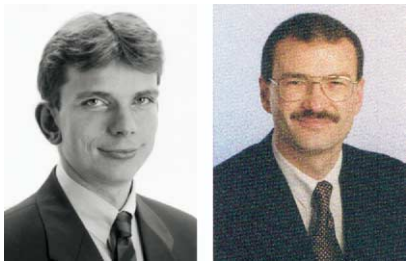
Comparing the global mRNA expression profile of human atrial and ventricular myocardium with high-density oligonucleotide arrays

Peter Ellinghaus, PhD,[a] Robert J. Scheubel, MD,[b] Dobromir Dobrev, MD,[c] Ursula Ravens, MD,[c] Juergen Holtz, MD,[d] Joachim Huetter, MD,[a] Ulrich Nielsch, PhD,[a] and Henning Morawietz, PhD[e]



Ellinghaus and Morawietz (*left to right*)

Objectives: The knowledge of chamber-specific gene expression in human atrial and ventricular myocardium is essential for the understanding of myocardial function and the basis for the identification of putative therapeutic targets in the treatment of cardiac arrhythmia and heart failure. In this study the gene expression pattern of human left atrial and ventricular myocardium was analyzed.

Methods: Global mRNA expression patterns with high-density oligonucleotide arrays between left atrial and left ventricular myocardium of 6 patients with heart failure undergoing heart transplantation were compared. Clustering of microarray data confirmed chamber-specific gene expression profiles. Genes similarly expressed in all patients were further analyzed, and data were confirmed by means of real-time polymerase chain reaction and Western blot analysis.

Results: Of 22,215 genes examined, 7115 transcripts were found to be expressed in all 12 human myocardial samples. One hundred twenty-five genes were differentially expressed between left atrial and left ventricular specimens in all patients examined. Novel genes preferentially expressed in human atria were identified. Interestingly, several potassium channels of subfamily K are more highly expressed in atria than in ventricles. Members of the potassium inwardly rectifying channel of subfamily J were found to be more highly expressed in human ventricular myocardium. Finally, chronic atrial fibrillation was associated with reduced atrial expression of the potassium channel TWIK-1, suggesting potential contribution of the corresponding current to electrical remodeling.

Conclusions: Human atria and ventricles show specific gene expression profiles. Our data provide the basis of a comprehensive understanding of chamber-specific gene expression in diseased human hearts and will support the identification of therapeutic targets in the treatment of arrhythmia and heart failure.

From the Institute of Cardiovascular Research,[a] Bayer AG, Wuppertal, Germany; the Department of Cardiothoracic Surgery[b] and the Institute of Pathophysiology,[d] Martin Luther University, Halle-Wittenberg, Germany; and the Departments of Pharmacology and Toxicology[c] and Vascular Endothelium and Microcirculation,[e] University of Technology, Dresden, Germany.

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Address for reprints: Henning Morawietz, PhD, Department of Vascular Endothelium and Microcirculation, Medical Faculty Carl Gustav Carus, University of Technology Dresden, Fetscherstr. 74, D-01307 Dresden, Germany (E-mail: Henning.Morawietz@mailbox.tu-dresden.de).

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The functional difference between atrial and ventricular myocardium could lead to chamber-specific gene expression. Markers of the atrial myocardium (eg, atrial natriuretic peptide)[1] and ventricular myocardium (eg, ventricular myosin light chain 1)[2] have been established. However, a comprehensive knowledge of chamber-specific gene expression in the human heart is essential for the understanding of myocardial function and the basis for the identification of putative novel drug targets in the specific treatment of cardiac arrhythmia, such as atrial fibrillation (AF) and heart failure.[3]

AF is the most frequent sustained arrhythmia, especially in the elderly and postoperatively as a common complication of cardiac surgery, and has been associated with an increased incidence of other complications and increased hospital length of stay. Most antiarrhythmic drugs are ion channel blockers.[4] However, those tested in a large clinical trial did not reduce mortality. In contrast, their proarrhyth-

mic side effects limit their use to those patients without any structural heart disease. In this context the identification of atrium-specific or at least atrium-enriched expression of genes is a first step in understanding the molecular basis of AF and identifying new drug targets for AF.

Recently, the Human Genome Project supported the development of microarrays containing most of the currently known well-annotated genes of the entire genome, allowing the identification of differentially expressed genes. Therefore, we used high-density oligonucleotide arrays (Affymetrix HG-U133A) to compare the expression of more than 22,000 transcripts between the left atrial and left ventricular myocardium of 6 patients undergoing heart transplantation. We determined the number of genes expressed in the human heart, found chamber-specific gene expression pattern, and identified putative new drug targets in the treatment of arrhythmias and heart failure.

Methods

Patients

Left atrial and ventricular specimens were obtained from 6 male patients of the Halle Cardiac Transplant Program undergoing orthotopic cardiac transplantation. After explantation, hearts were immediately dissected into segments from the left atrial or left ventricular free lateral wall appearing to consist of myocardium and avoiding regions with scar tissue and excessive fat or connective tissue and snap-frozen in liquid nitrogen. The patients were 57 ± 3.2 years old. The hemodynamic data were obtained from the date at which the last echocardiographic determination of the ejection fraction ($20.5\% \pm 2.3\%$) before transplantation was performed (mean arterial pressure, 78.8 ± 4.9 mm Hg; pulmonary capillary wedge pressure, 19.8 ± 2.4 mm Hg; cardiac index, 2.5 ± 0.1 L $\cdot$ min^{-1} $\cdot$ m^{-2}). The patients had end-stage heart failure (New York Heart Association functional class IV; 3 with ischemic cardiomyopathy and 3 with dilated cardiomyopathy) and were treated with angiotensin-converting enzyme inhibitors, angiotensin II receptor type 1 (AT_1) antagonists, β-blockers, nitric oxide donors, heart glycosides, lipid-lowering drugs, and diuretics.

Right atrial appendages were obtained from 8 patients with sinus rhythm (SR) and 5 patients with chronic AF (>6 months) undergoing cardiac surgery for coronary artery bypass grafting. The 2 groups of patients did not differ in terms of sex, age, body mass index, hypertension, diabetes, hyperlipidemia, or concomitant therapy with angiotensin-converting enzyme inhibitors, AT_1 blockers, lipid-lowering drugs, β-blockers, diuretics, nitrates, or glycosides. The study was approved by the local ethics committee. Each patient provided written informed consent. The investigation conforms to the principles outlined in the Declaration of Helsinki (1997).

Gene Array Analysis

RNA and protein isolation. Left atrial and ventricular specimens of patients were dissected immediately after explantation, snap-frozen, and stored in liquid nitrogen until RNA or protein preparation. Total RNA from myocardial tissue was isolated with the RNeasy total RNA isolation system (Qiagen). Protein from atrial and ventricular specimens was isolated by means of mechanical homogenization (Ultraturrax, Janke and Kunkel) in phosphate-buffered saline containing 1% (vol/vol) Triton X-100 and Complete protease inhibitor cocktail (Roche Diagnostics), followed by centrifugation to remove insoluble material (30 minutes, 14,000 rpm, 4°C). The protein concentration was determined with the BCA protein assay reagent (Pierce).

Array hybridization. First- and second-strand cDNA synthesis was carried out with 10 μg of total RNA according to the Affymetrix Expression Analysis Technical Manual with the High Yield RNA Transcript Labelling Kit (Enzo). The obtained cRNA was purified with RNeasy Mini columns (Qiagen) concentrated by means of ethanol precipitation and quantified by means of UV spectrophotometry. Yield and size distribution of the labeled transcripts were determined on a Bioanalyzer (Agilent). Each Affymetrix HG-U133A array was hybridized overnight at 45°C with 15 μg of labeled and fragmented cRNA. After hybridization, the gene chips were automatically washed and stained with streptavidin-phycoerythrin by using a fluidic station (Affymetrix). The arrays were analyzed with a Genechip System (Agilent).

Microarray analysis. Affymetrix Microarray Suite software version 5.0 (MAS 5.0) was used to analyze the relative abundance of each gene on the basis of the intensity of the signal from each probe set. A global scaling approach was used to normalize signal intensities. A target signal value of 500 was used for all arrays. The MAS 5.0 data files were then transferred into a database and analyzed with Microsoft Excel and GeneSpring 6.0 software (Silicon genetics). The detection algorithm of MAS 5.0 software was used to obtain a comprehensive overview of how many transcripts can be detected in each individual sample. We used significance levels of $P < .04$ (present) and $P > .06$ (absent) to define the cutoff points of P values for detection calls. MAS 5.0 software allows a comparative analysis of 2 or more microarray datasets. Only genes considered to be differentially expressed (increase or decrease) between atrial and ventricular specimens of the same donor were further analyzed. Instead of calculating the fold change by analyzing raw signal intensities, we used the signal log ratio (SLR) generated by MAS 5.0 software, allowing a more sensitive detection of smaller changes in gene expression, and calculated the fold change as follows: $FC = 2SLR$.

Validation with real-time polymerase chain reaction. The differential expression of genes identified by using oligonucleotide arrays was confirmed by means of real-time polymerase chain reaction (PCR) with the TaqMan protocol by using an ABI Prism 7700 sequence detection instrument, as described previously.[5] Because the calculated expression level between different samples was independent of the housekeeping gene used, we decided to normalize all mRNA expression data to ribosomal protein L32. The resulting expression shows the mean of 3 independent experiments and is presented in arbitrary units.

Western blot analysis. Western blot analysis was performed by using 10 μg of protein per sample, 4% to 12% NUPAGE polyacrylamide gels (Invitrogen), and polyvinylidene difluoride membranes. Expression of TASK-1 protein was determined by using anti-human TASK-1 antibody (Sigma), the ECL system (Amersham), and AIDA software (Raytest, Isotopenmessgeraete GmbH).

TABLE 1. Genes more than 2-fold higher expressed in atrial myocardium compared with ventricular myocardium

Gene	Fold change* (mean), n = 6	Accession no.	*P* value
Sarcolipin	9.62	NM_003063	.0001
Myosin, light polypeptide 4, alkali; atrial, embryonic	9.19	M36172	<.0001
A kinase (PRKA) anchor protein 3	8.67	NM_006422	.0124
Potassium channel, subfamily K, member 1 (TWIK-1)	6.73	U90065	<.0001
Myosin, heavy polypeptide 6, cardiac muscle	5.59	D00943	<.0001
Titin immunoglobulin domain protein (myotilin)	5.34	NM_006790	.0022
Signal transducer and activator of transcription 4	5.22	NM_003151	.0002
Nuclear receptor subfamily 2, group F, member 1 (COUP-TF 1)	4.87	AI951185	.0001
NADP-dependent retinol dehydrogenase-reductase	4.24	NM_005771	.0008
Natriuretic peptide precursor B	4.24	NM_002521	<.0001
Desmocollin 1	4.24	NM_004948	<.0001
Potassium voltage-gated channel, shaker-related subfamily, member 5	4.09	NM_002234	<.0001
Secreted frizzled-related protein 1	4.05	NM_003012	.0001
Phospholipase A2, group IIA (platelets, synovial fluid)	3.95	NM_000300	.0108
Keratin 18	3.78	NM_000224	.0042
dickkopf homolog 3 (*Xenopus laevis*)	3.73	NM_013253	.0001
Natriuretic peptide precursor A	3.69	M30262	.0006
Guanine nucleotide-binding protein (G protein), β5	3.52	NM_006578	.0001
Potassium channel, subfamily K, member 3 (TASK-1)	3.36	NM_002246	<.0001
H factor 1 (complement)	3.32	X04697	.0030
Upregulated by BCG-CWS	3.10	NM_022154	.0001
Phosphodiesterase 8B	2.96	AK023913	<.0001
Cartilage oligomeric matrix protein	2.86	NM_000095	.0018
Complement component 3	2.70	NM_000064	.0135
Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican)	2.70	AF231124	.0002
Phospholipase C, β1 (phosphoinositide-specific)	2.70	AL049593	<.0001
Chromosome 1 open reading frame 15	2.64	AF288395	<.0001
Corin	2.58	NM_006587	.0012
Myosin light chain 2a	2.49	NM_021223	<.0001
Transmembrane 6 superfamily member 1	2.43	NM_023003	<.0001
FK506 binding protein 11, 19 kd	2.41	NM_016594	<.0001
Visinin-like 1	2.41	NM_003385	.0002
Angiotensin II receptor, type 1	2.41	NM_004835	.0004
H factor (complement)-like 2	2.32	X56210	.0085
NY-REN-58 antigen	2.30	NM_016122	<.0001
Similar to neuralin 1	2.22	AL049176	.0171
Duffy blood group	2.07	NM_002036	.0108
Transgelin	2.05	NM_003186	.0005
Potassium intermediate/small conductance calcium-activated, KCNN2	2.02	NM_021614	.0006
Endothelin receptor type A	2.02	NM_001957	.0009

*Atrial versus ventricular myocardium.

Statistical analysis. Values are presented as means ± SD. Statistical analysis was performed with the SigmaStat statistical software (SPSS Inc).

Results

Chamber-specific Gene Expression in the Human Heart

The Affymetrix HG-U133A microarray allows, in principle, the simultaneous quantification of expression of 22,215 mRNA species. To determine the number of transcripts expressed in the failing human myocardium, we quantified the mRNAs expressed in all 12 left atrial or left ventricular biopsy specimens. The detection algorithm of Affymetrix MAS software allows a potential vote for each single array experiment in determining whether the measured transcript is detected (present) or not detected (absent). By using this algorithm, 7115 transcripts were found to be expressed in all 12 human myocardial samples. However, the number of mRNA species detected at least once in a single human myocardial biopsy specimen was much higher (12,522). This last

TABLE 2. Genes more than 2-fold higher expressed in ventricular myocardium compared with atrial myocardium

Gene	Fold change* (mean), n = 6	Accession no.	*P* value
Delta-like 1 homolog (Drosophila)	0.07	U15979	.0002
Myosin, light polypeptide 3, alkali; ventricular, skeletal, slow	0.10	NM_000258	<.0001
HSKM-B protein	0.10	AF070592	.0025
Ankyrin repeat domain 2 (stretch-responsive muscle)	0.16	NM_020349	.0017
KIAA1733 protein	0.19	AW054711	<.0001
Four-and-a-half LIM domain–containing protein 2	0.20	NM_001450	.0006
Carboxypeptidase, vitellogenic-like	0.27	NM_031311	.0001
Protein tyrosine phosphatase, nonreceptor type 3	0.28	NM_002829	<.0001
Myosin, light polypeptide 2, regulatory, cardiac, slow	0.31	AF020768	.0001
Gamma-aminobutyric acid (GABA) A receptor, α 4	0.33	NM_000809	<.0001
Dihydropyrimidinase-like 4	0.35	NM_006426	.0349
Hypothetical protein FLJ20156	0.36	NM_017691	.0069
Hypothetical protein FLJ14054	0.37	NM_024563	<.0001
Potassium inwardly-rectifying channel, subfamily J, member 2	0.39	AF153820	<.0001
Hypothetical protein FLJ32389	0.41	AL551046	.0001
Ribosomal protein L3-like	0.41	NM_005061	.0001
NDRG family member 4	0.41	AV724216	.0004
Hairy/enhancer-of-split related with YRPW motif 2	0.43	NM_012259	.0062
IMAGE:3862861, mRNA, complete cds	0.44	BG332462	<.0001
Isocitrate dehydrogenase 2 (NADP+), mitochondrial	0.44	U52144	<.0001
Likely ortholog of mouse limb-bud and heart gene	0.45	NM_030915	.0001
Hypothetical protein FLJ21901	0.45	NM_024622	<.0000
Phospholipase C-like 1	0.46	NM_006226	.0002
Lipoprotein lipase	0.46	NM_000237	.0001
LRP16 protein	0.47	NM_014067	<.0001
Phosphofructokinase, muscle	0.49	U24183	<.0001

*Atrial versus ventricular myocardium.

number also includes genes expressed in atrial myocardium but with an expression level of less than the detection limit in the ventricle and vice versa.

Interestingly, the number of expressed genes detected in human atrial specimens is consistently higher than that detected in the ventricular specimen from the same donor. In atrial myocardium an average of 46.2% ± 1.2% of the 22,215 mRNA species on the Affymetrix HG-U133A array could be detected, whereas the average percentage for ventricular specimens was only 42.2% ± 1.4% ($P < .05$). To perform a comprehensive analysis of all genes differentially expressed between human atrial and ventricular myocardium, we used the change call algorithm of Affymetrix software for subsequent gene selection. The change call algorithm is totally independent from the absent-present detection algorithm and allows a reproducible decision as to whether a transcript level is increased or decreased to a minor or major degree or not changed by comparing 2 different array experiments. Thus, by using the change call algorithm, it is possible to identify changes in the transcript level between the atrium and ventricle of the same donor, even if the expression is too low for a present call on one array. Only genes consistently differentially expressed between duplicate arrays were further analyzed. One hundred twenty-five genes were identified to be differentially expressed between left atrial and ventricular specimens in all 6 patients examined. Of those, Table 1 shows 40 genes with a more than 2.0-fold higher expression in the left atrium compared with that seen in the left ventricle, whereas Table 2 shows 26 genes with a more than 2.0-fold change in the left ventricle compared with that seen in the left atrium.

Hierarchical Clustering of Array Experiments

Hierarchical clustering of all array experiments was carried out to further confirm the chamber-specific gene pattern. In the agglomerative approach for hierarchical clustering, the chamber-specific expression profile of each patient is joined with the profiles of the other patients to form groups, resulting in a hierarchical tree.[6] For this hierarchical clustering, we selected only those 7115 mRNA species detected in all 12 myocardial samples. By using this approach, the software allowed a correct classification into atrial and ventricular specimens. However, when comparing only atrial or ventricular specimens, no correlation of similarity in the gene expression pattern with cause (eg, differences between hearts from patients with ischemic or dilated car-

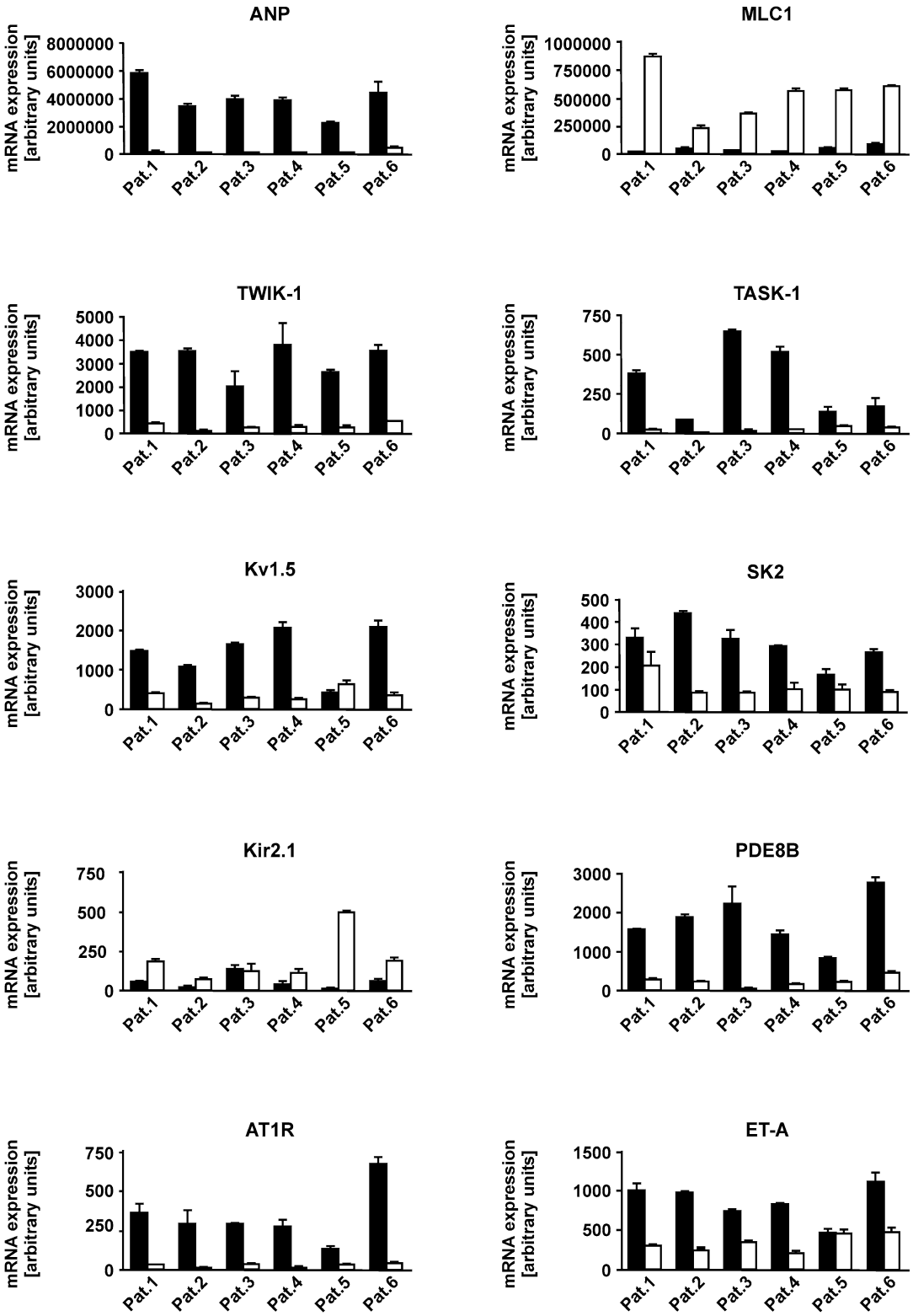

Figure 1. Real-time PCR (TaqMan) verification of selected genes consistently differentially expressed between atrial and ventricular specimens from all 6 patients. *Solid bars* represent normalized mRNA expression in the left atrium; *open bars* represent the expression level in the left ventricles (± SD calculated from 3 independent measurements).

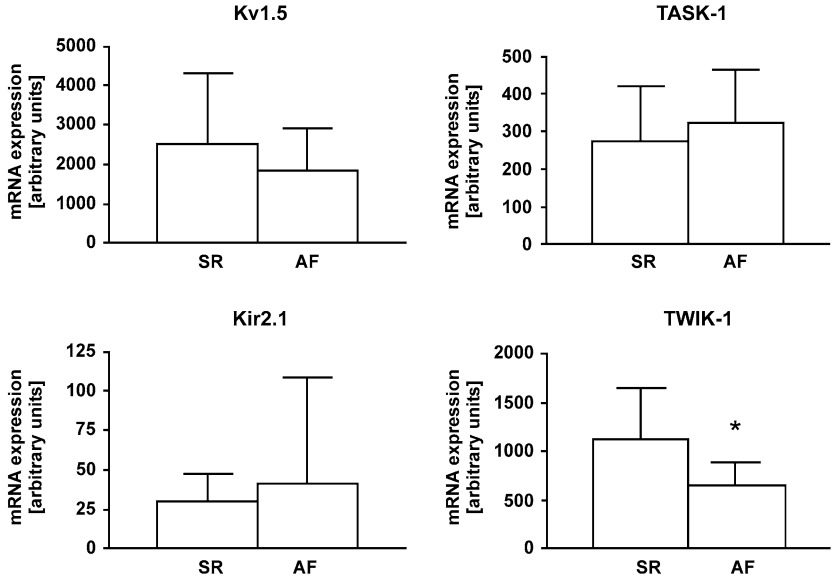

Figure 2. Expression of potassium channels in atrial myocardium of patients with SR and AF. The mRNA expression of the potassium channels Kv1.5, TASK-1, Kir2.1, and TWIK-1 was quantified by means of real-time PCR (TaqMan) in the atrial myocardium of 13 patients with coronary artery disease undergoing coronary artery bypass grafting surgery and with SR or chronic AF. *Bars* represent normalized mRNA expression (± SD; *$P < .05$ vs SR).

diomyopathy) could be observed by means of unsupervised hierarchical clustering of microarray data.

The chamber-specific expression pattern was not caused by a different content of vascular tissue in the specimen selected because the expression of marker genes for endothelial cells (eg, von Willebrand factor and endothelial cell-specific Tie2 receptor tyrosine kinase) or vascular smooth muscle cells (eg, smooth muscle–specific myosin heavy chain 1 and smooth muscle isoform of myosin regulatory light chain 2) did not differ between left atrial or ventricular samples in microarray data. Furthermore, markers of neuronal tissue (eg, neuronal specific transcription factor DAT1 and neuronal amiloride–sensitive cation channels 1 and 2) and markers of increased infiltration of inflammatory cells (eg, macrophage antigen α polypeptide CD11b) were not differentially expressed in specific myocardial samples.

Specific Genes with a Preferential Atrial or Ventricular Expression

The correct classification of all specimens into left atria and ventricles was furthermore examined by means of real-time PCR analysis of the atrial marker gene atrial natriuretic peptide and the ventricular marker gene ventricular myosin light chain 1. Chamber-specific marker gene expression confirmed the anatomic and histologic classification. Chamber-specific expression of several putative drug targets in the treatment of arrhythmia or heart failure was confirmed by means of real-time PCR. We found a preferred atrial expression of putative or established drug targets (eg, phosphodiesterase 8B, endothelin receptor type A, and AT_1; Figure 1). Several potassium channels, including TWIK-1 (KCNK1), TASK-1 (KCNK3), Kv1.5 (KCNA5), and SK2 (KCNN2), are more highly expressed in the atrium than in the ventricle, whereas a higher ventricular expression of potassium channel Kir2.1 (KCNJ2) was observed (Figure 1). For TASK-1, a commercially available antibody allowed the confirmation of differential expression also on the protein level.

Potassium Channel Expression in Patients with Chronic AF

Next, we studied whether expression of the potassium channels Kv1.5, TASK-1, Kir2.1, and TWIK-1 is modified in the atria of patients with chronic AF. We found no change in Kv1.5, TASK-1, and Kir2.1 expression between the SR and AF groups. However, TWIK-1 mRNA was less abundant in the AF than in the SR group (Figure 2).

Discussion

In this study we compared the chamber-specific expression pattern in failing human hearts. The number of more than 22,000 mRNA species that can be detected with the Affymetrix HG-U133A array allows a comprehensive view of the number of genes expressed in human atrial and ventricular myocardium. We found more than 7000 mRNA species to be expressed in all 12 human myocardial samples. This number highlights the number of active genes in the human heart but might be due to the detection limit of the array technology, even an underestimation.[7] Furthermore, individual genetic or clinical differences between patients might reduce the number of genes consistently expressed in all atrial or ventricular specimens. For the specific gene expression profile, the anatomic and functional differences be-

tween atrial and ventricular myocardium will account. These differences might include chamber-specific hemodynamic parameters and tissue composition. However, we did not find a different expression of markers of endothelial, vascular smooth muscle, neuronal, or inflammatory cells between left atrial and left ventricular specimens.

We could confirm a chamber-specific expression pattern for several genes that have recently been identified to be expressed in murine hearts in a similar manner (eg, preferred atrial expression of sarcolipin,[8] dickkopf homolog 3 [*Xenopus laevis*], and myosin light chain 2a[9]) and a preferred ventricular expression of four-and-a-half LIM domain–containing protein 2.[10] The chamber-specific expression pattern of "up-regulated by BCG-CWS," dickkopf homolog 3 (*X laevis*), and ankyrin repeat domain 2 found by us is in agreement with recent studies using RZPD filters preferentially directed to identify genes expressed in human congenitally malformed hearts.[11] The 2-fold higher atrial expression of endothelin receptor A is in agreement with our previous findings in the atrial and ventricular myocardium of patients with heart failure.[12,13]

Recently, in an excellent study Ruel and colleagues[14] described the changes in gene expression profile in right atrial myocardium and skeletal muscle before and approximately 1 hour after cardiopulmonary bypass and cardioplegic arrest. In this study we compared the gene expression pattern between the left atria and left ventricles of hearts explanted from patients undergoing heart transplantation and identified a different set of genes. This further supports the specificity of our findings.

Regarding putative new drug targets for the treatment of AF, we found a higher expression of several potassium channels in atrial myocardium compared with that seen in ventricular myocardium. This is of special interest because it has long been known that blockade of cardiac potassium channels and the resulting prolongation of repolarization and refractoriness is the mode of action of class III antiarrhythmic drugs, which are currently used for the treatment of AF. Potassium channels encoded by HERG underlie the delayed rectifier current (I_{Kr}), a sensitive target for most class III antiarrhythmic drugs, including methanesulfonamides, such as sotalol. However, their clinical use is limited by their side effects in the form of ventricular tachycardia because prolongation of ventricular repolarization seems to be invariably associated with proarrhythmia. Not much is known about the differences in the regional expression of the corresponding molecular targets for these drugs. In general, blockade of a cardiac current that is exclusively present in the atria is highly desirable because it is expected to be devoid of ventricular proarrhythmic effects.[15]

In the human heart the ultrarapid delayed rectifier potassium current (I_{Kur}) and its molecular correlate, the cardiac Kv1.5 channel, were identified in atrial but not in ventricular tissue by means of electrophysiologic experiments. Therefore, our finding that the Kv1.5 channel has a preferred atrial mRNA expression supports the idea that this potassium channel is an attractive target in the search for novel and safer atrial antiarrhythmic drugs.[16] In accordance with our findings and with electrophysiologic experiments, an atrium-enriched expression of Kv1.5 mRNA was previously described.[17] Moreover, expression of Kv1.5 channel subunits and current density were lower in the atria of patients with chronic AF compared with those of patients with SR.[18] Here the mRNA levels of Kv1.5 did not differ between patients with SR and those with AF. Therefore, the reduction of I_{Kur} might occur only in a subpopulation of patients with chronic AF.[19]

We furthermore identified 2-pore domain potassium channels to be preferentially expressed in atrial myocardium. For TASK-1, a preferred atrial expression has thus far only been demonstrated in rats.[20] In patients with chronic AF, TASK-1 mRNA levels were similar to those seen in patients with SR, which contrasts with the reduced abundance of several K^+ currents during AF.[19] Because contribution of TASK-1 to action potential repolarization should be larger in AF than in SR, blocking this channel might prolong action potential duration in these patients and represents a new therapeutic option for AF treatment.

Interestingly, the mRNA levels of the inward rectifier K^+ channel TWIK-1 were significantly lower in patients with AF than in patients with SR, suggesting potential contribution of the corresponding current to electrical remodeling.

Regarding ventricular-enriched genes, microarray data revealed that the potassium channel Kir2.1 (KCNJ2) is more abundantly expressed in ventricular myocardium compared with in atrial myocardium in all patients. Because of the limited availability of corresponding tissues, we could analyze Kir2.1 expression in the atrial myocardium of patients with AF only. Atrial Kir2.1 mRNA expression was unchanged. These findings contrast with recent results showing that current density of the inward rectifier K^+ current (I_{K1}) and mRNA levels of one of its channel subunits (Kir2.1) were 2-fold higher in right atrial tissue of patients with AF.[21]

We found a predominant expression of AT_1 in left atrial myocardium. The role of the AT_1 receptor in AF is not clear. Although initial studies showed a downregulation of atrial AT_1 mRNA and protein expression in patients with AF,[22] an increased AT_1 protein expression was recently described in the left atrial tissue of patients with AF.[23] In contrast, the expression of endothelin receptor type A is not altered in patients with AF.[24]

In conclusion, a specific gene expression profile was found in human atrial and ventricular myocardium. Our data provide the basis of a comprehensive understanding of chamber-specific gene expression in the human heart and

will support the identification of therapeutic targets in the treatment of cardiac arrhythmia and heart failure.

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