



Article

# Atrial Fibrillation in Heart Failure Is Associated With High Levels of Circulating microRNA-199a-5p and 22-5p and a Defective Regulation of Intracellular Calcium and Cell-to-Cell Communication

Anna Garcia-Elias <sup>1,2,\*</sup>, Marta Tajés <sup>2,3</sup>, Laia Yañez-Bisbe <sup>1,2</sup>, Cristina Enjuanes <sup>3,4</sup>, Josep Comín-Colet <sup>3,4,5</sup>, Selma A. Serra <sup>6</sup>, José M. Fernández-Fernández <sup>6</sup>, Kathryn W. Aguilar-Agon <sup>7</sup>, Svetlana Reilly <sup>7</sup>, Julio Martí-Almor <sup>2,8,9</sup> and Begoña Benito <sup>1,2,9,10,11,\*</sup>

## SUPPLEMENTARY TABLES

**Table S1.** Characteristics of the first cohort.

	permAF (n = 9)	SR (n = 9)	p-Value
<b><i>Patient Characteristics</i></b>			
Sex, men (%)	5 (55.5)	5 (55.5)	NS
Age, y (SD)	74.9 (8.2)	75.2 (7.0)	NS
BMI, kg/m <sup>2</sup> (SD)	26.8 (2.6)	26.8 (2.8)	NS
<b><i>Medical history</i></b>			
Tobacco history (%)	4 (44.4)	5 (55.5)	NS
Hypertension (%)	7 (77.8)	7 (77.8)	NS
Diabetes (%)	2 (22.2)	6 (66.7)	NS
Hypercholesterolemia (%)	4 (44.4)	7 (77.8)	NS
COPD (%)	2 (22.2)	1 (11.1)	NS
CKD (%)	2 (22.2)	2 (22.2)	NS
Previous stroke (%)	2 (22.2)	2 (22.2)	NS
<b><i>HF parameters</i></b>			
Ischemic etiology (%)	4 (44.4)	4 (44.4)	NS
Heart rate, bpm (SD)	70.2 (13.4)	80.0 (16.3)	NS
NYHA class (%):			NS
- I-II	6	6	
- III-IV	3	3	
LVEF, % (SD)	32.0 (6.5)	28.1 (5.6)	NS
Log Pro-BNP, mean (SD)	3.4 (0.5)	3.2 (0.6)	NS
<b><i>HF treatment</i></b>			
ACEI/ARB (%)	8 (88.9)	8 (88.9)	NS
Betablockers (%)	9 (100)	9 (100)	NS
Diuretics (%)	9 (100)	9 (100)	NS
<b><i>Heart rhythm</i></b>			
Permanent SR	0	9	
History of parox/pers AF*	0	0	
Permanent AF <sup>#</sup>	9	0	

BMI: body mass index; COPD: chronic obstructive pulmonary disease; CKD: chronic kidney disease; NYHA: New York Heart Association; LVEF: left ventricular ejection fraction; ACEI: angiotensin-converting-enzyme inhibitors; ARB: angiotensin-receptor blockers; SR: sinus rhythm; AF: atrial fibrillation. \*Includes episodes of paroxysmal or short-standing persistent AF; #includes long-standing (> 1 year) persistent or permanent AF. NS: non-significant.

**Table S2.** MicroRNAs differentially expressed in the discovery phase.

miRNA	p-Value	miRNA	p-Value	miRNA	p-Value
miR-505#	0.000701	miR-1	0.010444	miR-140	0.024151
miR-20a	0.002003	miR-151-5P	0.010454	miR-671-3p	0.024503
miR-539	0.002037	miR-645	0.011216	miR-664	0.024804
<b>miR-425#</b>	<b>0.002114</b>	miR-7#	0.011294	miR-195	0.025214
<b>miR-106a</b>	<b>0.002469</b>	miR-93#	0.011334	miR-9#	0.025847
miR-30d#	0.002633	miR-130b#	0.012461	miR-124a	0.026115
<b>miR-19a</b>	<b>0.004109</b>	miR-186	0.012493	miR-495	0.026444
<b>miR-16</b>	<b>0.004259</b>	miR-652	0.012506	miR-151-3p	0.026993
miR-628-3p	0.004991	<b>miR-26a</b>	<b>0.012758</b>	<b>miR-106b</b>	<b>0.029477</b>
<b>miR-199a-5p</b>	<b>0.006002</b>	miR-432	0.014040	miR-340	0.030723
<b>miR-301</b>	<b>0.006242</b>	let-7d	0.014543	miR-185	0.033889
<b>miR-374</b>	<b>0.006285</b>	<b>miR-23a</b>	<b>0.014962</b>	miR-411	0.035389
<b>miR-133a</b>	<b>0.006676</b>	miR-636	0.015891	miR-590-3P	0.035525
miR-374-5p	0.007119	<b>miR-20a#</b>	<b>0.016181</b>	miR-98	0.035920
miR-330	0.007449	<b>miR-324-5p</b>	<b>0.016251</b>	miR-30e-3p	0.035936
<b>miR-126#</b>	<b>0.007465</b>	miR-191#	0.016514	miR-1180	0.036382
<b>miR-17</b>	<b>0.007740</b>	miR-140-3p	0.016646	miR-27a	0.037929
miR-142-5p	0.007847	miR-625	0.017292	miR-331-5p	0.040697
miR-1255B	0.008360	miR-483-3p	0.017459	miR-656	0.042341
miR-598	0.008435	miR-744	0.018172	miR-107	0.043088
miR-26a-1#	0.008641	miR-489	0.018316	miR-331	0.043250
<b>miR-27b</b>	<b>0.008667</b>	miR-24	0.019166	miR-326	0.043429
<b>miR-22-5p</b>	<b>0.008810</b>	miR-28	0.019784	miR-146b	0.043554
miR-148b	0.009361	miR-380-3p	0.021854	miR-221	0.046365
miR-191	0.010162	miR-580	0.023425	miR-15b	0.048038
<b>miR-125a-5p</b>	<b>0.010217</b>				

miRNA selected for the replication phase are marked in bold.

**Table S3.** Characteristics of the donors of atrial tissue.

Tissue Collection Site	LAA		RAA	
	No AF	AF	No AF	AF
<b>Total number of patients</b>	6	6	5	5
Mean age, years ( $\pm$ SD)	60 $\pm$ 14	66 $\pm$ 12	64 $\pm$ 10	68 $\pm$ 12
Men, n (%)	4 (67)	5 (83)	4 (80)	5 (100)
<b>Surgical procedure, n (%)</b>				
CABG $\pm$ AVR/MVR	2 (33)	1 (17)	2 (40)	1 (20)
AVR/MVR	4 (67)	5 (83)	3 (60)	4 (80)
<b>Medical history, n (%)</b>				
Smoker/ex-smoker	4 (67)	2 (33)	3 (60)	1 (20)
Hypertension	3 (50)	3 (50)	2 (40)	3 (60)
Diabetes mellitus	2 (33)	0 (0)	0 (0)	0 (0)
Previous MI	1 (17)	2 (33)	0 (0)	0 (0)
COPD/asthma	0 (0)	0 (0)	0 (0)	0 (0)
<b>Medications, n (%)</b>				
Anticoagulants	1 (17)	4 (67)	0 (0)	3 (60)
$\beta$ -Blockers	2 (33)	5 (83)	3 (60)	5 (100)
Statins	2 (33)	3 (50)	1 (20)	3 (60)
Calcium-channel blockers	0 (0)	0 (0)	0 (0)	1 (20)
ACEIs and ARBs	1 (17)	4 (67)	1 (20)	3 (60)
Diuretics	0 (0)	2 (33)	2 (40)	2 (40)

LAA, left atrial appendage; RAA, right atrial appendage; noAF, sinus rhythm; AF, atrial fibrillation; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; AVR, aortic valve replacement; CABG, coronary artery bypass surgery; COPD, chronic obstructive pulmonary disease; MI, myocardial infarction; MVR, mitral valve replacement. Percentage in parentheses (%) indicates percentage within the same group.

**Table S4.** MiRNA primer sequences for atrial tissue.

No AF	AF
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Assay Name	Assay ID	Target Sequence 5'-3'
hsa-miR-22-5p	477987_mir	AGUUCUUCAGUGGCAAGCUUUA
hsa-miR-199a-5p	478231_mir	CCCAGUGUUCAGACUACCUGUUC
hsa-miR-191-5p	477952_mir	CAACGGAAUCCCAAAAGCAGCUG
hsa-miR-26a-5p	477995_mir	UUCAAGUAAUCCAGGAUAGGCU

## SUPPLEMENTARY FIGURES

FIG.S1

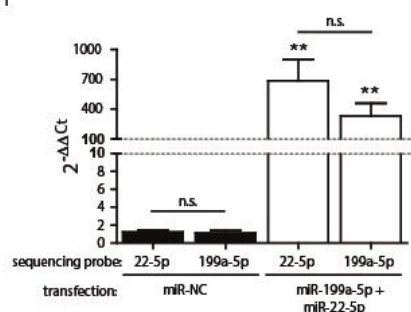


FIG.S2

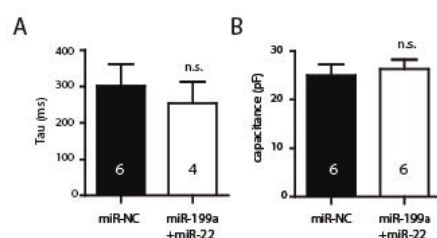
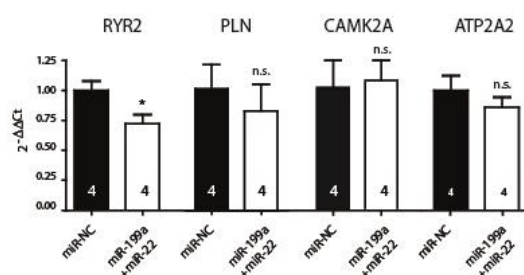


FIG.S3



**Figure S1.** RNA expression levels of miR-22-5p and miR-199a-5p in HL1 cells transfected with miR-199a+miR-22 or miR-NC. Specific probes for each miRNA were used. Data are expressed as the fold change ( $2^{-\Delta\Delta C_t}$ ) relative to the negative control, miR-NC. **Figure S2.** A. Fast inactivation kinetics (Tau) of  $I_{CaL}$  currents and, B. cell capacitance of HL-1 cells transfected with miR-199a+miR-22 (white) or miR-NC (black). Numbers within the columns represent number of patched cells. **Figure S3.** RYR2, PLN, CAMK2A and ATP2A2 mRNA expression levels in miR-199a+miR-22 HL-1 cells, normalized to miR-NC. n.s.: non-significant, \*  $p < 0.05$ , \*\*  $p < 0.005$ .