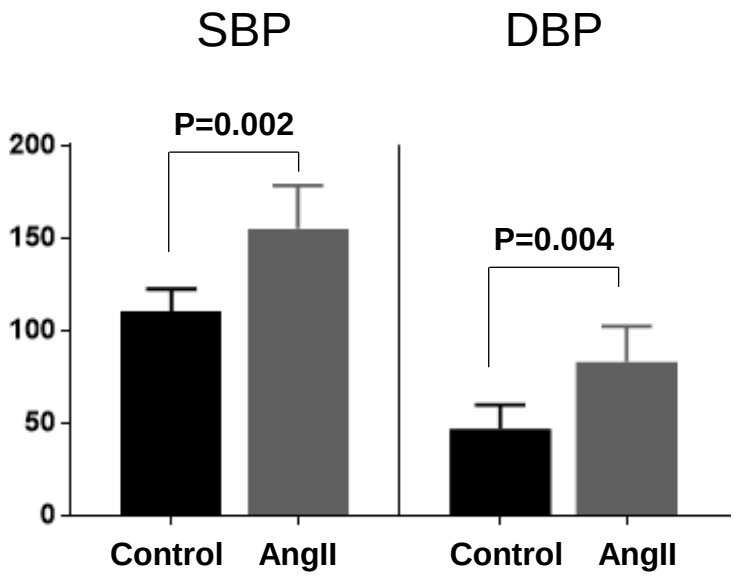


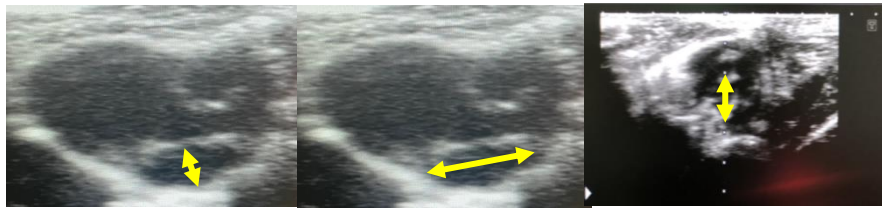


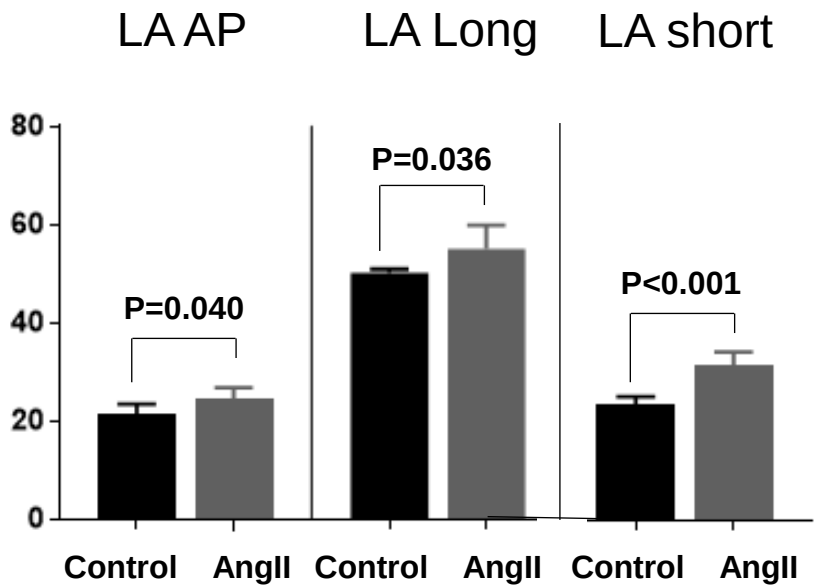


Pilot experiment – 3wks BP and LA size increment

- BP elevation and cardiac hypertrophy after 3wks of Ang II infusion (1000 ng/kg/min)



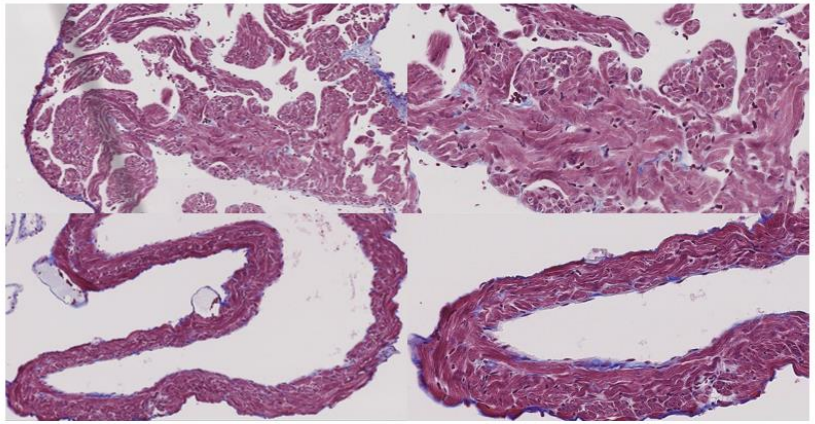




Pilot experiment – Histology of heart after 3wks

Control group

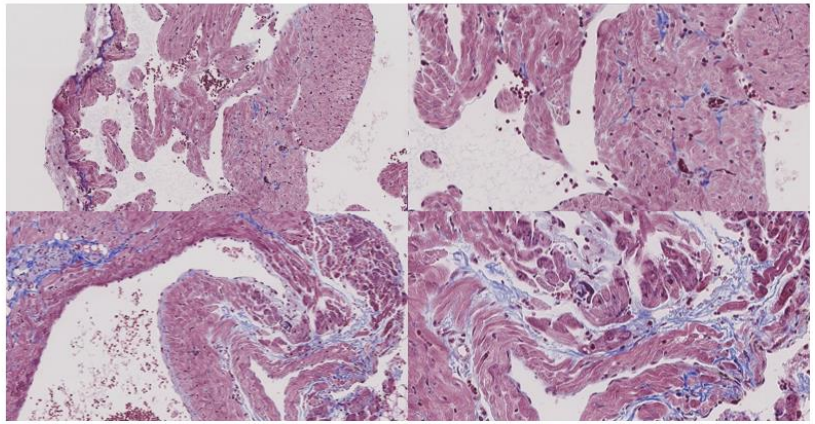
RAA



LAA

x200 x400

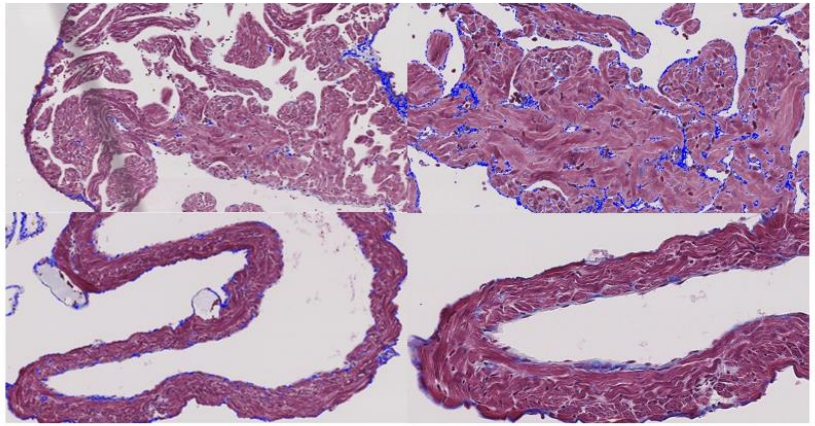
Ang II infusion group



x200 x400

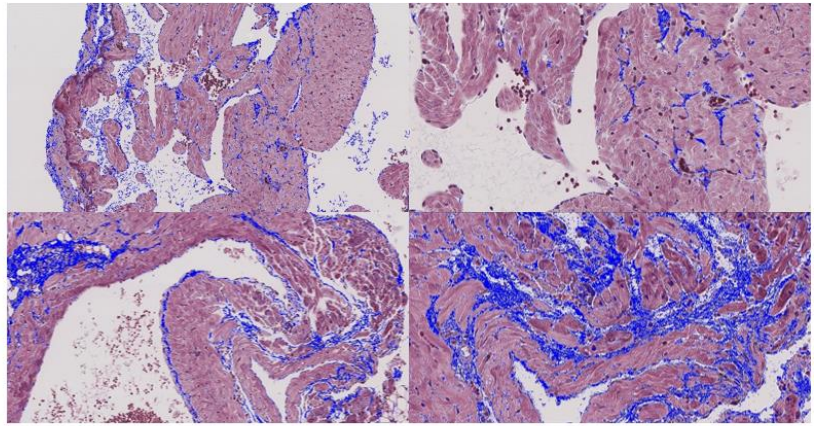
Analysis by ImagePro, ImageJ

RAA

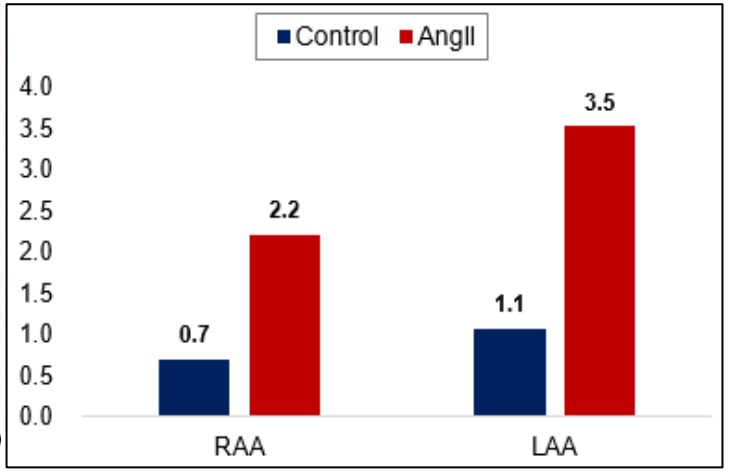


LAA

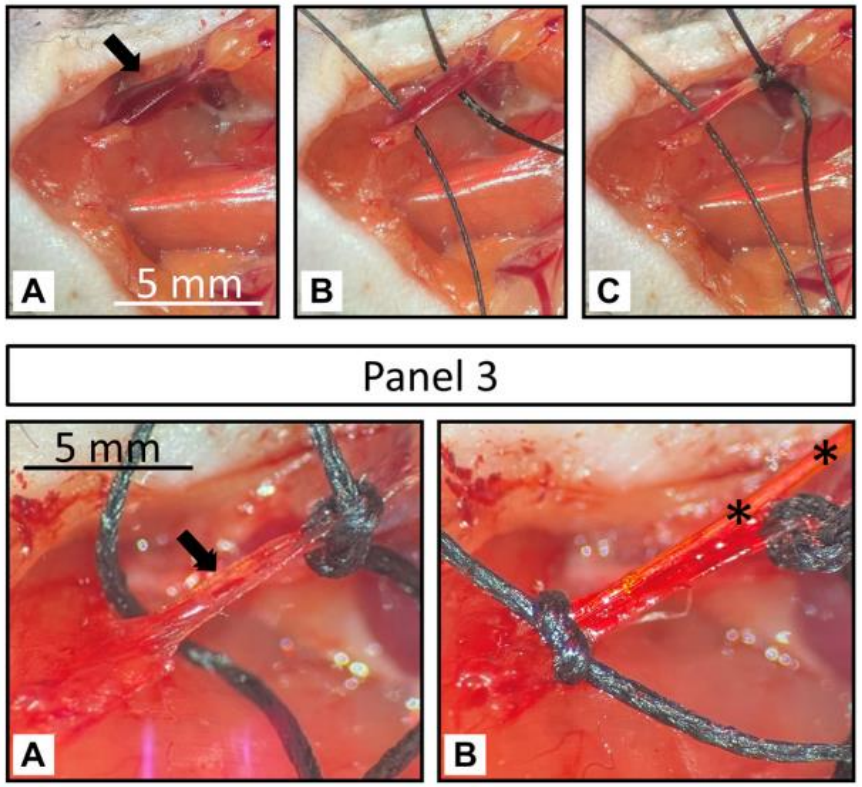
x200 x400

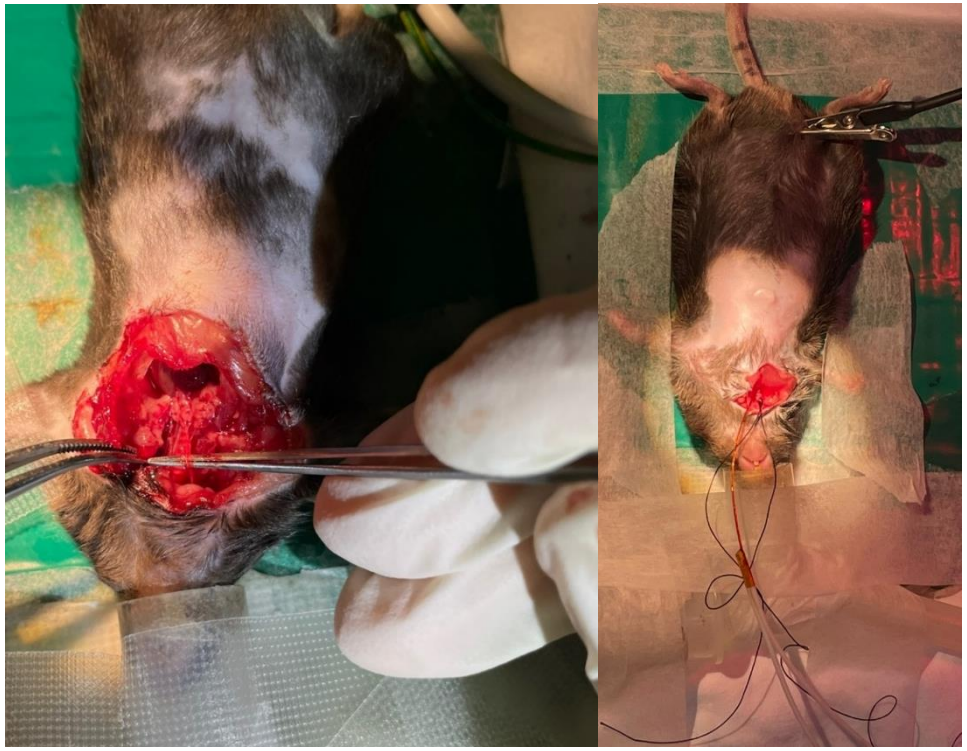


x200 x400

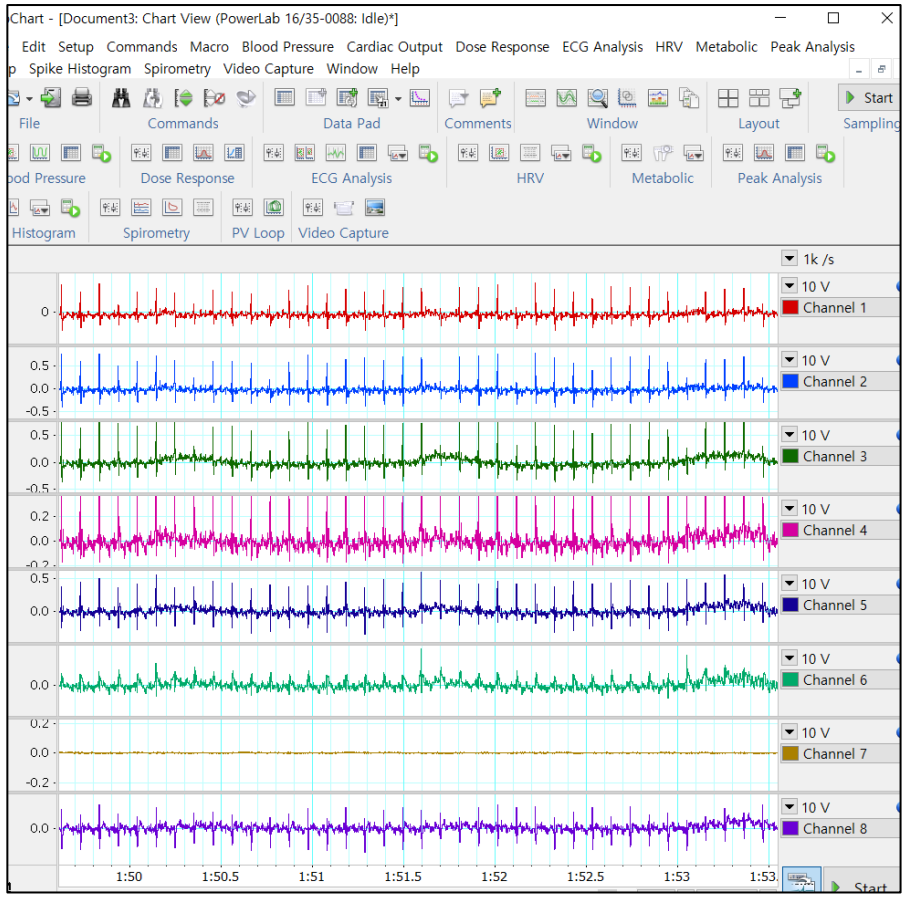


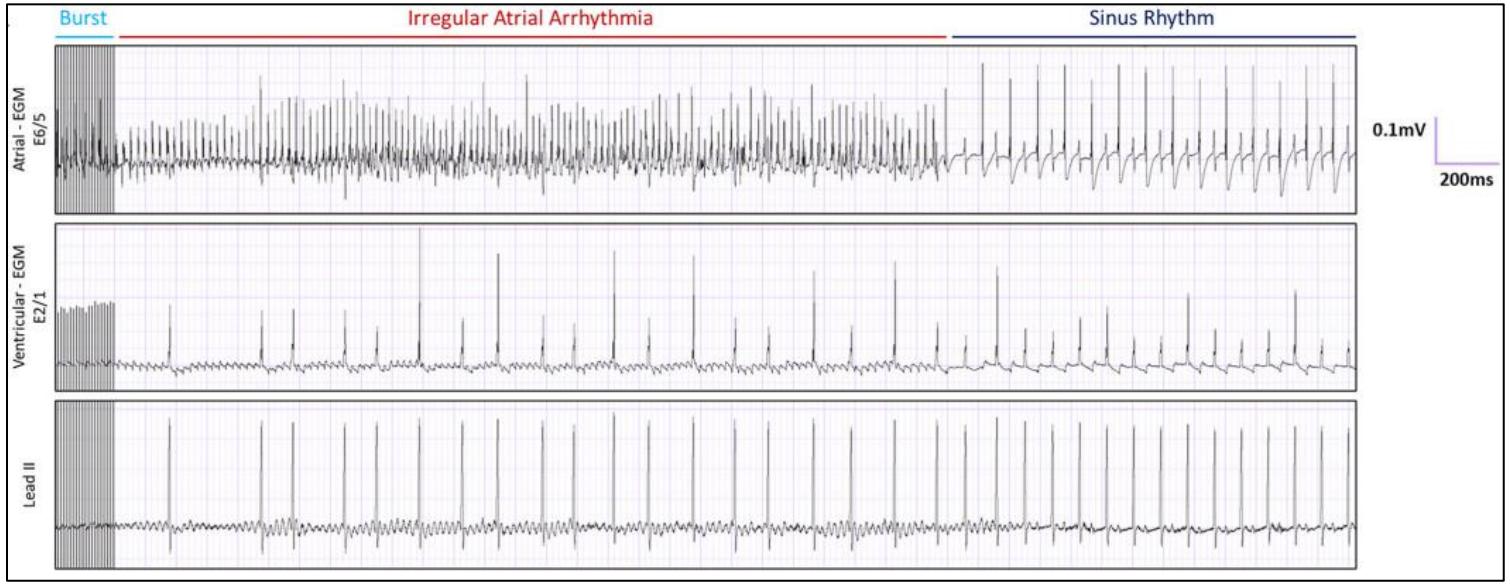
Pilot experiment – AF inducibility





Pilot experiment – AF inducibility





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Favere K, et al. Am J Physiol Heart Circ Physiol. 2022 Oct 1;323(4):H763-H773.
Pilot experiment, @ SNUH Biomedical Research Institute, In 2022/12-2023/6

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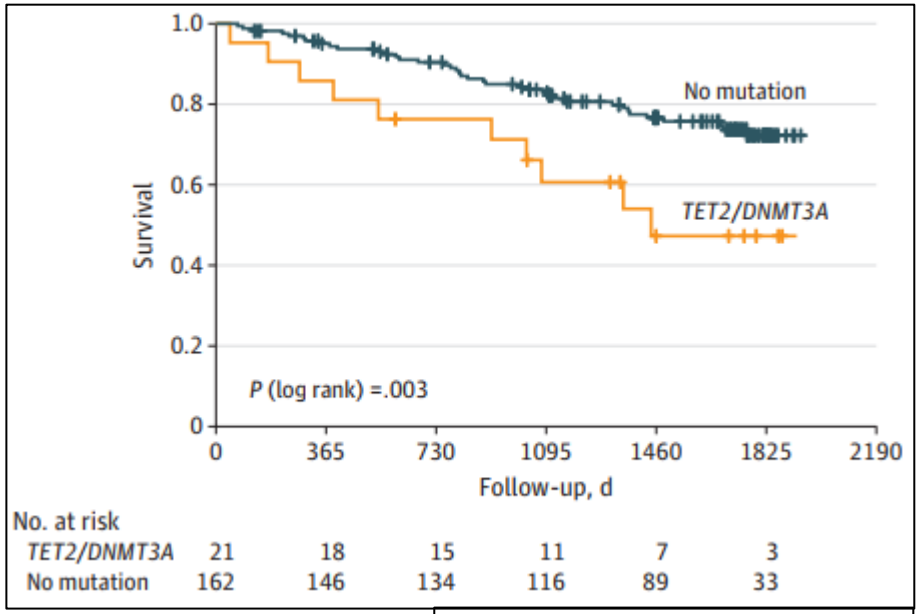
Contents

- Background
- Clinical research
- (Basic research)
- **Expected clinical implications and future research**
- **Conclusion**

Clinical perspective and translational outlook

- Ongoing research:
  - **Basic research: validation in mice model**
  - **Follow-up of 1004 AF cohort:** RFCA recurrence + stroke/heart failure hospitalization/death



Lena D, et al. JAMA cardiology. 2019

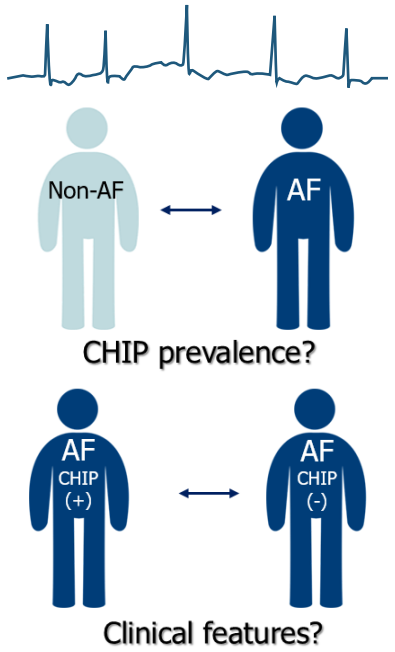
Such as this graph?

- ✓ *AF recurrence/burden after catheter ablation*
- ✓ *Stroke*
- ✓ *Heart failure hospitalization*
- ✓ *Death …*

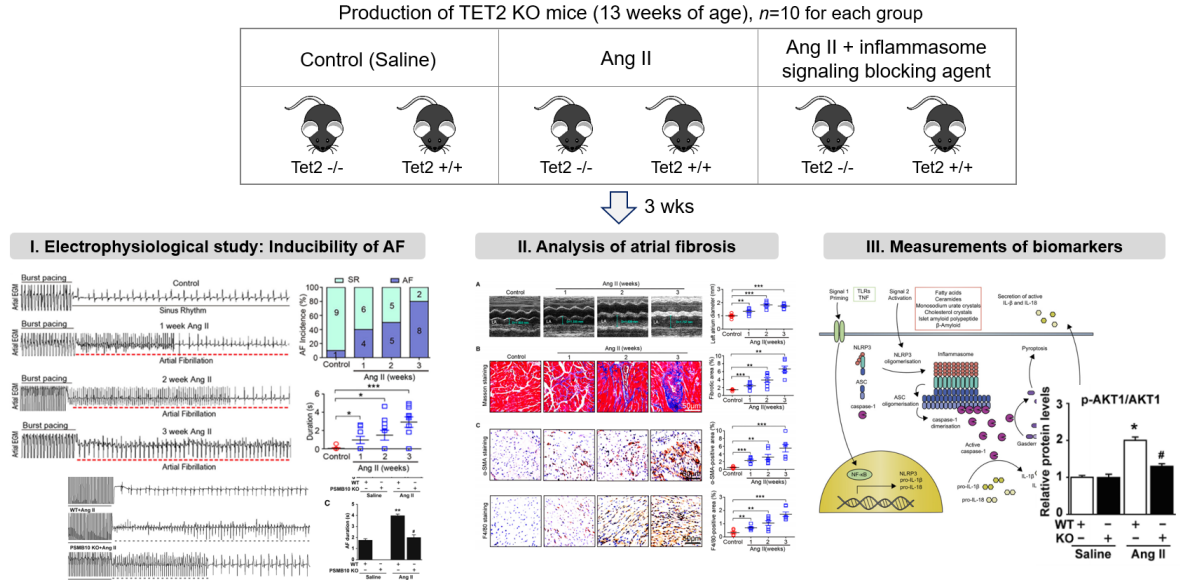
- ✓ *Stratified by*
  Inflammasome blocking agent +/- ?

Clinical perspective and translational outlook

Part I. Clinical research



Part II. Clinical research



Better understanding of AF: Pathophysiology, Clinical differentiation, Prognosis

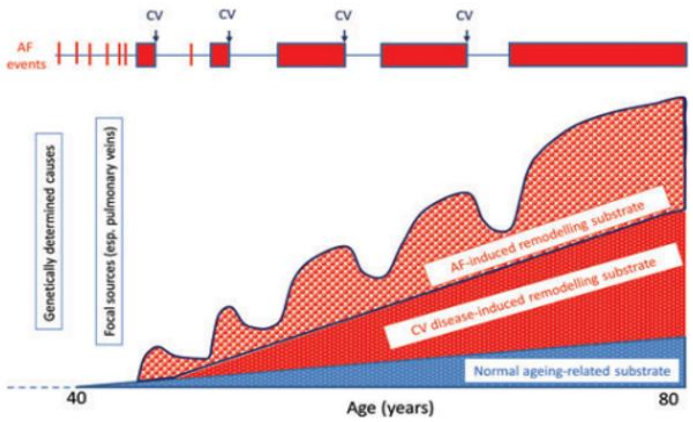
New treatment paradigm of AF

Conclusion

From our clinical research, in AF;

- CHIP mutations are ***1.4-fold*** more frequent
- The m/c somatic mutations are ***DNMT3A***, ***TET2 (1.7-fold)***, and ***ASXL1***
- CHIP(+) are ***older,*** more likely to have ***diabetes***, and have ***a longer AF duration*** and ***greater E/E' value*** than CHIP(-)
- Severe LA enlargement: *TET2* mutation x 1.9 ↑

  → CHIP might be associated with ***development*** and ***progression*** of AF

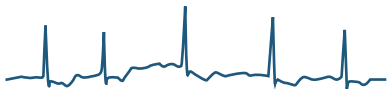


Conclusion

Our future research will…

- Compare angiotensin II–induced LA fibrosis, inflammation, and AF inducibility according to the presence of CHIP in mice
- Provide better understanding of AF from pathophysiology to new treatment strategy

AF, the m/c cardiac arrhythmia causing enormous healthcare burden

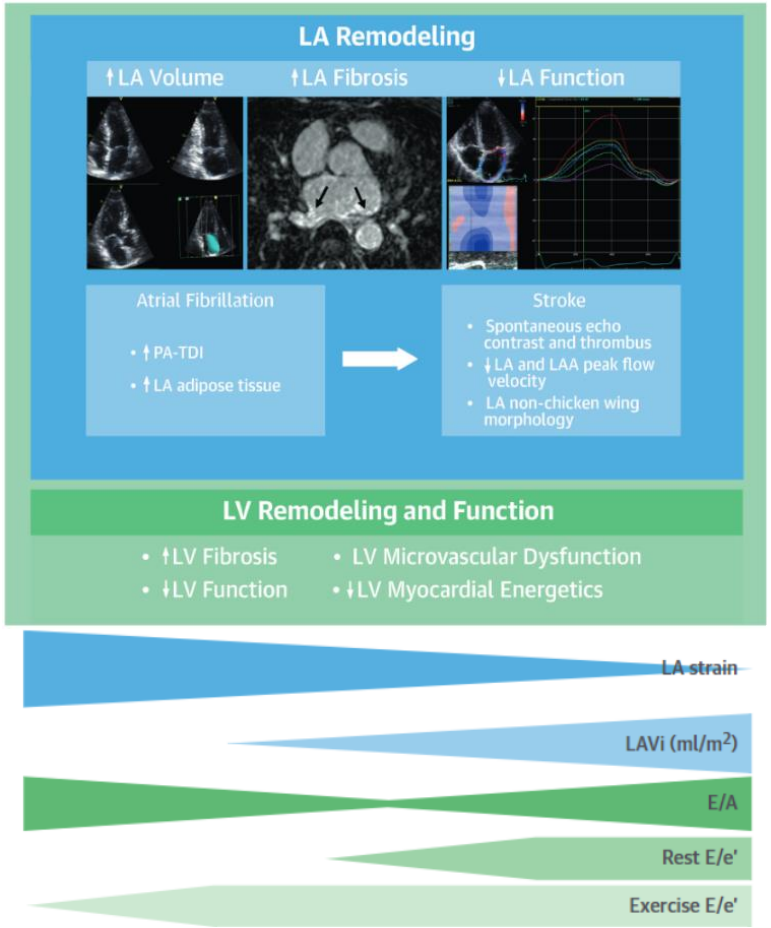


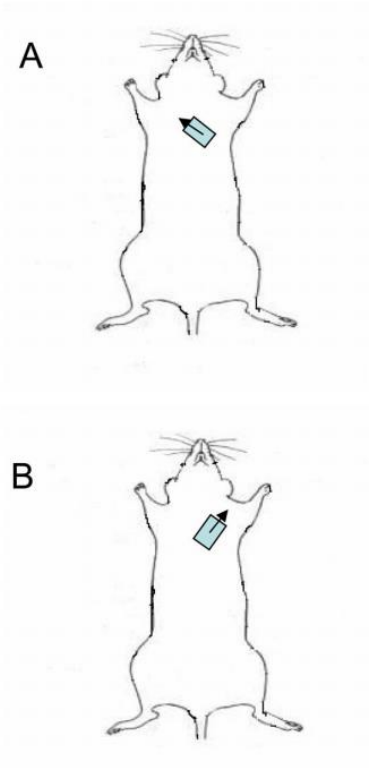
Mechanism of AF development → Clinical phenotype Stratified prognosis → New treatment target

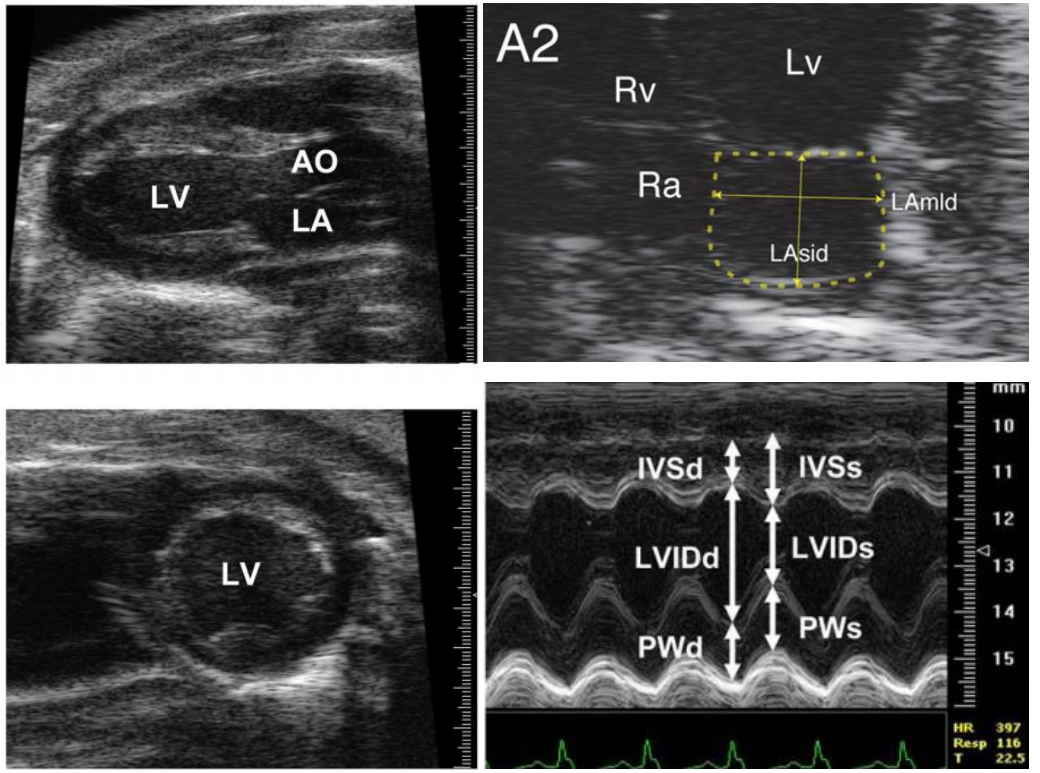
Thank you for your attention

Method – Blood pressure/Transthoracic echocardiography

- LA remodeling: AF development



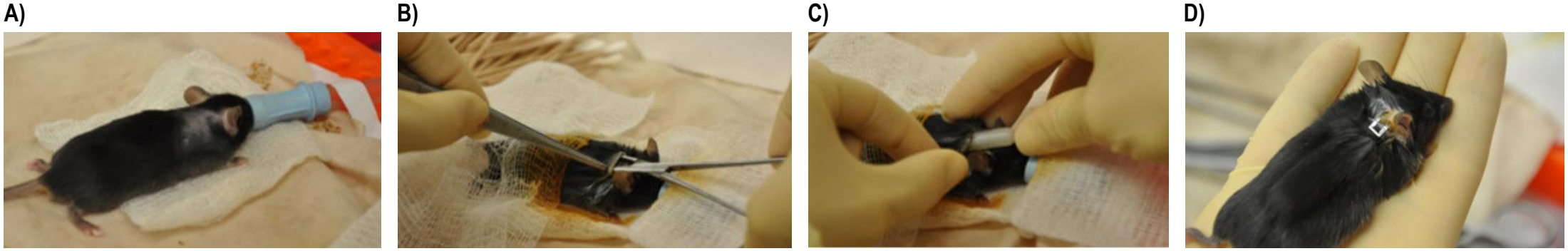




- LA diameter, LV dimension, LVEF, LVFS

Thomas L, et al. J Am Coll Cardiol. 2019; Delgado V, et al. J Am Coll Cardiol. 2017
Gao S, et al. Curr Protoc Mouse Biol. 2011; Colazzo F, et al. PLoS One. 2015

Method – Continuous Ang II infusion (induce atrial fibrosis)



- Via osmotic mini-pump
- Ang II (Sigma-Aldrich, St Louis, MO)
- 1500 ~2000 ng/kg/min, 3 wks

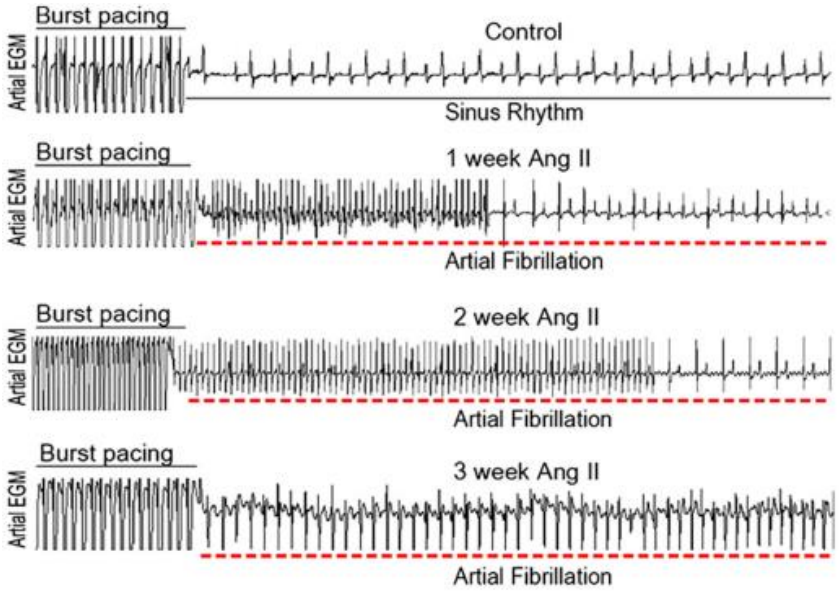
* Optimal Ang II concentration to evaluate the effect of CHIP mutations to be determined.

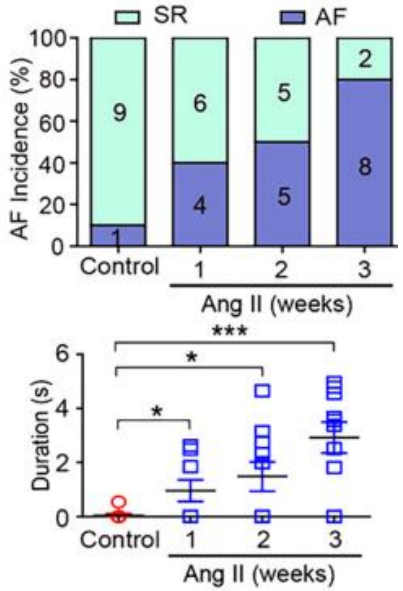
Method – Evaluation of AF inducibility

- Atrial burst pacing by Millar 1.1F octapolar EP catheter via Rt. jugular vein.
- 1-lead body surface ECG, 4 intracardiac bipolar electrograms (GY6328B; HeNan HuaNan Medical Science. & Technology Ltd.)
- 5 second bursts through the catheter electrodes

1) Burst pacing started at a 40 ms cycle length, decreasing by 2 ms in each successive burst to a cycle length of 20 ms
2) Burst pacing was performed for a total of 3 times in each mouse (with a 5-min break)

* ***AF***: period of rapid irregular atrial rhythm for at least 1 second



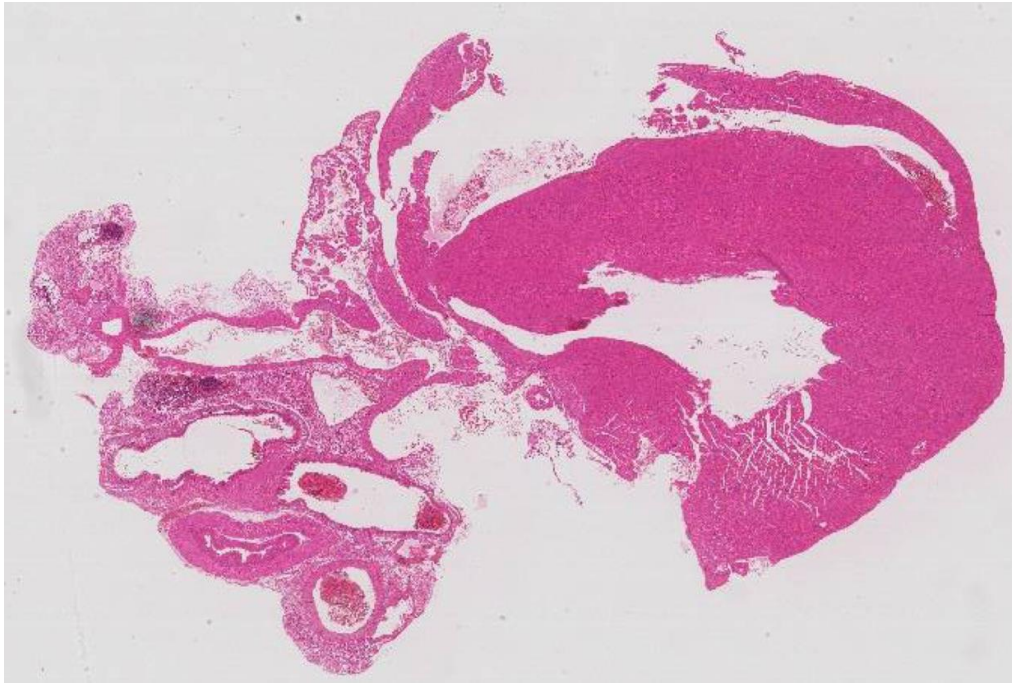


40 → 38 → 36 … → 20 ms

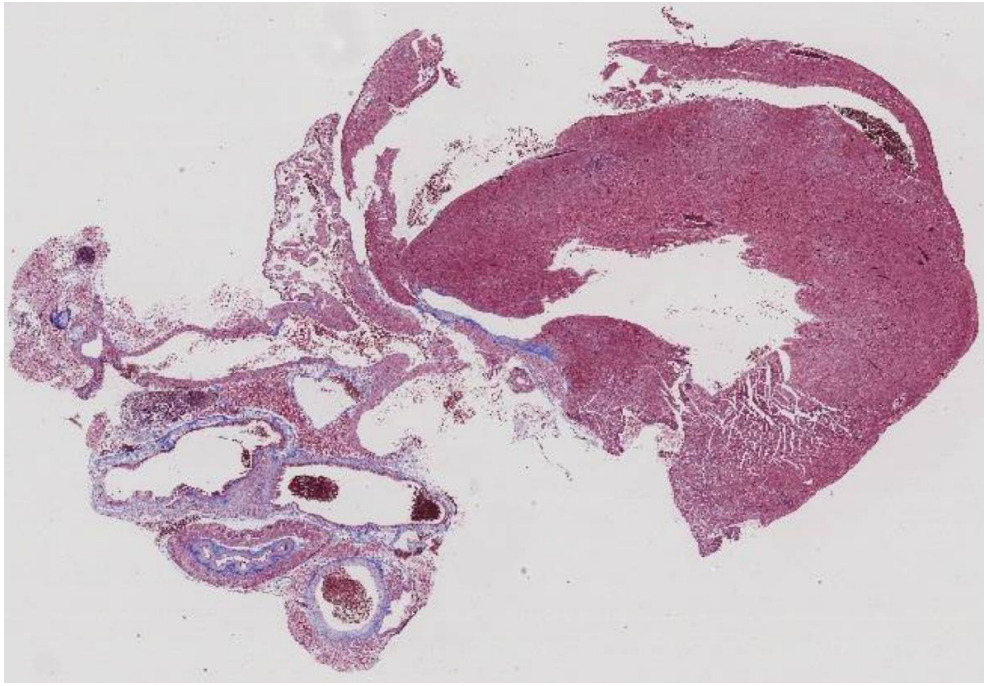
Li J et al, Hypertension. 2019; Li J, et al. Hypertension, 2018; Wu YX, et al. Front Physiol. 2019

Method – Evaluation of atrial fibrosis

- **Day 22**, after AF induction test
- Whole atrial tissue: 4% paraformaldehyde immersion (24 hours) → embed in paraffin → sliced into 5-mm thick sections → H&E, Masson's trichrome, IHC



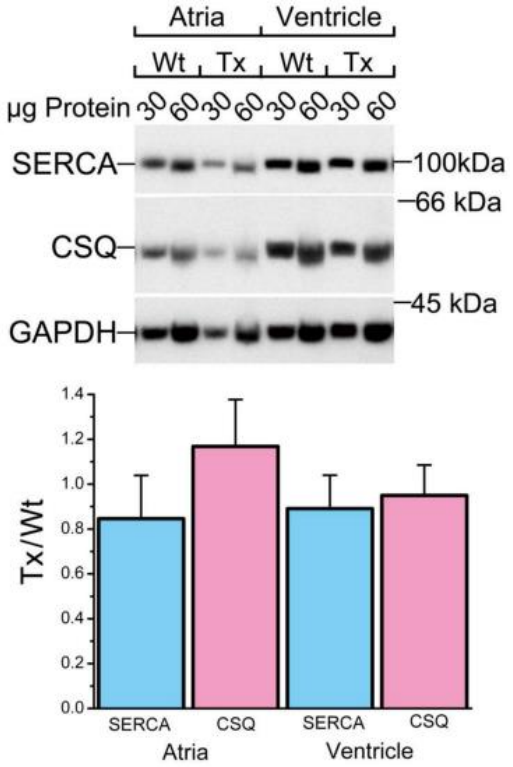
H&E



Masson's trichrome

Method – Analysis of immune biomarker

- **mRNA expression**
  - Profibrotic signals: Collagen 1, collagen 3, a-SMA
  - LA inflammation: IL-1b, IL-6, TNF-a, TGF-a
- **Protein analysis**
  - From frozen atrial tissue
  - Western blot: TNF-α, IL-6, MCP-1,
  - Collagen 1, Collagen 3, α-SMA, TGF-β, Smad2/3



Choi EK, et al. CircJ, 2012; Li J, et al. Hypertension. 2019; Li J, et al. Hypertension, 2018; Wu YX, et al. Front Physiol. 2019

Method – Summary of parameters

Role of clonal hematopoiesis of indeterminate potential in angiotensin II-induced atrial fibrosis in mice

- **Physiological profile**
  - ✓ Body/heart weight
  - ✓ Blood pressure
  - ✓ Surface ECG
  - ✓ Heart function by transthoracic echocardiography, etc.

- **AF inducibility and duration**
  - ✓ In vivo electrophysiology by burst pacing

- **Inflammation**
  - ✓ Histology: H&E staining, IHC (against for macrophage)
  - ✓ mRNA (qRT-PCR): IL-1β, IL-6, TNF-α, MCP-1,
  - ✓ Protein (western blot): TNF-α, IL-6, MCP-1,
- **Fibrosis**
  - ✓ Histology: MT or Picrosirius red staining
  - ✓ mRNA (qRT-PCR): Collagen 1, Collagen 3, α-SMA, TGF-β
  - ✓ Protein (western blot): Collagen 1, Collagen 3, α-SMA, TGF-β, Smad2/3