

Age-related regulation and region-specific distribution of ion channel subunits promoting atrial fibrillation in human left and right atria

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Aims

Age-induced changes and electrical remodelling are important components of the atrial fibrillation (AF) substrate. To study regional distribution and age-dependent changes in gene expression that may promote AF in human atria.

Methods and results

Human left atrial (LA) and right atrial (RA) tissue samples were obtained from donor hearts unsuitable for transplantation and from patients undergoing mitral valve repair. Atrial fibrillation was mimicked *in vitro* by tachypacing of human atrial tissue slices. Ionic currents were studied by the whole-cell patch-clamp technique; gene expression was analysed by real-time qPCR and immunoblotting. Both healthy RA and RA from older patients showed greater CACNA1c mRNA and CaV1.2 protein expression than LA. No age-dependent changes of Kir2.1 expression in both atria were seen. Remodelling occurred in a qualitatively similar manner in RA and LA. I_{K1} and Kir2.1 protein expression increased with AF. miR-1, miR-26a, and miR-26b were down-regulated with AF in both atria. $I_{Ca,L}$ was decreased. CACNA1c and CACNA2b expression decreased and miR-328 increased in RA and LA during AF. *Ex vivo* tachypacing of human atrial slices replicated these findings. There were age-dependent increases in miR-1 and miR-328, while miR-26a decreased with age in atrial tissues from healthy human donor hearts.

Conclusion

Features of electrical remodelling in man occur in a qualitatively similar manner in both human atria. Age-related miR-328 dysregulation and reduced $I_{Ca,L}$ may contribute to increased AF susceptibility with age.

Keywords

Ageing • Atrial fibrillation • Ion channel • miRNA • mRNA • Protein • Remodelling

Introduction

Atrial fibrillation (AF) represents a major cardiovascular healthcare challenge. Understanding cellular remodelling processes is important to generate further, effective approaches to treat the arrhythmia. Involved factors may represent novel therapeutic targets.^{1,2}

Reduced L-type calcium ($I_{Ca,L}$) and increased inward-rectifier currents (I_{K1}) are mechanistically involved in action potential shortening and alterations of intracellular calcium-homeostasis associated with

AF.³ Attenuation of Ca^{2+} current and augmentation of K^+ currents have been linked to aging in a canine model.⁴ MicroRNAs regulate gene expression levels of the main pore-forming subunits and are involved in AF pathogenesis.⁵ Among others, microRNA-1 regulate expression of the I_{K1} subunit Kir2.1 and microRNA-328 is involved in the regulation of the calcium channel subunit CaV1.2.^{6,7} The microRNA-26 family consists of three distinct genes miR-26a-1, miR-26a-2, and miR-26b that encode mature microRNAs with identical seed sequences and the same target mRNAs. MicroRNA-26 has

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What's new?

- First comprehensive study illustrating that previously known changes in right or left atria indeed occur similarly in both atria of a single patient.
- First demonstration of reduced $I_{Ca,L}$ and respective microRNA in elderly patients with atrial fibrillation.

been shown to be dysregulated in AF and regulate the expression of Kir2.1.⁵ MicroRNAs also regulate age-dependent processes.⁸ Down-regulation of $I_{Ca,L}$ has been reported in ageing canines.⁹ As AF has an age-dependent demography in man, it is of interest to fill respective knowledge gaps.

Regionally different ion channel expression may underlie specific electrophysiological properties of cardiac chambers.¹⁰ Alterations in spatial distribution may additionally contribute to the AF substrate. Only few studies have looked into regional differences in human ion channel expression.^{11,12} No previous study systematically compared changes in matched left atrial (LA) and right atrial (RA) tissues from one patient.

Therefore, the present work sought to analyse region- and age-dependent components in gene expression remodelling associated to AF.

Methods

Patient population

Pairs of LA and RA tissue were obtained from healthy donor hearts and from patients undergoing surgical mitral valve repair (MVR). The study complies with the Declaration of Helsinki and local ethics committees at Goethe-Universität, Frankfurt, Germany (Nr.54/05) and University of Szeged, Hungary (Nr. 63/97) approved the study.

Donor hearts were obtained from six female and nine male organ donors with a mean age of 50.8 ± 9.6 years ranging from 31 to 68, whose non-diseased hearts were explanted to obtain pulmonary and aortic valves for transplant surgery as no matching recipient was available for whole hearts. Before cardiac explantation, organ donors did not receive medication apart from dobutamine, furosemide, and plasma expanders. After explantation, each heart was perfused with cardioplegic solution and kept cold ($4-6^\circ\text{C}$) for 2–4 h prior to dissection.

Surgical patients were older than 18 years and had an indication for MVR. All patients provided written information consent to participate. Exclusion criteria were paroxysmal AF, haemodynamic instability, active endocarditis, systemic inflammation, or treatment with immunosuppressive agents. In patients undergoing MVR LA tissue was collected from the site of incision at the roof to anterior LA. RA tissue was from the site of insertion of the heart lung machine at the right atrial appendage (RAA) to RA roof. Donor heart tissue samples were from comparable regions in the anterior LA and RA roof.

Patients were classified as having persistent/permanent AF if a previous diagnosis of AF was noted in medical records and AF was present on the admission electrocardiogram, whereas sinus rhythm (SR) patients had no mention of AF and were in SR on admission. Patients in SR with a previous diagnosis of AF were classified as having paroxysmal AF. Additional details on surgical patients are provided in *Table 1*.

Table 1 Characteristics of surgical patients

	SR	AF	P-value
Patients	8	14	
Male	5 (62%)	9 (64%)	P = NS
Mean age (years)	55 ± 11	70 ± 9	P < 0.05
CAD	1 (12%)	2 (14%)	P = NS
Additional bypass surgery	0 (0%)	0 (0%)	P = NS
Degree of MVD	3.2 ± 0.4	3.2 ± 0.7	P = NS
Hypertension	1 (12%)	3 (21%)	P = NS
Diabetes	1 (12%)	1 (7%)	P = NS
Hyperlipidaemia	3 (37%)	4 (28%)	P = NS
LVEF (%)	60 ± 7	53 ± 14	P = NS
LVEDD (mm)	59 ± 9	58 ± 8	P = NS
LA (mm)	49 ± 13	57 ± 5	P < 0.05
Digitalis	1 (12%)	3 (21%)	P = NS
ACE-I/ATRB	5 (62%)	8 (57%)	P = NS
β -blockers	5 (62%)	9 (64%)	P = NS
Calcium channel blockers	1 (12%)	1 (7%)	P = NS
Amiodarone	0 (0%)	1 (7%)	P = NS
Statins	4 (50%)	5 (36%)	P = NS

ACE-I, angiotensin-converting enzyme inhibitor; AF, atrial fibrillation; ATRB, angiotensin receptor blocker; CAD, coronary artery disease; LA, left atrium; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; MVD, mitral valve disease; NS, not significant; SR, sinus rhythm.

Electrophysiological and molecular biological methods

Details of these methods are provided in the [Supplementary material online](#).

Statistical analysis

Molecular data were analysed with GraphPad Prism 5 using the Student's *t*-tests when comparing two conditions, or an analysis of variance with Bonferroni or Newman–Keuls correction for multiple comparisons. A *P*-value < 0.05 was considered significant and tests were performed two-sided. Data were tested for outliers using the Grubbs outlier test and, if outliers were present, they were removed. Clampfit (Molecular Devices, Ismaning, Germany) and GraphPad Prism (GraphPad Software, San Diego, CA, USA) were used for patch-clamp data analysis. Data are presented as mean \pm SEM.

Results

Regional expression of ion channels, related mRNA, and microRNA—healthy hearts

Regional distribution was studied in pairs of LA and RA tissue obtained from each donor heart. miR-1 and miR-26a,b expressions were not different in LA and RA samples (*Figure 1A*). Respective mRNA and protein levels of Kir2.1 and 2.3 were similar in both atria (*Figure 1B* and *C*). Despite greater CACNA1c mRNA and CaV1.2 protein levels in RA tissue compared to LA, the relative expression of miR-328 was not significantly different in the two regions,

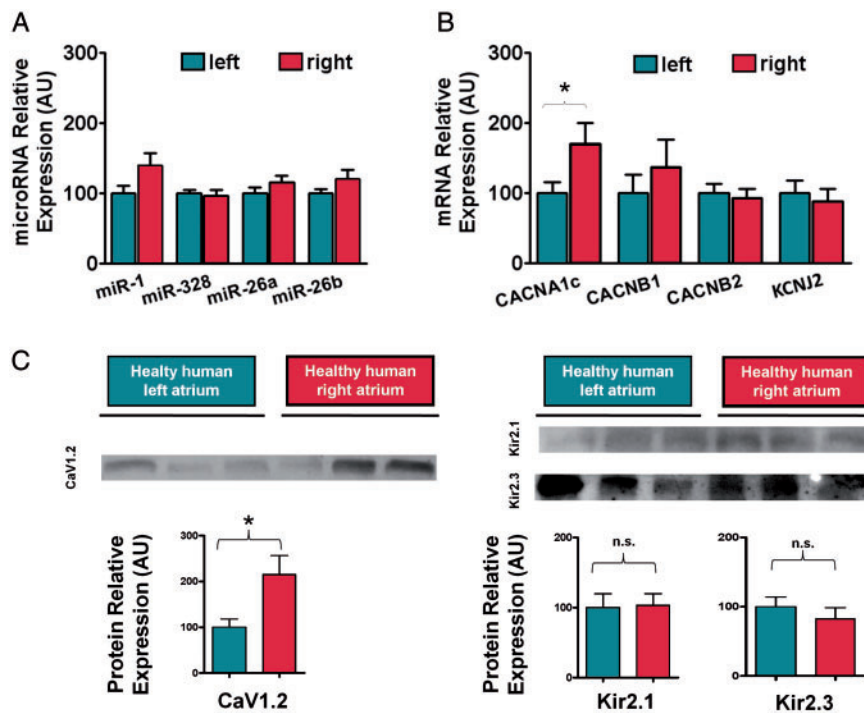


Figure 1 Regional expression of ion channels, related mRNA and microRNA—healthy hearts. (A) MicroRNA expression levels are measured by quantitative real-time PCR in healthy human LA and RA atrial tissue samples ($n = 15$ each) from donor hearts. (B) mRNA expression levels from the same atrial tissue samples are illustrated. (C) Protein quantification from these samples is illustrated. Representative western blots and semiquantitative mean \pm SEM data are shown for calcium (CACNA1c) and I_{K1} channel subunits (Kir2.1 and Kir2.3). Membrane protein fractions were used, actin served as a loading control (not shown). Data are presented as mean \pm SEM and RA levels were normalized to LA levels. β -actin and U6 served as internal standards for mRNA and microRNA measurements, respectively. Antibodies were applied at dilution of 1:1000. * $P < 0.05$. AU, arbitrary units; LA, left atrial; n.s., not significant; RA, right atrial; PCR, polymerase chain reaction; SEM, standard error of the mean.

suggesting that miR-328 may not be the only factor determining basal levels of CaV1.2 expression [$P =$ not significant (NS); Figure 1A].

The mRNA encoding for the pore-forming α -subunit of I_{CaL} channels (CACNA1c) was ~ 1.7 -fold more abundant in RA than in LA ($P < 0.05$). mRNA expression for the β -1 and β -2 Ca^{2+} channel regulatory subunits did not differ (Figure 1B). I_{K1} channel subunit mRNA KCNJ2, encoding Kir2.1 was also similar between atria (Figure 1B). In accordance with mRNA levels, immunoblot studies showed ~ 2.1 -fold higher protein amount for CaV1.2 in RA as compared to LA ($P < 0.05$, Figure 1C). Relative expression of I_{K1} -subunits Kir2.1 and Kir2.3 was not different in the two regions (Figure 1C).

Ion channel proteins, mRNA, and microRNA in mitral valve repair atria

Next, we studied ion channel distribution in paired LA and RA samples from patients in SR undergoing MVR.

Here, CACNA1c mRNA was also more abundantly expressed in RA compared with LA samples (~ 1.9 -fold greater expression level in RA vs. LA; $P < 0.05$). CACNB1, CACNB2, and KCNJ2 mRNA expression was not different (Figure 2B). In line with these findings, protein expression of CaV1.2 was ~ 2.4 -fold greater in RA compared with LA ($P < 0.05$), while I_{K1} subunits Kir2.1 and Kir2.3 were evenly expressed (Figure 2C). The expression levels for miR-26a,b and miR-

328 in SR (Figure 2A) were similar, but miR-1 ($P < 0.05$) was less abundantly expressed in RA samples (Figure 2A).

Right-left differences in mitral valve repair samples from patients with atrial fibrillation

Regional distribution of RNA transcripts and protein expression were compared between RA and LA in paired samples obtained from MVR patients with AF.

In tissue from patients with AF, expression levels of miR-26a, miR-26b, and miR-328 were not different between RA and LA, but expression level of miR-1 was decreased in RA vs. LA ($P < 0.05$, Figure 3A). CACNA1c mRNA levels were similar between RA and LA ($N = 14/14$, $P =$ NS), and CACNB1, CACNB2, and KCNJ2 mRNA levels were comparable (Figure 3B). In contrast to mRNA levels, there was a regional gradient of ~ 1.6 -fold greater protein expression in RA compared with LA for CaV1.2—present also in SR— ~ 2.9 -fold greater Kir2.1 protein expression ($P < 0.05$, Figure 3C) in AF samples.

Atrial fibrillation-induced remodelling in right and left atria

Compared with SR levels, CACNA1c mRNA was reduced by AF in both regions ($P < 0.05$ and $P =$ NS, respectively). Relative mRNA

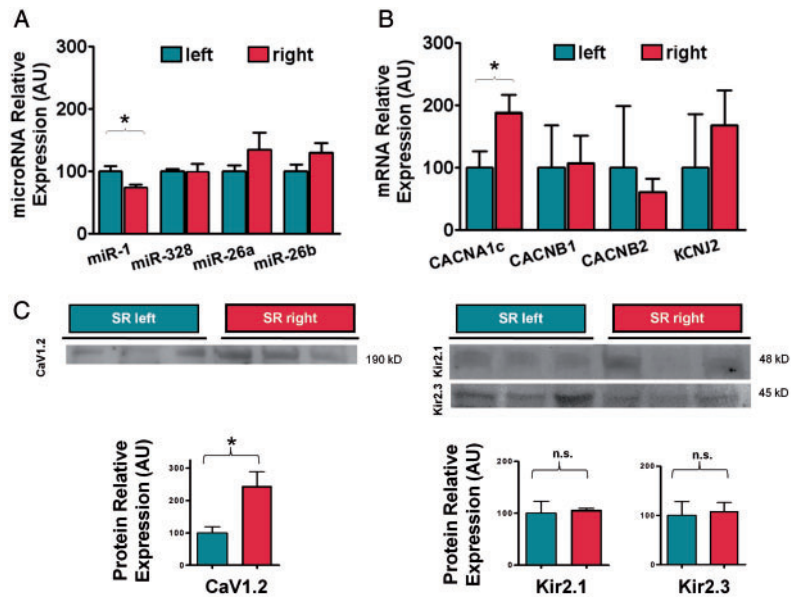


Figure 2 Ion channel proteins, mRNA, and microRNA in MVR hearts. (A) MicroRNA expression levels in SR human LA and RA tissue samples ($n = 8$ each) from patients undergoing MVR are illustrated. (B) mRNA expression levels in respective atrial tissue samples ($n = 8$ each). (C) Protein quantification in identical tissue samples is illustrated. Representative western blots and semiquantitative mean \pm SEM data are shown for calcium (CACNA1c) and I_{K1} channel subunits (Kir2.1 and Kir2.3). Membrane protein fractions were used, actin served as a loading control (not shown). Data are presented as mean \pm SEM and RA levels were normalized to LA levels. Antibodies were applied at dilution of 1:1000. * $P < 0.05$. AU, arbitrary units; LA, left atrial; MVR, mitral valve repair; n.s., not significant; RA, right atrial; SEM, standard error of the mean; SR, sinus rhythm.

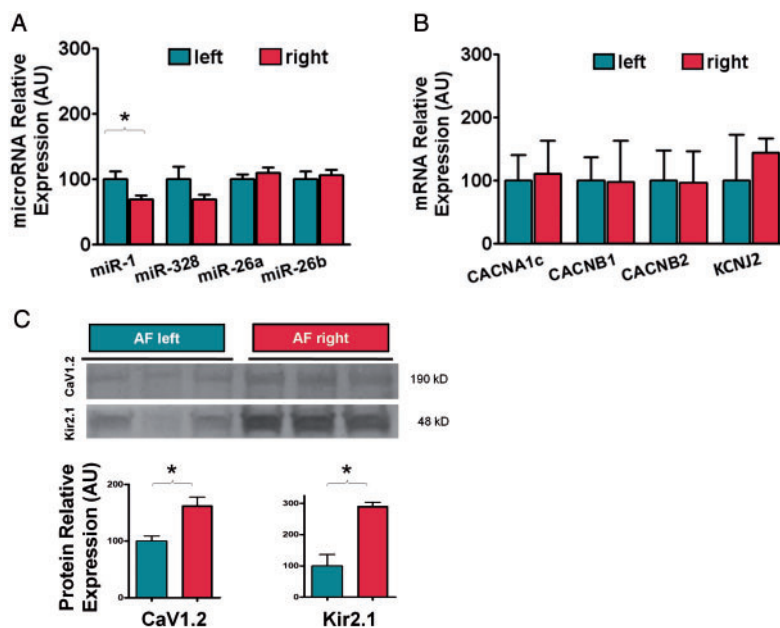


Figure 3 Right-left differences in MVR samples from patients with AF. (A) MicroRNA expression levels in AF human LA and RA tissue samples ($n = 14$ each) from patients undergoing MVR are illustrated. (B) mRNA expression levels in respective atrial tissue samples ($n = 14$ each). (C) Protein quantification in identical tissue samples is illustrated. Representative western blots and semiquantitative mean \pm SEM data are shown for calcium (CACNA1c) and I_{K1} channel subunits (Kir2.1 and Kir2.3). Membrane protein fractions were used, actin served as a loading control (not shown). Data are presented as mean \pm SEM and RA levels were normalized to LA levels. Antibodies were applied at dilution of 1:1000. * $P < 0.05$. AF, atrial fibrillation; AU, arbitrary units; LA, left atrial; MVR, mitral valve repair; RA, right atrial; SEM, standard error of the mean.

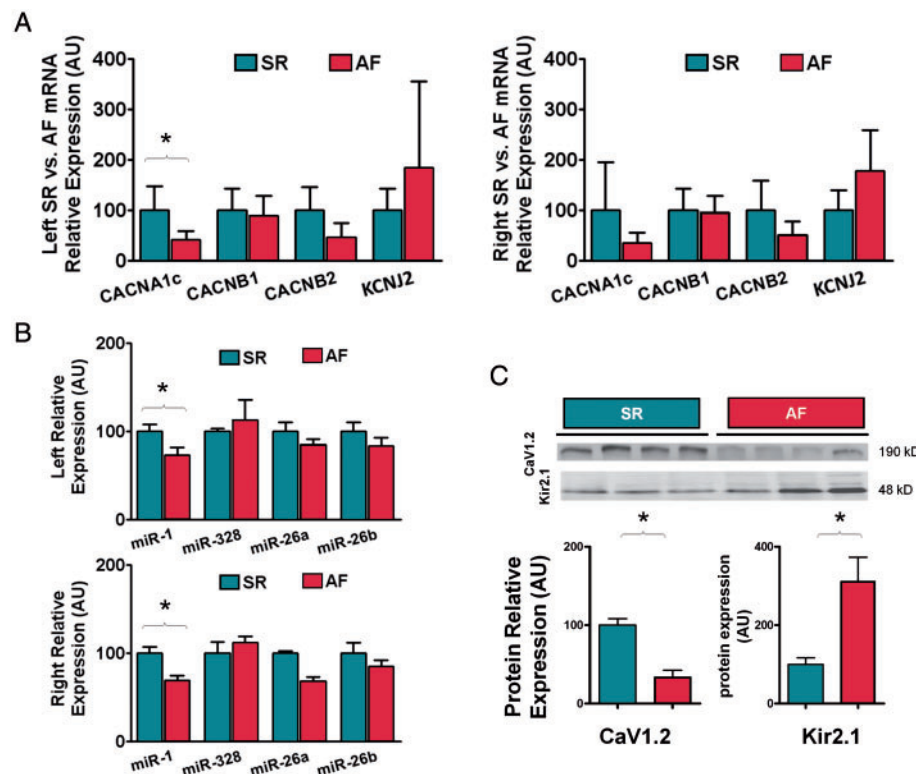


Figure 4 AF-induced remodeling in right and left atria. This figure illustrates differences in RNA and protein expression between SR and AF atria from patients undergoing MVR. (A) mRNA ion channel subunit expression levels in the RA from AF ($n = 14$) and SR ($n = 8$) patients illustrate a decrease in CACNA1c expression and a tendency towards an increase in Kir2.1 mRNA expression. The right-sided panel is an analogous representation of mRNA expression changes occurring in the LA from the same AF and SR patients as in panel A. A similar signature of remodeling can be appreciated. (B) Changes in microRNA expression in RA (top) and LA (bottom) tissue samples are illustrated. (C) Representative western blots and mean \pm SEM data for L-type calcium and inward-rectifier channel subunits (CACNA1c, Kir 2.1) expression in AF relative to SR patients ($n = 4$ each). Representative western blots and semiquantitative mean \pm SEM data are shown for calcium (CACNA1c) and I_{K1} channel subunits (Kir2.1 and Kir2.3). Membrane protein fractions were used, actin served as a loading control (not shown). Data are presented as mean \pm SEM and RA levels were normalized to LA levels. Antibodies were applied at dilution of 1:1000. * $P < 0.05$. AF, atrial fibrillation; AU, arbitrary units; LA, left atrial; MVR, mitral valve repair; RA, right atrial; SEM, standard error of the mean; SR, sinus rhythm.

expression of KCNJ2 remained largely unchanged ($P = \text{NS}$; Figure 4A). While mRNA levels in LA and RA showed a numerical trend to less CACNA1c and more KCNJ2 mRNA ($P = \text{NS}$), immunoblots showed significantly lower amounts for CaV1.2 protein and greater Kir2.1 protein in AF samples ($P < 0.05$ for both, Figure 4C).

MicroRNA expression was consistently altered in AF. In relation to levels in SR miR-1 expression was reduced ($P < 0.05$, Figure 4B). miR-26a,b expression also nominally decreased in both atria without statistical significance. miR-328 expression showed a non-significant trend to increase with AF both in the LA and RA regions ($P = \text{NS}$, Figure 4B). These trends are consistent with the suggested role for these microRNAs in regulating CaV1.2 and Kir2.1 expression in AF and AF-induced changes were qualitatively similar in LA and RA.

Atrial fibrillation-induced ionic current alterations were studied by whole-cell current recordings in cardiomyocytes isolated from LA tissue (SR and AF). In analogy to changes in protein expression of Kir2.1, I_{K1} density recorded at -100 mV increased in AF cells compared with SR ($P < 0.05$, Figure 5A). Similarly, as suggested by reduced CaV1.2 protein expression, I_{CaL} density in AF ($P < 0.05$, Figure 5B).

Ex vivo tachypacing in human left atrial cardiomyocytes

We cultured atrial tissue slices obtained from SR patients and electrically stimulated them *ex vivo* (0.6 Hz vs. 4 Hz). Under these conditions, 0.6 Hz ought to mimic SR and 4 Hz simulated AF. Tachypacing led to a non-significant tendency in reduced miR-1 levels by $\sim 26\%$ and mildly increased miR-328 by $\sim 25\%$ ($N = 7/7$; $P = \text{NS}$; Figure 6A). These changes qualitatively replicated tendencies of changes *in vivo* although they only indicate a trend.

Age-dependent changes in ion channel expression

For the purpose of this analysis, samples of patients were dichotomized into two groups at the median age (51 years).

MicroRNA-328 expression was $\sim 35\%$ greater in the 'old' (mean age 59 ± 2 years) compared to the 'young' (mean age 44 ± 3 years) group ($N = 8/7$, $P < 0.05$, Figure 7A), coinciding with a numerical trend towards decreased mRNA levels of CACNA1c, CACNB1; and

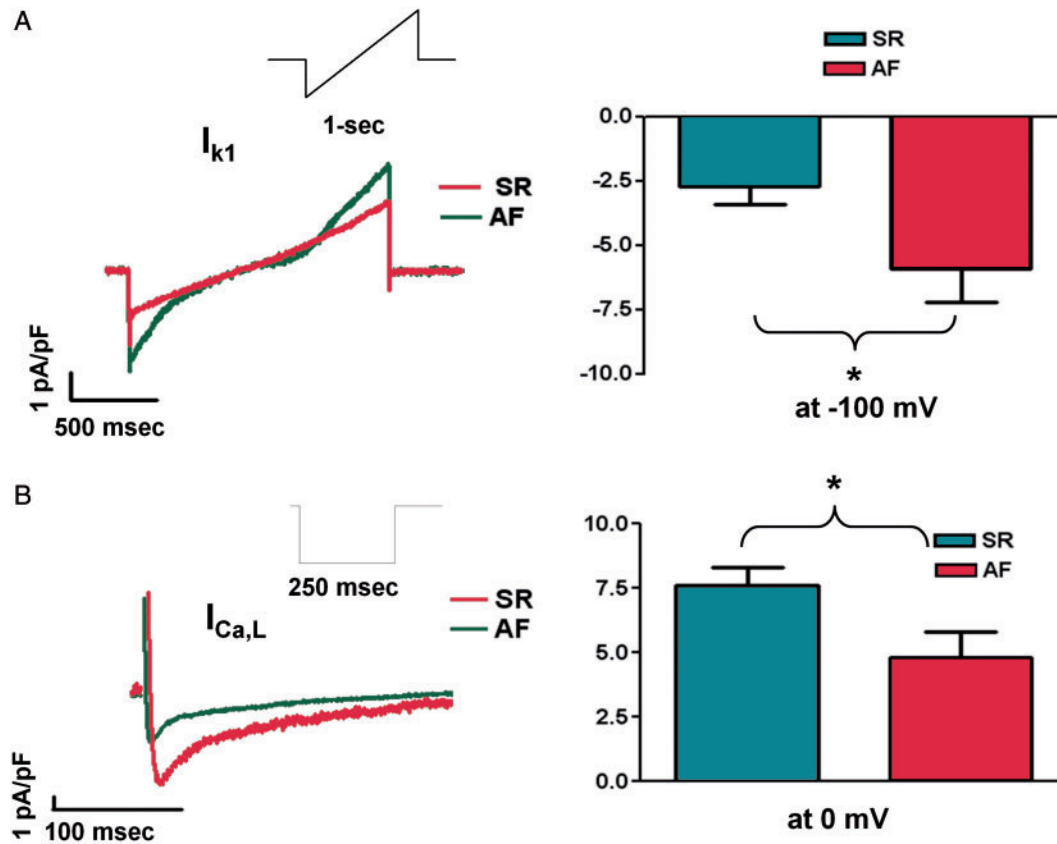


Figure 5 Ionic current changes in AF. Representative recordings and mean \pm SEM current densities from LA cardiomyocytes of patients in SR and AF. (A) I_{K1} was recorded with a ramp protocol (inset) in the presence of tertiapin-Q (100 nM) to avoid contamination by $I_{K_{ACh}}$. I_{K1} density was increased in LA cells in AF compared to SR (at -100 mV: -5.9 ± 1.3 vs. -2.7 ± 0.7 , $n = 5$ cells each, $*P < 0.05$). (B) $I_{Ca,L}$ was recorded with a step protocol (inset). $I_{Ca,L}$ density was decreased in AF compared to SR (at 0 mV: -7.6 ± 0.7 vs. -4.8 ± 1.0 , $n = 5$ and 3 cells, respectively, $*P < 0.05$). AF, atrial fibrillation; AU, arbitrary units; LA, left atrial; MVR, mitral valve repair; RA, right atrial; SEM, standard error of the mean; SR, sinus rhythm.

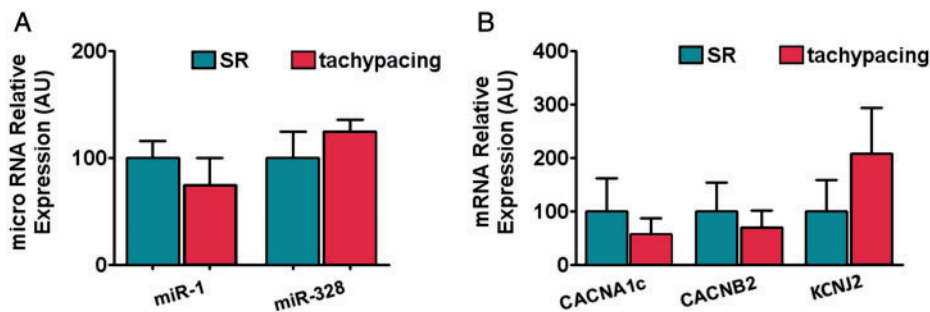


Figure 6 Ex vivo tachypacing induced remodelling in human LA cardiomyocytes. Quantitative real-time PCR results from 4 Hz-paced control (pacing, $n = 7$) and 1 Hz-paced control (SR, $n = 7$) human atrial tissues slices mimics results obtained comparing SR and AF tissue samples. Data are presented as mean \pm SEM, relative to 'SR'. (A) Changes in microRNA expression are illustrated. miR-1 was significantly reduced and miR-328 increased in tachypaced tissue samples. (B) Changes in calcium and inward-rectifier channel subunits demonstrate a tendency towards changes observed *in vivo*. Representative western blots and semiquantitative mean \pm SEM data are shown for calcium (CACNA1c) and I_{K1} channel subunits (Kir2.1 and Kir2.3). Membrane protein fractions were used, actin served as a loading control (not shown). Data are presented as mean \pm SEM and RA levels were normalized to LA levels. β -actin and U6 served as internal standards for mRNA and microRNA measurements, respectively. Antibodies were applied at dilution of 1:1000. $*P < 0.05$. AF, atrial fibrillation; AU, arbitrary units; LA, left atrial; PCR, polymerase chain reaction; SEM, standard error of the mean.

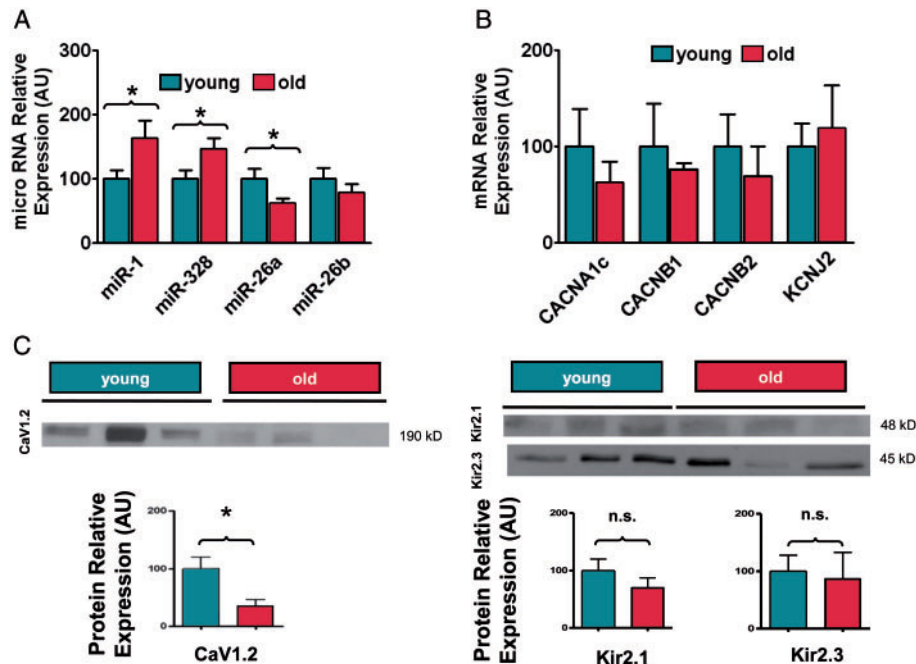


Figure 7 Age-dependent changes in ion channel expression. This figure illustrates changes in relation to age in healthy donor heart tissue. Quantitative real-time PCR results from healthy young ($n = 8$) and old ($n = 7$) human atrial tissue samples are represented. (A) MicroRNA expression is illustrated. (B) Ion channel coding mRNA expression is illustrated. Patient age was dichotomized at the mean and the 'young' group had an average age of 44 ± 3 years vs. 59 ± 2 years for the 'old' group, $P < 0.05$. (C) Protein expression levels of $n = 6$ each for CACNA1c, $n = 6$ each for Kir2.1, and $n = 3$ each for Kir2.3. Representative western blots and semiquantitative mean \pm SEM data are shown for calcium (CACNA1c) and I_{K1} channel subunits (Kir2.1 and Kir2.3). Membrane protein fractions were used, actin served as a loading control (not shown). Data are presented as mean \pm SEM and RA levels were normalized to LA levels. β -actin and U6 served as internal standards for mRNA and microRNA measurements, respectively. Antibodies were applied at dilution of 1:1000. * $P < 0.05$. AU, arbitrary units; n.s., not significant; PCR, polymerase chain reaction; SEM, standard error of the mean.

CACNB2 (Figure 7B) expression. CaV1.2 protein levels were ~ 3.1 -fold reduced ($P < 0.05$, Figure 7C). Relative expression of miR-1 increased with age ($\sim 56\%$, $P < 0.05$, Figure 7A), expression of miR-26a also decreased with age ($P < 0.05$, Figure 7A). MiR-26b showed a mild tendency towards less expression with age ($P = \text{NS}$, Figure 7A). Protein levels for Kir2.1 and Kir2.3 showed a trend to decrease (Kir2.1 $\sim 30\%$; Kir2.3 $\sim 13\%$, $P = \text{NS}$, Figure 7B and C).

Discussion

This study sought to determine several aspects of AF remodelling: (i) to establish whether there is a difference in expression between right and left atria from each patient. (ii) To study if both atria are subjected to similar remodelling processes during human AF. (iii) To identify rapid heart rate as a determinant of the remodelling process in human atrial tissue. (iv) To evaluate changes related to age as a potential underlying modulator of the substrate for AF in left and right atria.

Main findings

Human atria exhibit regional heterogeneity regarding expression of calcium channel subunits but not inward-rectifier subunits. In healthy

RA there is $\sim 70\%$ greater abundance of CACNA1c mRNA and ~ 2 -fold more CaV1.2 protein—underlying calcium currents—than in LA. This pattern is qualitatively similar in diseased hearts. In mitral valve disease patients, RA CACNA1c mRNA expression is $\sim 90\%$ greater than LA mRNA and protein is $\sim 2.4\times$ fold more abundant.

We observed an age-dependent increase in expression of miR-328, along with down-regulated CACNA1c mRNA and CaV1.2 protein potentially underlying changes that may facilitate AF occurrence with age.

Regional ion channel distribution

The current knowledge about the cellular and molecular basis of AF in man is largely based on studies using RAAs, with only a few studies comparing cellular electrophysiology or gene expression in the RAAs and left atrial appendages (LAAs).¹³ Even more, there are few studies examining changes in regions other than appendages from patients. If changes that have been described in RA could similarly be observed in LA remained largely unknown.

Greater I_{K1} -densities in LAA than RA have been reported in patients with paroxysmal AF yielding a left to right gradient in action potential duration.¹² This finding is not readily consistent with our data showing similar remodelling processes in both atria during persistent/permanent AF. However, among the 95 patients in the

previous study, matched RA and LA samples were only available from four individual patients, while all our samples had an individual match from both atria. Furthermore, results obtained in patients with paroxysmal AF could not be replicated in persistent/permanent AF in the study by Voigt *et al.* Accordingly, our study and previous work might look at different stages of the remodelling process, which could explain discrepancies.

Regional expression differences in mRNA expression were studied in human tissue (RA, LA, ventricles, and Purkinje fibres).¹¹ This study used human donor heart tissue unsuitable for transplantation similar to our study. There was no statistically significant overall gene expression difference between right- and left-sided chambers but tissue differences (i.e. atrium vs. ventricle vs. Purkinje fibres) were strong determinants of ion channel subunit expression pattern. In an earlier paper, mRNA transcripts of a set of ion channels and calcium modulating proteins were studied.¹⁴ Right atrial appendage tissue samples of patients with valvular heart disease were compared these with cardiac tissue of patients with coronary disease.

Relevance for atrial fibrillation mechanisms

In this study, we have shown that previously identified key mechanistic features of electrical remodelling underlying AF¹⁵ can be similarly observed in human LA and RA tissue. I_{K1} augmentation is among the key mechanisms of action potential duration shortening, which critically stabilizes atrial rotors maintaining AF.^{16,17} According to our data up-regulation of this channel occurs in both atria with relatively greater changes in RA. This finding deserves further study.

We have shown, that both $I_{Ca,L}$ and Ca^{2+} channel protein expression are down-regulated in AF. We found elevated Kir2.1 mRNA and protein levels and greater I_{K1} density in AF samples. Changes in miR-1, a regulator of Kir2.1, are coinciding with this pathology.⁶ Notably, miR-1 has been shown to regulate multiple genes including the main cardiac connexin isoform Cx43, the I_{Ks} channel regulatory subunit minK encoded by *KCNE1* or calcium handling proteins, such as the Na^+/Ca^{2+} exchanger *NCX1*,⁵ which genes may also contribute to AF pathogenesis. According to our data in healthy human atrial tissue there is regional heterogeneity between LA and RA expression of calcium channel subunits with larger expression in RA. Less calcium channel and related current could lead to shorter action potential durations. This difference may contribute to explain why the LA is the natural habitat for AF.

Age-dependent changes

To date, no data regarding the influence of ageing on human electrophysiology exist. Work in animals found decreased Ca^{2+} current function and augmented potassium currents in aging dogs,⁴ and indirect evidence for a reduction in $I_{Ca,L}$ as a potential contributor (besides increased fibrosis) to AF vulnerability in old canines.⁹

The results of our study confirm a reduction in calcium channel subunits in elderly humans and suggest that age-dependently increased expression of microRNAs (miR-1 and miR-328) and subsequently down-regulated mRNA and protein levels of related genes could play a role in AF development. In particular, age-dependent down-regulation of calcium channel protein expression, as a consequence of increased miR-328 levels may lead to decreased L-type

Ca^{2+} current and could provide a contributory mechanistic explanation for the increased susceptibility for AF development with older age.

Limitations

As this study was performed in human atrial tissue, sample numbers are small and accordingly, statistical power is limited. Several changes were only documented as a trend owing to small sample numbers and large variability of measurements. This study could not address many nowadays all unanswered issues regarding human atrial electrophysiology and cellular aetiology of AF.

Conclusions

Healthy human LA exhibits less calcium channel subunit expression than RA—potentially owing to greater LA microRNA 328 expression. Atrial fibrillation remodelling of inward-rectifier and calcium channel subunits occurs to a qualitatively similar extent in both atria, while RA has a relatively larger increase in inward rectifier subunits in response to AF. We found an age-dependent increase in microRNAs (miR-1 and miR-328) and down-regulated mRNA and protein levels of calcium channel genes that could play a role in AF development with older age.

Gene symbols

mRNA	GenBank ID	Function
CACNA1c	NM_000719	Encodes CaV1.2, the α -subunit of the cardiac L-type calcium channel
CACNB1	NM_000723	Encodes β 1 regulatory subunit of calcium channels
CACNB2	NM_000724	Encodes β 2 regulatory subunit of calcium channels
KCNJ2	NM_000891	Encodes Kir2.1, the pore-forming subunit of cardiac inward-rectifier potassium channel
Protein	GenBank GeneID	Function
CaV1.2	775	The α -subunit of the cardiac L-type calcium channel
Kir2.1	3759	Pore-forming subunit of cardiac inward rectifier
Kir2.3	3761	Pore-forming subunit of cardiac inward rectifier
microRNA	miRBase accession	
miR-1	MI0000651	
miR-26a	MI0000083	
miR-26b	MI0000084	
miR-328	MI0000804	

Supplementary material

Supplementary material is available at *Europace* online.

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Conflict of interest: none declared.

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