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Molecular remodeling of ion channels, exchangers and pumps in atrial and ventricular myocytes in ischemic cardiomyopathy

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Key words: ischemic cardiomyopathy, molecular profiling, atrial myocytes, ventricular myocytes, ion channels, phospholemman, Na⁺,K⁺-ATPase, CLIC

Abbreviations: ICM, ischemic cardiomyopathy; CE&P, channels, exchangers and pumps

Existing molecular knowledge base of cardiovascular diseases is rudimentary because of lack of specific attribution to cell type and function. The aim of this study was to investigate cell-specific molecular remodeling in human atrial and ventricular myocytes associated with ischemic cardiomyopathy. Our strategy combines two technological innovations, laser-capture microdissection of identified cardiac cells in selected anatomical regions of the heart and splice microarray of a narrow catalog of the functionally most important genes regulating ion homeostasis. We focused on expression of a principal family of genes coding for ion channels, exchangers and pumps (CE&P genes) that are involved in electrical, mechanical and signaling functions of the heart and constitute the most utilized drug targets. We found that (1) CE&P genes remodel in a cell-specific manner: ischemic cardiomyopathy affected 63 CE&P genes in ventricular myocytes and 12 essentially different genes in atrial myocytes. (2) Only few of the identified CE&P genes were previously linked to human cardiac disfunctions. (3) The ischemia-affected CE&P genes include nuclear chloride channels, adrenoceptors, cyclic nucleotide-gated channels, auxiliary subunits of Na⁺, K⁺ and Ca²⁺ channels, and cell-surface CE&Ps. (4) In both atrial and ventricular myocytes ischemic cardiomyopathy reduced expression of *CACNG7* and induced overexpression of *FXYP1*, the gene crucial for Na⁺ and K⁺ homeostasis. Thus, our cell-specific molecular profiling defined new landmarks for correct molecular modeling of ischemic cardiomyopathy and development of underlying targeted therapies.

Introduction

Cardiovascular disease is the leading cause of human morbidity and mortality. Ischemic cardiomyopathy¹ (ICM) affects approximately one out of 100 people in the United States, most often middle-aged to elderly men. ICM is the most common form of cardiomyopathy leading to dilation of the cardiac chambers and congestive heart failure. In spite of great efforts, molecular markers and targets of ICM are not currently well understood.² The tissue-specific pathogenesis pathways in the human heart reportedly include approximately 20 genes³ coding for structural proteins and those associated with Ca²⁺ homeostasis and energy metabolism. However, some of the implicated genes may be secondary to ICM because intrinsic noise of investigative platforms precludes from detecting low-signal cell-type-specific critical targets at the

whole-tissue level. Different cell types remodel in the development and disease following their unique program. Moreover, this remodeling is anatomically heterogeneous. To overcome these problems and characterize ICM-induced molecular remodeling in a cell-type and anatomical region-specific fashion, we combined two technological innovations, laser-capture microdissection of cells of interest and microarray optimized for detection of alternative RNA splicing events in a narrow catalog of genes coding for ion channels, exchangers and pumps (CE&P genes), which comprise the most acclaimed target classes for top-selling prescription drugs. Because CE&P molecules are involved in electrical, signaling and mechanical functions of the heart, their remodeling in ICM may result in decreased cardiac contractile functions.⁴ It is likely that pathophysiological conditions of ICM affect regulation of expression of CE&P splice variants.⁵ Thus,

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molecular profiling of disease-induced CE&P remodeling may narrow the search for crucial players and enable better understanding of underlying disease mechanisms and effective ICM prevention and treatment. We focused our research on CE&P molecular remodeling in atrial and ventricular cardiomyocytes because of their ultimate role in cardiac contraction targeted by ICM. This research strategy addressed the complexity of cardiovascular system and tissue heterogeneity complicated by differential gene expression and splice variation. Our findings associate ICM with altered expression of *CACNG7* and *FXYDI* in atrial and ventricular myocytes and show that these cells remodel in ICM following their unique programs involving different subsets of CE&P genes.

Results

Laser capture microdissection is an established method for isolation with confidence of histochemically identified cells from heterogeneous cell populations allowing the rational design of comprehensive splice variant profiling of CE&P remodeling in cardiomyocytes. Results of microarray analysis are presented in Table 3 and summarized in Table 4. We found that ICM caused remodeling of 12 genes in atrial myocytes. Increased expression in relation to healthy hearts was found for the *FXYDI* gene coding for protein regulator of Na⁺,K⁺-ATPase phospholemman (2.3-fold) and intracellular chloride channels *CLIC1* and *CLIC4* (both 1.6-fold). The other nine identified genes were significantly downregulated including *FXYD7*, the inward rectifier K_{ir}2.4 channel, the acetylcholine receptor α 10 subunit, the Ca²⁺ channel Ca_v γ subunits, the AMPA-selective glutamate receptor 3, the K_v4.3 K⁺ channel involved in initial phase of repolarization and setting the plateau voltage of the action potential, the polyunsaturated fatty acids-activated and mechano-sensitive K_{2p}4.1 channel, the Na⁺ channel Na_v β 1 subunit. In ventricular myocytes we identified 63 genes affected by ICM. An increase (2.5-fold) was found only for *FXYDI*. The other 62 CE&P genes were downregulated 1.5–3.5 fold. Some of them belong to the same functional groups that are affected by ICM in atrial myocytes (Table 4) including *FXYD3*, three A-type K⁺ channels, two inward rectifiers, three tandem-pore-domain K⁺ channels, three Ca_v γ subunits, six acetylcholine receptor subunits, and the AMPA1 glutamate receptor. In addition, we identified three delayed rectifiers, the Ca²⁺-activated K_{Ca}3.1 channel, seven modifiers and β -subunits of K⁺ channels, six voltage-gated Ca²⁺ channels, two ENaC and trp channels, cyclic nucleotide-gated channels HCN2 and CNGA3, three connexins, NMDA and glycine receptors, GABA receptor subunits and three adrenoceptors.

To find out whether the splice microarray results reported in Table 3 are disease-type specific, we supplemented the same splice microarray of ICM ventricular myocytes with one additional sample prepared from left ventricle of a 41-years old *dilated* cardiomyopathy male donor. The top-list ANOVA gene score annotation for combined altered CE&P genes in ventricular myocytes (Table 3) was reduced from 63 to just 4 genes (*FXYDI* ($G_{\text{fold}} = +2.4$), *HCN2* (-1.5), *GLRA1* (-1.8) and *GJCI* (-2.3)). This result suggests that dilated and ischemic cardiomyopathy may affect

different CE&P subsets and only four indicated genes may be common among the ones affected by these diseases, overexpression of *FXYDI* being the most obvious characteristic feature.

Discussion

Our study is based on the precept that to characterize the ICM-induced molecular remodeling it is essential to investigate it at the level of cells and molecules responsible for altered cardiac function. To achieve this goal, we developed the investigative approach combining laser capture microdissection of identified cardiac cells from tissue biopsies with splice microarray of custom-narrowed sets of CE&P genes that play ultimate role in cardiac contraction targeted by ICM. Our approach for the first time enabled direct comparison of altered gene expression caused by ICM in atrial and ventricular myocytes and identified new essential players.

First of all, we found that atrial and ventricular myocytes remodel following their unique programs where ICM affects different CE&P genes. Although some of these genes were already reported to be expressed in the heart^{4,79} (see also refs. in Tables 3 and 4), only few of them were previously linked to known cardiac pathologies.^{21,39,64,80–84} These include (1) α_{1B} , α_{2C} and β_1 -adrenoceptors whose downregulation in cardiomyopathy was linked to pathological remodeling in failing ventricular myocardium.^{42,51,85} (2) *KCND2*. In diabetic ventricle, a switch from K_v4.2 to K_v1.4 may underlie the slower kinetics of the I_{to} K⁺ current.⁴⁰ (3) *KCND3*. Downregulation of cardiac K_v4.3 and minK channels may be associated with arrhythmia and atrial fibrillation.^{14,61,62,86} It was reported that congestive heart failure and hypertrophy decrease K_v4.3 expression in terminally failing human hearts.⁸⁷ In line with this finding, *KCND3* gene transfer abrogates the hypertrophic response to aortic stenosis.⁸⁸ (4) *HCN2*. Transfer of this gene was also tested as gene therapy for cardiac arrhythmias in experimental animals with positive results.⁸⁹ However, the existing knowledge base remains rudimentary in the absence of attribution to certain cardiac cell types and functions.

We found that ICM does not alter expression of Ca_v1.2 and Na_v5 channel isoforms and Na⁺,K⁺-ATPase but rather affects some of their accessory subunits. The most profound change in both atrial and ventricular myocytes was overexpression of phospholemman. Binding of phospholemman to Na⁺,K⁺-ATPase induces a decrease in the affinity of α 1- β 1 and α 2- β 1 isozymes to external K⁺ and approximately 2-fold decrease in the affinity to internal Na⁺.⁹⁰ Inhibition of Na⁺/Ca²⁺ exchanger by phospholemman⁹¹ may add to the disbalance of Na⁺, K⁺ and Ca²⁺ gradients across the plasma membrane and contribute to hypertrophy of ICM muscle cells due to overexpression of *FXYDI*. Another unexpected finding that may have profound functional consequences is underexpression of Na_v β 1. Although the functional role of this single-membrane-spanning-repeat protein in the heart remains uncertain, it co-localizes with the Na_v1.5 pore-forming α 1 subunit in the T-tubule system and intercalating discs levels in cardiomyocytes¹⁷ and modulates Na_v1.5 channels in the heart by increasing the Na⁺ current density.⁹² Confirming its crucial role in the heart, *SCN1B* knock-out caused prolongation of QT and RR intervals⁹³

and development of cardiomyopathy.⁹⁴ Post-transcriptional gene silencing of Na_vβ1 reduced mRNA and protein levels of Na_v1.5, KChIP2 mRNA and K_v4.3 resulting in markedly decreased Na⁺ and I_{to} currents.⁹⁵ Thus, underexpression of Na_vβ1 may lead to a suppression of I_{to}, action potential prolongation and increased susceptibility of the heart to ventricular arrhythmia.

Other new potential targets for drug discovery are *CLICs*.^{8,35} Members of the p64 family, *CLIC* proteins localize to the cell nucleus and exhibit both nuclear and plasma-membrane chloride channel activity, but their functions are not well defined. *CLIC2*, which shares sequence similarity with *CLIC1*, modulates cardiac ryanodine receptors and inhibits Ca²⁺ release from the sarcoplasmic reticulum.⁹⁶ Thus, ICM-induced remodeling of *CLICs* in cardiomyocytes may affect membrane potential, intracellular pH and cell volume.

The four identified Ca_vγ calcium channel subunits downregulated in ICM show a broad spectrum of modulating activities that may have a role in cardiac myocytes. The γ7, which is homologous to γ5, regulates stability of certain mRNAs⁹⁷ and, along with γ2 and γ8, controls trafficking and gating of AMPA receptors.^{98,99} It remains to be studied whether remodeling of AMPA receptors in ICM is associated with Ca_vγs. Acetylcholine,⁷² AMPA and NMDA receptors,⁷³ also downregulated in ICM, are present in cardiac neuromuscular junctions and intercalating disk, but little is known about their non-neuronal expression and roles in the heart.

Our study is the first step in molecular characterization of ICM with organ- and cell-specific annotation of altered expression of CE&P genes. Our study does not determine whether upregulation or downregulation of the identified CE&P genes are the primary drivers of ICM or reflect pathophysiological response to the disease. However, it provides an objective context within which it would be easier to find therapeutic targets among the elucidated markers of ICM. Future extension of this study may clarify links between CE&P genes expression and drug therapy, duration of disease, age, gender and race as potential factors in ICM and define key aspects of the principal gene network in relation to the development of ICM at the cellular and specific intercellular levels. In conjunction with protein and immunohistochemical analyses this may yield a more robust approach to better understanding mechanisms and pathophysiology of ICM—a critical need in the clinical utilization of this field.

Methods

Human cardiac tissue samples. Healthy (Table 1) and ICM hearts (Table 2) of anonymous donors were obtained from the Cardiac Transplantation Center at the Washington University

Table 1. Characteristics of the healthy heart donors

Patient #	Age	Sex	Cause of death	Cardiac tissue studied
H1	58	F	Intracerebral hemorrhage	LV
H2	70	M	Intracerebral hemorrhage	LV, LAA
H3	40	M	Brain tumor	LV
H4	72	F	Intracerebral hemorrhage	LAA
H5	58	M	Intracerebral hemorrhage	LAA

LV, left ventricle; LAA, left atrium appendage.

in St. Louis, MO. Healthy hearts were excluded from transplantation after exceeding 6 h-limit of allograft ischemic time. Showing no evidence of hemorrhagic stroke, they retained normal structure and function evidenced by bimodal biophotonic imaging and optical mapping of the atrioventricular junction.¹⁰⁰ There was no delay in collecting donor's heart tissue. Decision on suitability for transplantation was made prior to harvest of organs, our team members were notified in advance (at least 2 h before cross clamping) and were present during organ harvest. Heart was cardioplegically arrested, harvested and transferred to us immediately after removal from the chest. Tissue samples (0.5–2 g) were dissected from the area outside of scar burden (if any) of left ventricle and left atrial appendages under cold cardioplegic arrest conditions within approximately 30–40 min after removal of the heart from donor's chest. Tissue was washed in 4°C saline, immersed in Tissue-Tek OCT Compound (Electron Microscopy Sciences, Hatfield, PA), flash frozen in liquid nitrogen and stored at -80°C until use. Although availability of donors suitable for this study was limited, the number of selected human hearts (Tables 1 and 2) is in full compliance with the NCBI-recommended MIAME guidelines to microarray¹⁰¹ and enabled us to analyze all individual donor's samples, grouped by cell type, simultaneously on one microarray slide, thus excluding possible slide-to-slide variations.

Laser-capture microdissection and isolation of mRNA. Serial cryostat sections (7–8 μm thick) were cut from the frozen tissue samples using Minotome Plus microtome cryostat. Before laser-capture microdissection, cardiomyocytes in the sections were quickly (10 s) stained with Eosin Y (Sigma-Aldrich, St. Louis, MO) according to standard procedure. Given the notably large size of cardiac muscle cells relative to other cells in the tissue, this method of identification is sufficient to distinguish stained cardiomyocytes from other cells under microscope. Laser-capture microdissection was performed with PixCell II system (Arcturus, Mountain View, CA) using a 7.5-μm laser spot as described earlier.⁵ The excised cardiomyocytes were picked up from the slide

Table 2. Characteristics of the ICM heart patients

Patient #	Age	Sex	Complications and prior treatment	PR (ms)	HR (bpm)	QRS (ms)	EF (%)
D1	54	M	CABG, VT, ICD	184	100	202	ND
D2	46	M	VF, ICD	216	91	106	19
D3	50	M	ICD	128	128	144	25

CABG, coronary artery bypass graft; ICD, implantable cardioverter defibrillator; QRS, a waveform on EKG which represents excitation of the ventricles; EF, ejection fraction; ND, not determined.

Table 3. Top-list ANOVA gene-score annotation for CE&P genes altered by ICM in atrial and ventricular myocytes as compared to healthy subjects

Gene	Genbank #	Variant accession #	p-value* (ICM/H)	G _{fold} change* (ICM/H)
Atrial myocytes				
<i>FXYD1</i> ^{6,7}	NM_021902	H75358	0.02859	2.3
<i>CLIC1</i> ⁸	NM_001288	BU173816	0.04412	1.6
<i>CLIC4</i> ⁸	NM_013943	BG436443	0.04047	1.6
<i>KCNJ14</i> ^{9,10}	NM_013348	na	0.01289	-1.6 [†]
<i>FXYD7</i>	NM_022006	CQ722304	0.01907	-1.6
<i>CHRNA10</i> ¹¹	NM_020402	CR744383	0.02780	-1.6
<i>CACNG7</i> ¹²	NM_031896	H19702	0.01039	-1.6
<i>GRIA3</i> ¹³	NM_000828	DA531074	0.01945	-1.7
<i>KCND3</i> ¹⁴	NM_004980	NM_172198	0.00837	-1.7
<i>KCNK4</i> ^{9,15}	NM_016611	BE900958	0.01063	-1.8
<i>SCN1B</i> ¹⁶⁻¹⁸	NM_001037	DA062026	0.03059	-1.8
<i>CACNG2</i>	NM_006078	na	0.02046	-4.7
Ventricular myocytes				
<i>FXYD1</i> ^{6,7}	NM_021902	H75358	0.00511	2.5
<i>KCNN4</i>	NM_002250	AL552182	0.00551	-1.5
<i>CLCN2</i>	NM_004366	BM789394	0.00391	-1.5
<i>CACNG7</i>	NM_031896	H19702	0.00069	-1.5
<i>CACNG8</i>	NM_031895	CQ718803	0.00092	-1.5
<i>CACNA1A</i>	NM_000068	BF529475	0.01245	-1.5
<i>KCNH6</i>	NM_173092	CQ730511	0.00758	-1.5
<i>CACNG5</i>	NM_145811	AX101266	0.03112	-1.5
<i>GRIN2A</i>	NM_000833	BG718790	0.01562	-1.5
<i>SCNN1D</i>	NM_002978	AK093372	0.00046	-1.6 [†]
<i>GABRA3</i>	NM_000808	DA801686	0.00407	-1.6
<i>CACNA1E</i> ^{19,20}	NM_000721	L27745	0.00148	-1.6
<i>KCNH1</i> ^{9,21,22}	NM_172362	DB021985	0.00092	-1.6
<i>KCNV2</i> ²³	NM_133497	CQ724488	0.00935	-1.6
<i>CHRNA6</i>	NM_004198	DA415543	0.00817	-1.6
<i>KCNK6</i> ^{9,24}	NM_004823	AW883970	0.02392	-1.6
<i>CFTR</i> ²⁵	NM_000492	BG386556	0.00212	-1.6
<i>GLRA3</i>	NM_006529	BG186165	0.00657	-1.6
<i>TRPC4</i> ^{26,27}	NM_016179	AF421361	0.02904	-1.6
<i>FXYD3</i>	NM_021910	DR006067	0.01264	-1.6 [†]
<i>GRIN2D</i>	NM_000836	AB209292	0.00249	-1.6 ^{†,‡}
<i>CACNA1D</i> ^{9,28,29}	NM_000720	CQ731466	0.00347	-1.7
<i>HCN2</i> ^{9,30}	NM_001194	BX281160	0.00039	-1.7
<i>KCNS1</i> ⁹	NM_002251	DA231979	0.02663	-1.7
<i>KCNK18</i> ³¹	NM_181840	AX319992	0.00469	-1.7
<i>VMD2</i> ³²	NM_004183	AA205892	0.03238	-1.7
<i>TRPM1</i> ³³	NM_002420	na	0.02334	-1.7
<i>CHRND</i> ³⁴	NM_000751	BF306695	0.01945	-1.7 [†]
<i>CLIC3</i> ³⁵	NM_004669	BE902424	0.01425	-1.7 [†]
<i>CACNA1H</i> ^{36,37}	NM_021098	DB100395	0.00183	-1.7
<i>CACNA1G</i> ²⁹	NM_198396	BM451648	0.00045	-1.7

Table 3. (continued)

<i>KCND2</i> ³⁸⁻⁴⁰	NM_012281	DA125095	0.01268	-1.7
<i>CNGA3</i>	NM_001298	AK131300	0.02203	-1.7
<i>GABRG2</i>	NM_198904	BI819259	0.02867	-1.8
<i>KCNA4</i> ^{9,28,39-41}	NM_002233	CQ741592	0.02019	-1.8
<i>ADRA1B</i> ⁴²	NM_000679	na	0.02796	-1.8 [†]
<i>CHRNA4</i>	NM_000744	BC096291 [†]	0.00353	-1.8
<i>CACNA1I</i>	NM_021096	AX068892	0.00270	-1.8
<i>GRIA1</i>	NM_000827	DA749477	0.01665	-1.8
<i>KCNJ1</i> ^{9,43}	NM_153767	NM_000220	0.00360	-1.8
<i>KCNC3</i> ^{9,28}	NM_004977	BM474777	0.00913	-1.8
<i>KCNQ4</i> ^{9,44}	NM_004700	AK074957	0.02336	-1.9
<i>KCNMB2</i> ⁴⁵	NM_181361	BG185231	0.01402	-1.9
<i>GABRG1</i>	NM_173536	CQ714573	0.02843	-1.9
<i>KCNG1</i> ⁴⁶	NM_172318	DA497732	0.02389	-1.9
<i>KCNE1</i> ^{14,47,48}	NM_000219	AY789480	0.01744	-1.9
<i>GLRA1</i>	NM_000171	BP208426	0.01999	-2.0
<i>CHRNA1</i>	NM_000079	CD013888	0.02807	-2.0 [†]
<i>NOX1</i> ⁴⁹	NM_013954	NM_013955	0.00356	-2.0
<i>GRID2</i>	NM_001510	DB052812	0.00299	-2.0
<i>CHRNA9</i>	NM_017581	BF513332 [†]	0.00625	-2.0
<i>ADRA2C</i> ⁵⁰⁻⁵³	NM_000683	T39448	0.00030	-2.1 [†]
<i>GJB1</i>	NM_000166	BF571436	0.01233	-2.1
<i>GABRR1</i>	NM_002042	CB959800	0.01415	-2.1
<i>SCNN1G</i> ⁹	NM_001039	CQ721445	0.00501	-2.2
<i>CNGB3</i>	NM_019098	BX104558	0.00254	-2.5
<i>CHRN3</i>	NM_000749	DA127065	0.00064	-2.5
<i>GJC1</i> ⁵⁴	NM_152219	na	0.00582	-2.5
<i>ADRB1</i> ^{52,55,56}	NM_000684	na	0.00674	-2.5
<i>KCNG4</i>	NM_172347	CQ728641	0.01073	-2.8
<i>KCTD11</i>	NM_00100291	na	0.00300	-3.1 [†]
<i>KCNA2</i> ^{9,16,28,57}	NM_004974	BI907383	0.00795	-3.1
<i>GJB6</i>	NM_006783	AY789474	0.00040	-3.5

*A two-way ANOVA model was used to compare ICM vs. healthy (H) cardiac samples as described in splice microarray data analysis. p-values, geometric mean (G_{mean}), and the respective G_{fold} Change values were calculated. [†]Partial (tiling) human cardiac cDNA clones: AI082799 (*KCNJ14*); DA560890 (*SCNN1D*); AI138642, AI144215 and AI183491 (*FXYD3*); AA455065 (*GRIN2D*); AA716738 and AI367583 (*CHRN3*); W69541, AI146716 and W69457 (*CLIC3*); AA010313 (*ADRA1B*); W81677 (*CHRNA1*); AAB31164 (*ADRA2C*); AJ709249 and AJ711264 (*KCTD11*). [‡]CK845061.1 cDNA from rat ventricle (*GRIN2D*). *This sequence may represent a bonafide polyA tail.

surface and captured on LCM Caps. To confirm the quality of cell isolation, the sectional images taken before and after microdissection were thoroughly inspected to exclude contamination with non-muscle cells, and only then the captured samples from serial sections were joined together. High-quality cellular RNA was recovered from the collected cells using PicoPure[™] RNA isolation kit (Arcturus) and treated with RNase-free DNase (Qiagen, Valencia, CA). Quality of RNA was tested right before the labeling for microarray by measuring the OD and 28S/18S

Table 4. CE&P genes altered by ICM in atrial and ventricular myocytes

CE&P class protein	Atrial myocytes	Ventricular myocytes
Cl ⁻ channel	<i>CLIC1</i> ⁸ , <i>CLIC4</i> ⁸	<i>CLIC3</i> ³⁵ , <i>CLCN2</i> [*] , <i>CFTR</i> ^{25,58,59} , <i>VMD2</i> ³²
Transient outward (I _{to}) K ⁺ channel, A-type	<i>K_v4.3 (KCND3)</i> ¹⁴	<i>K_v1.4 (KCNA4)</i> ^{9,28,39-41} , <i>K_v3.3 (KCNC3)</i> ^{9,28} , <i>K_v4.2 (KCND2)</i> ^{*38-40}
Delayed rectifier K ⁺ channel		<i>K_v1.2 (KCNA2)</i> ^{9,16,28,57} , <i>K_v7.4 (KCNQ4)</i> ^{9,44} , <i>K_v10.1 (KCNH1)</i> ^{9,21,22}
K ⁺ channel modifiers and β subunits		<i>K_v6.1 (KCNG1)</i> ⁴⁶ , <i>K_v6.3 (KCNG4)</i> , <i>K_v8.2 (KCNV2)</i> ^{9,23} , <i>K_v9.1 (KCNK9)</i> ⁹ , <i>minK (KCNE1)</i> ^{*14,47,48,60-62} , tetramerization domain containing 11 (<i>KCTD11</i>)
Inward rectifier K ⁺ channel	<i>K_{ir}2.4 (KCNJ14)</i> ^{9,10}	<i>K_{ir}1.1 (KCNJ1)</i> ^{9,43} , <i>K_v11.2 or HERG2 (KCNH6)</i>
Tandem pore domain K ⁺ channel	<i>K_{2p}4.1 (KCNK4)</i> ^{9,15}	<i>K_{2p}6.1 (KCNK6)</i> ^{9,24} , <i>K_{2p}18.1 (KCNK18)</i> ³¹
Ca ²⁺ -activated K ⁺ channel		<i>K_{Ca}3.1 (KCNN4)</i> [*] , β2 subunit of maxik (<i>KCNMB2</i>) ⁴⁵
Voltage-gated Ca ²⁺ channel α ₁ subunit ^{63,64}		<i>Ca_v1.3α_{1D} (CACNA1D)</i> ^{9,28,29} , <i>Ca_v2.1α_{1A} (CACNA1A)</i> [*] , <i>Ca_v2.3α_{1E} (CACNA1E)</i> ^{19,20} , <i>Ca_v3.1α_{1G} (CACNA1G)</i> ^{65,66} , <i>Ca_v3.1α_{1H} (CACNA1H)</i> ^{36,37,67} , <i>Ca_v3.1α_{1I} (CACNA1I)</i>
Ca ²⁺ channel γ subunit	<i>Ca_vγ₂ (CACNG2)</i> , <i>Ca_vγ₇ (CACNG7)</i> ¹²	<i>Ca_vγ₅ (CACNG5)</i> , <i>Ca_vγ₇ (CACNG7)</i> ¹² , <i>Ca_vγ₈ (CACNG8)</i>
Na ⁺ channel subunits	<i>Na_vβ₁ (SCN1B)</i> ^{17,18}	<i>ENaC-γ (SCNN1G)</i> ⁹ , <i>ENaC-δ (SCNN1D)</i>
TRP channel		<i>TRPC4</i> ^{26,27,68} , <i>melastatin-1 (TRPM1)</i> ³³
Cyclic nucleotide-gated channel ⁶⁹		<i>HCN2</i> ^{9,30} , <i>CNGA3</i>
Connexin		<i>Cx32 (GJB1)</i> , <i>Cx30 (GJB6)</i> , <i>Cx31.9 (GJC1)</i> ^{54,70,71}
Acetylcholine receptor ⁷²	<i>α10 (CHRNA10)</i> ¹¹	<i>α1 (CHRNA1)</i> , <i>α4 (CHRNA4)</i> , <i>α6 (CHRNA6)</i> , <i>α9 (CHRNA9)</i> , <i>β3 (CHRNB3)</i> , <i>δ (CHRNδ)</i> ³⁴
Glycine receptor		<i>α1 (GLRA1)</i> , <i>α3 (GLRA3)</i>
GABA receptor		<i>α3 (GABRA3)</i> [†] , <i>γ1 (GABRG1)</i> , <i>γ2 (GABRG2)</i> , <i>ρ (GABRR1)</i>
NMDA receptor receptor ^{73,74}		<i>2A (GRIN2A)</i> , <i>2D (GRIN2D)</i>
Glutamate receptor receptor ^{73,74}	<i>AMPA3 (GRIA3)</i> ¹³	<i>AMPA1 (GRIA1)</i> , <i>δ2 (GRID2)</i>
Adrenergic receptor ⁷⁵		<i>α_{1B} (ADRA1B)</i> ^{42,76-78} , <i>α_{2C} (ADRA2C)</i> ^{50,52,53} , <i>β₁ (ADRB1)</i> ^{52,56}
H ⁺ channel		<i>NADPH oxidase (NOX1)</i> ⁴⁹
Na ⁺ ,K ⁺ -ATPase regulator	<i>FXYD1</i> ⁷⁷ , <i>FXYD7</i>	<i>FXYD1</i> ⁷⁷ , <i>FXYD3</i>

* Mouse Genome Database gene expression data (www.informatics.jax.org) confirming expression of the marked genes in mouse heart.

RNA ratios. On average, the OD ratio at 260/280 nm and 260/230 nm was >1.8 and the ratio of 28S/18S in the RNA samples selected for experiments was ≥1.6. Individual samples of total RNA were optionally amplified without 3'-bias using the TransPlex™ Whole Transcriptome Amplification kit (Rubicon Genomics, Ann Arbor, MI). (The non-bias 3'-amplification was confirmed by the automated RT-PCR with capillary electrophoretic quantification of amplicons executed on a commercial human cardiac total RNA samples (Sigma) with primers to *CACNA1C* and *CACNB2*, calcium channel genes characterized by complex alternative splicing). The amplified products were purified using QIAquick PCR purification kit (Qiagen).

cDNA labeling and microarray. The PCR products were fragmented with DNase I, denatured and end-labeled with Cy-3 fluorescent dye. The individual donor's samples, grouped according to cell type, were analyzed simultaneously on Human Ion Channel Splice Arrays 8-pack 4 x 44K slides (ExonHit Therapeutics, Gaithersburg, MD) manufactured on the Ion Channel Splice Array sv1.1 platform representing 287 human CE&P, including 248 alternatively spliced ones in total 1,655 splicing events and supplemented with additional capabilities to recognize connexins and ryanodine receptors.

Microarray data analysis. The statistically significant differential expression patterns between ICM and healthy atrial and ventricular cell samples was analyzed using long- to short-form ratio statistics and expression level statistics to identify genes and splice events affected by ICM. All analyses were performed at ExonHit using Partek Genomics Suite. Principal Component Analysis was carried out to illustrate the level of spread between samples and experimental groups. A two-way ANOVA model was used to perform statistical tests on the probe set level intensities, to compare ICM vs. healthy cells. A Source of Variation plot was generated from this data to find the relative level of difference contributed by each factor. A cutoff level was determined to generate "top hit lists" of probe sets that indicate the most statistically significant differences between the sample groupings. The raw and transformed data sets are submitted to Gene Expression Omnibus, accession # GSE17294 and GSE17530. The method accounted for specific splice variants shown in the results, but did not evaluate the ICM-induced changes in alternative splicing. Low variability between the individual RNA samples in meaningful ($p < 0.01$) probesets, estimated as average of individual probesets standard deviations normalized to the respective mean values (0.08 ± 0.04 , mean \pm st.dev), suggests that differences in

donor's age and gender in our study have not notably affected the results of microarray. Additional details are presented in Supplementary Methods.

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Note

Supplementary materials can be found at: www.landesbioscience.com/supplement/GronichCHAN4-2-Sup.pdf

References

- Felker GM, Shaw LK, O'Connor CM. A standardized definition of ischemic cardiomyopathy for use in clinical research. *J Am Coll Cardiol* 2002; 39:210-8.
- Kittleson MM, Minhas KM, Irizarry RA, Ye SQ, Edness G, Breton E, et al. Gene expression analysis of ischemic and nonischemic cardiomyopathy: Shared and distinct genes in the development of heart failure. *Physiol Genomics* 2005; 21:299-307.
- Sanoudou D, Vafiadaki E, Arvanitis DA, Kranias E, Kontrogianni-Konstantopoulos A. Array lessons from the heart: focus on the genome and transcriptome of cardiomyopathies. *Physiol Genomics* 2005; 21:131-43.
- Mangoni ME, Nargeot J. Genesis and regulation of the heart automaticity. *Physiol Rev* 2008; 88:919-82.
- Tiwari S, Zhang Y, Heller J, Abernethy DR, Soldatov NM. Atherosclerosis-related molecular alteration of the human $Ca_v1.2$ calcium channel α_C subunit. *Proc Natl Acad Sci USA* 2006; 103:17024-9.
- Bibert S, Roy S, Schaefer D, Horisberger J-D, Geering K. Phosphorylation of phospholemman (FXD1) by protein kinases A and C modulates distinct Na,K-ATPase isoforms. *J Biol Chem* 2008; 283:476-86.
- Fuller W, Eaton P, Bell JR, Shattock MJ. Ischemia-induced phosphorylation of phospholemman directly activates rat cardiac Na/K-ATPase. *FASEB J* 2004; 18:197-9.
- Berryman M, Bretscher A. Identification of a novel member of the chloride intracellular channel gene family (*CLIC5*) that associates with the actin cytoskeleton of placental microvilli. *Mol Biol Cell* 2000; 11:1509-21.
- Harrell MD, Harbi S, Hoffman JF, Zavadil J, Coetzee WA. Large-scale analysis of ion channel gene expression in the mouse heart during perinatal development. *Physiol Genomics* 2007; 28:273-83.
- Liu GX, Derst C, Schlichthörl G, Heinen S, Seeböhm G, Brüggemann A, et al. Comparison of cloned K_v2 channels with native inward rectifier K^+ channels from guinea-pig cardiomyocytes. *J Physiol* 2001; 532:115-26.
- Sgard F, Charpantier E, Bertrand S, Walker N, Caput D, Graham D, et al. A novel human nicotinic receptor subunit, $\alpha 10$, that confers functionality to the $\alpha 9$ -subunit. *Molecular Pharmacology* 2002; 61:150-9.
- Chu P-J, Robertson HM, Best PM. Calcium channel γ subunits provide insights into the evolution of this gene family. *Gene* 2001; 280:37-48.
- Gill SS, Pulidoa OM, Muellera RW, McGuire PF. Molecular and immunohistochemical characterization of the ionotropic glutamate receptors in the rat heart. *Brain Res Bull* 1998; 46:429-34.
- Brundel BJJM, Van Gelder IC, Henning RH, Tieleman RG, Tuinenburg AE, Wieses M, et al. Ion channel remodeling is related to intraoperative atrial effective refractory periods in patients with paroxysmal and persistent atrial fibrillation. *Circulation* 2001; 103:684-90.
- Ozaita A, Vega-Saenz de Miera E. Cloning of two transcripts, HKT4.1a and HKT4.1b, from the human two-pore K^+ channel gene *KCNK4*. Chromosomal localization, tissue distribution and functional expression. *Mol Brain Res* 2002; 102:18-27.
- Dixon JE, Shi W, Wang H-S, McDonald C, Yu H, Wymore RS, et al. Role of the $K_v4.3$ K^+ channel in ventricular muscle: A molecular correlate for the transient outward current. *Circ Res* 1996; 79:659-68.
- Domínguez JN, de la Rosa Á, Navarro F, Franco D, Aránega AE. Tissue distribution and subcellular localization of the cardiac sodium channel during mouse heart development. *Cardiovasc Res* 2008; 78:45-52.
- Makita N, Bennett PB Jr, George AL Jr. Voltage-gated Na^+ channel β_1 subunit mRNA expressed in adult human skeletal muscle, heart and brain is encoded by a single gene. *J Biol Chem* 1994; 269:7571-9.
- Mitchell JW, Larsen JK, Best PM. Identification of the calcium channel $\alpha 1E$ ($Ca_v2.3$) isoform expressed in atrial myocytes. *Biochim Biophys Acta—Gene Str Expr* 2002; 1577:17-26.
- Weiergräber M, Henry M, Hescheler J, Schneider T. Ablation of the $Ca_v2.3$ containing E-type voltage-gated calcium channel results in cardiac arrhythmia and altered autonomic control within the murine cardiovascular system. *Heart Rhythm* 2005; 2:35-6.
- Ackerman MJ. Cardiac channelopathies: it's in the genes. *Nat Med* 2004; 10:463-4.
- Choi G, Kopplin LJ, Tester DJ, Will ML, Haglund CM, Ackerman MJ. Spectrum and frequency of cardiac channel defects in swimming-triggered arrhythmia syndromes. *Circulation* 2004; 110:2119-24.
- Czirják G, Tóth ZE, Enyedi P. Characterization of the heteromeric potassium channel formed by $K_v2.1$ and the retinal subunit $K_v8.2$ in *Xenopus* oocytes. *J Neurophysiol* 2007; 98:1213-22.
- Patel AJ, Maingret F, Magnone V, Fosset M, Lazdunski M, Honore E. TWIK-2, an inactivating 2P domain K^+ channel. *J Biol Chem* 2000; 275:28722-30.
- Yajima T, Nagashima H, Tsutsumi-Sakai R, Hagiwara N, Hosoda S, Quetermos T, et al. Functional activity of the CFTR Cl⁻ channel in human myocardium. *Heart Vessels* 1997; 12:255-61.
- Abramowitz J, Birnbaumer L. Physiology and pathophysiology of canonical transient receptor potential channels. *FASEB J* 2009; 23:297-328.
- Nakayama H, Wilkin BJ, Bodi I, Molkenkin JD. Calcineurin-dependent cardiomyopathy is activated by TRPC in the adult mouse heart. *FASEB J* 2006; 20:1660-70.
- Gaborit N, Le Bouter S, Szuts V, Varro A, Escande D, Nattel S, et al. Regional and tissue specific transcript signatures of ion channel genes in the non-diseased human heart. *J Physiol* 2007; 582:675-93.
- Mangoni ME, Couette B, Bourinet E, Platzer J, Reimer D, Striessnig J, et al. Functional role of L-type $Ca_v1.3$ Ca^{2+} channels in cardiac pacemaker activity. *Proc Natl Acad Sci USA* 2003; 100:5543-8.
- Xiao J, Yang B, Lin H, Lu Y, Luo X, Wang Z. Novel approaches for gene-specific interference via manipulating actions of microRNAs: Examination on the pacemaker channel genes *HCN2* and *HCN4*. *J Cell Physiol* 2007; 212:285-92.
- Kang D, Mariash E, Kim D. Functional expression of TRESK-2, a new member of the tandem-pore K^+ channel family. *J Biol Chem* 2004; 279:28063-70.
- O'Driscoll KE, Hatton WJ, Burkin HR, Leblanc N, Britton FC. Expression, localization, and functional properties of Bestrophin 3 channel isolated from mouse heart. *Am J Physiol Cell Physiol* 2008; 295:1610-24.
- Fonfria E, Murdock PR, Cusdin FS, Benham CD, Kelsell RE, McNulty S. Tissue distribution profiles of the human TRPM cation channel family. *J Recep Sign Transduct* 2006; 26:159-78.
- Kong B, Liu Y-L, Lü X-d. Microarray-bioinformatics analysis of altered genomic expression profiles between human fetal and infant myocardium. *Chinese Med J* 2008; 121:1257-64.
- Qian Z, Okuhara D, Abe MK, Rosner MR. Molecular cloning and characterization of a mitogen-activated protein kinase-associated intracellular chloride channel. *J Biol Chem* 1999; 274:1621-7.
- Chiang C-S, Huang C-H, Cheng H, Chang Y-T, Chang D, Chen J Jr, et al. The $Ca_v3.2$ T-type Ca^{2+} channel is required for pressure overload-induced cardiac hypertrophy in mice. *Circ Res* 2009; 104:522-30.
- Cribbs LL, Lee JH, Yang J, Satin J, Zhang Y, Daud A, et al. Cloning and characterization of $\alpha 1H$ from human heart, a member of the T-type Ca^{2+} channel gene family. *Circ Res* 1998; 83:103-9.
- Guo W, Jung WE, Marionneau C, Aimond F, Xu H, Yamada KA, et al. Targeted deletion of $K_v4.2$ eliminates I_{to} and results in electrical and molecular remodeling, with no evidence of ventricular hypertrophy or myocardial dysfunction. *Circ Res* 2005; 97:1342-50.
- Marionneau C, Brunet S, Flagg TP, Pilgram TK, Demolombe S, Nerbonne JM. Distinct cellular and molecular mechanisms underlie functional remodeling of repolarizing K^+ currents with left ventricular hypertrophy. *Circ Res* 2008; 102:1406-15.
- Nishiyama A, Ishii DN, Backx PH, Pulford BE, Birks BR, Tamkun MM. Altered K^+ channel gene expression in diabetic rat ventricle: isoform switching between $K_v4.2$ and $K_v1.4$. *Am J Physiol Heart Circ Physiol* 2001; 281:1800-7.
- Kashiwakura Y, Cho HC, Barth AS, Azene E, Marbán E. Gene transfer of a synthetic pacemaker channel into the heart: A novel strategy for biological pacing. *Circulation* 2006; 114:1682-6.
- Woodcock EA. Roles of α_{1A} - and α_{1B} -adrenoceptors in heart: Insights from studies of genetically modified mice. *Clin Exp Pharmacol Physiol* 2007; 34:884-8.
- Shuck ME, Bock JH, Benjamin CW, Tsai TD, Lee KS, Slightom JL, et al. Cloning and characterization of multiple forms of the human kidney ROM-K potassium channel. *J Biol Chem* 1994; 269:24261-70.
- Yeung SY, Pucovsky V, Moffatt JD, Saldanha L, Schwake M, Ohya S, et al. Molecular expression and pharmacological identification of a role for K_v7 channels in murine vascular reactivity. *Br J Pharmacol* 2007; 151:758-70.
- Wallner M, Meera P, Toro L. Molecular basis of fast inactivation in voltage and Ca^{2+} -activated K^+ channels: A transmembrane β -subunit homolog. *Proc Natl Acad Sci USA* 1999; 96:4137-42.
- Brahmajothi MV, Morales MJ, Liu S, Rasmussen RL, Campbell DL, Strauss HC. In situ hybridization reveals extensive diversity of K^+ channel mRNA in isolated ferret cardiac myocytes. *Circ Res* 1996; 78:1083-9.

47. Mustapha Z, Pang L, Nattel S. Characterization of the cardiac KCNE1 gene promoter. *Cardiovasc Res* 2007; 73:82-91.
48. Gaborit N, Steenman M, Lamirault G, Le Meur N, Le Bouter S, Lande G, et al. Human atrial ion channel and transporter subunit gene-expression remodeling associated with valvular heart disease and atrial fibrillation. *Circulation* 2005; 112:471-81.
49. Mollnau H, Oelze M, August M, Wendt M, Daiber A, Schulz E, et al. Mechanisms of increased vascular superoxide production in an experimental model of idiopathic dilated cardiomyopathy. *Arterioscler Thromb Vasc Biol* 2005; 25:2554-9.
50. Aggarwal A, Esler MD, Socratous F, Kaye DM. Evidence for functional presynaptic alpha-2 adrenoceptors and their downregulation in human heart failure. *J Am Coll Cardiol* 2001; 37:1246-51.
51. Brede M, Wiesmann F, Jahns R, Hadamek K, Arnolt C, Neubauer S, et al. Feedback inhibition of catecholamine release by two different α_2 -adrenoceptor subtypes prevents progression of heart failure. *Circulation* 2002; 106:2491-6.
52. Kardias S, Kelly R, Keddache M, Aronow B, Grabowski G, Hahn H, et al. Multiple interactions between the α_{1C} and β_1 -adrenergic receptors influence heart failure survival. *BMC Med Genet* 2008; 9:93.
53. Kitsios G, Zintzaras E. Genetic variation associated with ischemic heart failure: A HuGE review and meta-analysis. *Am J Epidemiol* 2007; 166:619-33.
54. Bukauskas FF, Kreuzberg MM, Rackauskas M, Bukauskiene A, Bennett MVL, Verselis VK, et al. Properties of mouse connexin 30.2 and human connexin 31.9 hemichannels: Implications for atrioventricular conduction in the heart. *Proc Natl Acad Sci USA* 2006; 103:9726-31.
55. Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R, et al. β_1 - and β_2 -Adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective β_1 -receptor downregulation in heart failure. *Circ Res* 1986; 59:297-309.
56. Dorn GW, II, Molkentin JD. Manipulating cardiac contractility in heart failure: Data from mice and men. *Circulation* 2004; 109:150-8.
57. Barry DM, Trimmer JS, Merlie JP, Nerbonne JM. Differential expression of voltage-gated K^+ channel subunits in adult rat heart: Relation to functional K^+ channels? *Circ Res* 1995; 77:361-9.
58. Davies WL, Vandenberg JI, Sayeed RA, Trezise AEO. Cardiac expression of the cystic fibrosis transmembrane conductance regulator involves novel exon 1 usage to produce a unique amino-terminal protein. *J Biol Chem* 2004; 279:15877-87.
59. Warth JD, Collier ML, Hart P, Geary Y, Gelband CH, Chapman T, et al. CFTR chloride channels in human and simian heart. *Cardiovasc Res* 1996; 31:615-24.
60. Sanguinetti MC, Curran ME, Zou A, Shen J, Specter PS, Atkinson DL, et al. Coassembly of K_v LQT1 and minK (IsK) proteins to form cardiac I_{Ks} potassium channel. *Nature* 1996; 384:80-3.
61. Restier L, Cheng L, Sanguinetti MC. Mechanisms by which atrial fibrillation-associated mutations in the S1 domain of KCNQ1 slow deactivation of I_{Ks} channels. *J Physiol* 2008; 586:4179-91.
62. Sampson KJ, Terrenoire C, Cervantes DO, Kaba RA, Peters NS, Kass RS. Adrenergic regulation of a key cardiac potassium channel can contribute to atrial fibrillation: evidence from an I_{Ks} transgenic mouse. *J Physiol* 2008; 586:627-37.
63. Vassort G, Talavera K, Alvarez JL. Role of T-type Ca^{2+} channels in the heart. *Cell Calcium* 2006; 40:205-20.
64. Pitt GS, Dun W, Boyden PA. Remodeled cardiac calcium channels. *J Mol Cell Cardiol* 2006; 41:373-88.
65. Mangoni ME, Traboulsie A, Leoni A-L, Couette B, Marger L, Le Quang K, et al. Bradycardia and slowing of the atrioventricular conduction in mice lacking $Ca_v3.1/\alpha_{1C}$ T-type calcium channels. *Circ Res* 2006; 98:1422-30.
66. Perez-Reyes E, Cribbs LL, Daud A, Lacerda AE, Barclay J, Williamson MP, et al. Molecular characterization of a neuronal low-voltage-activated T-type calcium channel. *Nature* 1998; 391:896-900.
67. Niwa N, Yasui K, Ophof T, Takemura H, Shimizu A, Horiba M, et al. $Ca_v3.2$ subunit underlies the functional T-type Ca^{2+} channel in murine hearts during the embryonic period. *Am J Physiol Heart Circ Physiol* 2004; 286:2257-63.
68. Ju Y-K, Chu Y, Chaulter H, Lai D, Gervasio OL, Graham RM, et al. Store-operated Ca^{2+} influx and expression of TRPC genes in mouse sinoatrial node. *Circ Res* 2007; 100:1605-14.
69. Biel M. Cyclic nucleotide-regulated cation channels. *J Biol Chem* 2009; 284:9017-21.
70. White TW, Srinivas M, Ripps H, Trovato-Salinaro A, Condorelli DF, Bruzzone R. Virtual cloning, functional expression and gating analysis of human connexin31.9. *Am J Physiol Cell Physiol* 2002; 283:960-70.
71. Söhl G, Nielsen PA, Eiberger J, Willecke K. Expression profiles of the novel human connexin genes hCx30.2, hCx40.1 and hCx62 differ from their putative mouse orthologues. *Cell Commun Adhes* 2003; 10:27-36.
72. Dvorakova M, Lips KS, Brüggmann D, Slavikova J, Kuncova J, Kummer W. Developmental changes in the expression of nicotinic acetylcholine receptor α -subunits in the rat heart. *Cell Tissue Res* 2005; 319:201-9.
73. Dai S, Hall DD, Hell JW. Supramolecular assemblies and localized regulation of voltage-gated ion channels. *Physiol Rev* 2009; 89:411-52.
74. Mueller RW, Gill SS, Pulido OM. The monkey (*Macaca fascicularis*) heart neural structures and conducting system: An immunohistochemical study of selected neural biomarkers and glutamate receptors. *Toxicol Pathol* 2003; 31:227-34.
75. Perrino C, Rockman HA. Reversal of cardiac remodeling by modulation of adrenergic receptors: a new frontier in heart failure. *Current Opinion in Cardiology* 2007; 22:443-9.
76. Weinberg DH, Trivedi P, Tan CP, Mitra S, Perkins-Barrow A, Borkowski D, et al. Cloning, expression and characterization of human α_1 adrenergic receptors α_{1A} , α_{1B} and α_{1C} . *Biochem Biophys Res Commun* 1994; 201:1296-304.
77. McCloskey DT, Turnbull L, Swigart P, O'Connell TD, Simpson PC, Baker AJ. Abnormal myocardial contraction in α_{1A} - and α_{1B} -adrenoceptor double-knockout mice. *J Mol Cell Cardiol* 2003; 35:1207-16.
78. Rivard K, Trepanier-Boulay V, Rindt H, Fiset C. Electrical remodeling in a transgenic mouse model of α_{1B} -adrenergic receptor overexpression. *Am J Physiol Heart Circ Physiol* 2009; 296:704-18.
79. Demolombe S, Marionneau C, Le Bouter S, Charpentier F, Escande D. Functional genomics of cardiac ion channel genes. *Cardiovasc Res* 2005; 67:438-47.
80. Borlak J, Thum T. Hallmarks of ion channel gene expression in end-stage heart failure. *FASEB J* 2003; 17:1592-608.
81. Moss AJ, Kass RS. Long QT syndrome: from channels to cardiac arrhythmias. *J Clin Invest* 2005; 115:2018-24.
82. Bezzina CR, Wilde AAM, Roden DM. The molecular genetics of arrhythmias. *Cardiovasc Res* 2005; 67:343-6.
83. Nattel S, Burstein B, Dobrev D. Atrial remodeling and atrial fibrillation: Mechanisms and implications. *Circ Arrhythmia Electrophysiol* 2008; 1:62-73.
84. Shieh C-C, Coghlan M, Sullivan JP, Gopalakrishnan M. Potassium channels: Molecular defects, diseases and therapeutic opportunities. *Pharmacol Rev* 2000; 52:557-94.
85. Bristow MR, Reynolds MVA, Port JDA, Rasmussen RA, Ray PEA, Feldman AM. Reduced beta1 receptor messenger RNA abundance in the failing human heart. *J Clin Invest* 1993; 92:2737-45.
86. Tsuji Y, Zicha S, Qi X-Y, Kodama I, Nattel S. Potassium channel subunit remodeling in rabbits exposed to long-term bradycardia or tachycardia: Discrete arrhythmogenic consequences related to differential delayed-rectifier changes. *Circulation* 2006; 113:345-55.
87. Zicha S, Xiao L, Stafford S, Cha TJ, Han W, Varro A, et al. Transmural expression of transient outward potassium current subunits in normal and failing canine and human hearts. *J Physiol* 2004; 561:735-48.
88. Lebeche D, Kaprielian R, del Monte F, Tomaselli G, Gwathmey JK, Schwartz A, et al. In vivo cardiac gene transfer of $K_v4.3$ abrogates the hypertrophic response in rats after aortic stenosis. *Circulation* 2004; 110:3435-43.
89. Donahue JK. Gene therapy for cardiac arrhythmias: A dream soon to come true? *J Cardiovasc Electrophysiol* 2007; 18:553-9.
90. Crambert G, Füzesi M, Garty H, Karlisch S, Geering K. Phospholemman (FXD1) associates with Na,K-ATPase and regulates its transport properties. *Proc Natl Acad Sci USA* 2002; 99:11476-81.
91. Zhang X-Q, Ahlers BA, Tucker AL, Song J, Wang J, Moorman JR, et al. Phospholemman inhibition of the cardiac Na^+/Ca^{2+} exchanger: Role of phosphorylation. *J Biol Chem* 2006; 281:7784-92.
92. Qu YS, Isom LL, Westenbroek RE, Rogers JC, Tanada TN, McCormick KA, et al. Modulation of cardiac Na^+ channel expression in *Xenopus* oocytes by $\beta 1$ subunits. *J Biol Chem* 1995; 270:25696-701.
93. Lopez-Santiago LF, Meadows LS, Ernst SJ, Chen C, Malhotra JD, McEwen DP, et al. Sodium channel *Scn1b* null mice exhibit prolonged QT and RR intervals. *J Mol Cell Cardiol* 2007; 43:636-47.
94. Hesse M, Kondo CS, Clark RB, Su L, Allen FL, Geary-Joo CTM, et al. Dilated cardiomyopathy is associated with reduced expression of the cardiac sodium channel *Scn5a*. *Cardiovasc Res* 2007; 75:498-509.
95. Deschênes I, Armoundas AA, Jones SP, Tomaselli GF. Post-transcriptional gene silencing of KChIP2 and $Na_v\beta 1$ in neonatal rat cardiac myocytes reveals a functional association between Na and I_{Na} currents. *J Mol Cell Cardiol* 2008; 45:336-46.
96. Dulhunty AF, Pouliquin P, Coggan M, Gage PW, Board PG. A recently identified member of the glutathione transferase structural family modifies cardiac RyR2 substrate activity, coupled gating and activation by Ca^{2+} and ATP. *Biochem J* 2005; 390:333-43.
97. Ferron L, Davies A, Page KM, Cox DJ, Leroy J, Waihe D, et al. The stargazin-related protein $\gamma 7$ interacts with the mRNA-binding protein heterogeneous nuclear ribonucleoprotein A2 and regulates the stability of specific mRNAs, including $Ca_v2.2$. *J Neurosci* 2008; 28:10604-17.
98. Tomita S, Chen L, Kawasaki Y, Petralia RS, Wenthold RJ, Nicoll RA, et al. Functional studies and distribution define a family of transmembrane AMPA receptor regulatory proteins. *J Cell Biol* 2003; 161:805-16.
99. Kato AS, Zhou W, Milstein AD, Knierman MD, Siuda ER, Drotzl JE, et al. New transmembrane AMPA receptor regulatory protein isoform, $\gamma 7$, differentially regulates AMPA receptors. *J Neurosci* 2007; 27:4969-77.
100. Hucker WJ, Ripplinger CM, Fleming CP, Fedorov VV, Rollins AM, Efimov IR. Bimodal biophotonic imaging of the structure-function relationship in cardiac tissue. *J Biomed Optics* 2008; 13:054012.
101. Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, Stoeckert C, et al. Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. *Nat Genet* 2001; 29:365-71.