



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

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SUPPLEMENTARY TABLES

Table S1. Characteristics of the first cohort.

	permAF (n = 9)	SR (n = 9)	p-Value
<i>Patient Characteristics</i>			
Sex, men (%)	5 (55.5)	5 (55.5)	NS
Age, y (SD)	74.9 (8.2)	75.2 (7.0)	NS
BMI, kg/m ² (SD)	26.8 (2.6)	26.8 (2.8)	NS
<i>Medical history</i>			
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
<i>HF parameters</i>			
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
<i>HF treatment</i>			
ACEI/ARB (%)	8 (88.9)	8 (88.9)	NS
Betablockers (%)	9 (100)	9 (100)	NS
Diuretics (%)	9 (100)	9 (100)	NS
<i>Heart rhythm</i>			
Permanent SR	0	9	<0.001
History of parox/pers AF*	0	0	
Permanent AF#	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
miR-425#	0.002114	miR-7#	0.011294	miR-195	0.025214
miR-106a	0.002469	miR-93#	0.011334	miR-9#	0.025847
miR-30d#	0.002633	miR-130b#	0.012461	miR-124a	0.026115
miR-19a	0.004109	miR-186	0.012493	miR-495	0.026444
miR-16	0.004259	miR-652	0.012506	miR-151-3p	0.026993
miR-628-3p	0.004991	miR-26a	0.012758	miR-106b	0.029477
miR-199a-5p	0.006002	miR-432	0.014040	miR-340	0.030723
miR-301	0.006242	let-7d	0.014543	miR-185	0.033889
miR-374	0.006285	miR-23a	0.014962	miR-411	0.035389
miR-133a	0.006676	miR-636	0.015891	miR-590-3P	0.035525
miR-374-5p	0.007119	miR-20a#	0.016181	miR-98	0.035920
miR-330	0.007449	miR-324-5p	0.016251	miR-30e-3p	0.035936
miR-126#	0.007465	miR-191#	0.016514	miR-1180	0.036382
miR-17	0.007740	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
miR-27b	0.008667	miR-24	0.019166	miR-326	0.043429
miR-22-5p	0.008810	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
miR-125a-5p	0.010217				

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
Total number of patients	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)
Surgical procedure, n (%)				
CABG \pm AVR/MVR	2 (33)	1 (17)	2 (40)	1 (20)
AVR/MVR	4 (67)	5 (83)	3 (60)	4 (80)
Medical history, n (%)				
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)
Medications, n (%)				
Anticoagulants	1 (17)	4 (67)	0 (0)	3 (60)
β -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	CAACGGAAUCCCAAAGCAGCUG
hsa-miR-26a-5p	477995_mir	UUCAAGUAAUCCAGGAUAGGCU

SUPPLEMENTARY FIGURES

FIG.S1

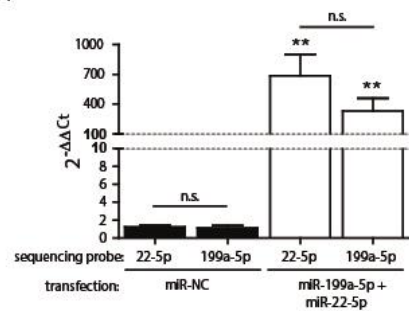


FIG.S2

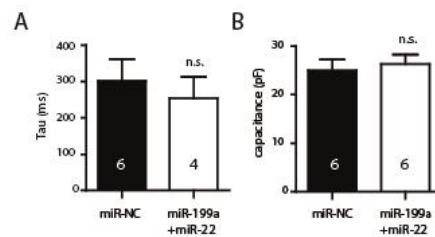


FIG.S3

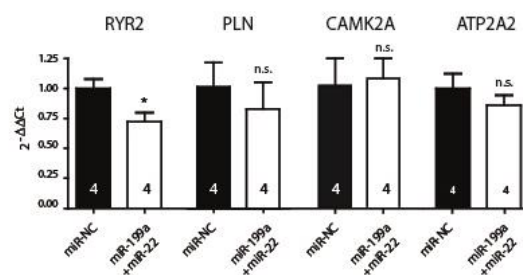


Figure S1. RNA expression levels of miR-22-5p and miR-199a-5p in HL1 cells transfected with miR-199a+miR-22 or miR-NC. Specific probes for each miRNA were used. Data are expressed as the fold change ($2^{-\Delta\Delta Ct}$) relative to the negative control, miR-NC. **Figure S2.** A. Fast inactivation 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, * $p < 0.05$, ** $p < 0.005$.