Characterization of the cardiac KCNE1 gene promoter

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Abstract

Background: KCNE1 encodes an essential cardiac slow delayed-rectifier potassium current (I\textsubscript{Ks}) β-subunit (minK). Varying minK expression is important in disease-related remodeling and species-dependent expression. This study addressed 5′-regulatory elements that potentially control KCNE1 transcription.

Methods and results: The transcriptional start site of human KCNE1 (HKCNE1) was determined with 5′-RACE. Of four isoforms, the putative promoter driving the isoforms constituting >80% expression in human hearts was further analyzed. A 1625-bp region 5′ to the transcriptional start site was subcloned into luciferase-reporter plasmid (PGL3-Basic). The full promoter sequence increased luciferase expression 31-fold in neonatal rat cardiomyocytes (NRMs). A much smaller 327-bp core promoter maintained activity 21–29 fold. The core promoter conferred cardiomyocyte-preferential expression, with an activity in NRMs 4.9-fold greater than in Chinese Hamster Ovary cells (CHOs), compared to ∼2.0 for the full-length promoter. Site-directed mutagenesis of all three GATA elements in the core promoter reduced its activity by >50% and attenuated cardiomyocyte-preferential expression. Mutagenesis of the second GATA element alone decreased promoter activity by ∼50%. GATA4 knockdown with siRNA inhibited ∼40% of core promoter activity in NRMs. Angiotensin-II increased HKCNE1 promoter activity, but only in the presence of intact GATA elements. The typically low-level I\textsubscript{Ks} expression in mouse and rabbit is related to low minK expression. Cloning of the mouse KCNE1 (MKCNE1) 5′-regulatory region showed ∼50% sequence identity to human. MKCNE1 had only 1 GATA element in the region corresponding to the human core promoter and had less promoter activity (11.7 vs 29.0-fold PGL3-Basic for human).

Conclusion: Promoter elements in the HKCNE1 5′-end, particularly GATA binding sites, may be important in tissue, disease and species-related transcriptional regulation of I\textsubscript{Ks}.

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Keywords: Arrhythmia (mechanisms); Gene expression; Ion channels; K⁺-channel; Long QT syndrome

1. Introduction

K⁺-channels play an important role in the regulation of resting membrane potential, heart rate and action potential configuration [1,2]. K⁺-channels contain pore-forming α-subunits that co-assemble with accessory β-subunits to stabilize channel structure, promote membrane insertion and regulate gating and conductance [1,3,4]. The KCNE gene family encodes single transmembrane-spanning β-subunit proteins [3]. The first KCNE-encoded protein to be described, minK (KCNE1), discovered in 1988 [5], is expressed in various tissues including heart, ear, colon, uterus and lymphocytes [6,7].

KCNE1 encodes a 129–130 amino-acid protein that associates with the α-subunit KvLQT1 to form the slow delayed-rectifier current I\textsubscript{Ks} [3]. I\textsubscript{Ks} plays a key role in cardiac action potential repolarization, particularly under repolarization stress [8,9]. Mutations in the human KCNE1 gene (HKCNE1) cause autosomal dominant Romano-Ward (RW) and rarer autosomal recessive Jervell and Lange-
Nielsen (JLN) forms of long QT syndromes [6,7,10,11]. Recently, \( I_{\text{ KC }} / \text{minK} \) remodeling has been observed in cardiac-disease models [12–15], with mRNA expression changes pointing to transcriptional regulation. HKCNE1 maps to human chromosome 21 (21q22.1–q22.2), containing three exons and two introns [16]. The first two exons encode \(^5\) untranslated regions (UTRs), whereas the third exon contains the entire coding region and \(^3\) UTR [16]. The \( KCNE1 \) coding region is relatively conserved: there is 92% amino-acid sequence identity between mouse and rat and ~76% between mouse and human. \( HKCNE1 \) is important in human cardiac physiology and pathophysiology [17,18], but minK expression is weak in mouse [19] and rabbit [20] hearts, corresponding to very small \( I_{\text{ KC }} \) density [21,22].

In the early 1990s, mouse and rat \( KCNE1 \) gene organization was characterized and putative promoter sequences identified but not analyzed [23,24]. Here, we describe experiments designed to define promoter regions important in controlling cardiac minK expression.

2. Experimental procedures

2.1. RNA and genomic DNA isolation

Total RNA was extracted from normal human and mouse heart with Trizol (Invitrogen). A human genomic clone (RPCI-11 Human BAC Clones) was obtained from Roswell Park Cancer Institute. Mouse genomic DNA was extracted from mouse heart. Human tissues were obtained from the Réseau de tissus pour études biologiques (RETEB) tissue bank under procedures approved by the Human Research Ethics Committee of the Montreal Heart Institute, tissue procurement and animal handling were consistent with NIH Guidelines.

2.2. Mapping of the HKCNE1 transcription start sites by \(^5\) RACE (rapid amplification of cDNA ends)

\(^5\)RACE was used to locate the transcription start site (TSS) of human cardiac \( KCNE1 \) transcripts with Ambion’s RNA ligase-mediated \(^5\)‘rapid amplification of cDNA ends (RLM-\(^5\)RACE), which reacts only with \(^5\)‘-capped-mRNA. Outer and Inner \( HKCNE1 \)-specific primers were based on the \( HKCNE1 \) sequence published by Splawski et al. [16], as shown in Table 1.

2.3. Quantification of \( HKCNE1 \) transcripts

To determine the abundance of various \( HKCNE1 \) transcripts, two-step real-time reverse transcription (RT)-quantitative polymerase chain reaction was performed with SYBR green I. The initial RT step was performed with random primers and MMLV reverse transcriptase (Invitrogen). For isoform quantification in human tissues, cDNAs were synthesized with RNA from normal human tissues

### Table 1

<table>
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<tr>
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<th>Sequences</th>
<th>Location</th>
<th>Application</th>
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Abbreviations: F: forward primer, R: reverse primer.
Primers for RT-QPCR crossed inter-exon junctions to avoid genomic-DNA contamination (Table 1). Absolute quantification was with a plasmid containing exon 1–2, exon 0.5–1A, and exon 2B–3 sequences. Plasmid DNA was serially diluted and used as a standard for real-time quantitative RT-PCR. Human GAPDH was the internal control. PCR products were verified based on dissociation curves and gel electrophoresis.

2.4. Construction of promoter-luciferase fusion plasmids

Variable lengths of the human and mouse KCNE1 promoter region were amplified by PCR. PCR primers were designed based on human GenBank sequence NT_011512 and mouse NT-082371 (Table 1). HKCNE1 5′-upstream regions were amplified with RPCI-11 human BAC clones and mouse (MKCNE1) 5′-upstream regions were amplified with mouse genomic DNA. PCR products were cloned into pCRII vector (Invitrogen) and then subcloned into the polylinker region of PGL3-Basic (Promega) with the appropriate restriction enzymes.

2.5. DNA sequencing and analysis

The promoter region sequences obtained by PCR were verified by DNA sequencing and the orientations of all constructs were confirmed by restriction endonuclease analysis and DNA sequencing. Sequencing results were analyzed with BLAST. Potential transcription factor binding sites were analyzed with TRANSFAC and alignment was performed with Clone manager V6.0.

2.6. Cell culture

Neonatal rat cardiomyocytes were isolated and cultured as described previously [25]. CHO cells were cultured in DMEM/F-12 (Invitrogen) plus 10% FBS and 1% penicillin-streptomycin in a humidified 5% CO2 incubator at 37 °C. 1 × 10⁵ cells/well were seeded in a 24-well plate for transfection.

2.7. Transfection and promoter activity measurements

KCNE1 promoter-luciferase fusion plasmids (1 μg) and 100 ng of PRL-TK plasmid (Renilla luciferase expression vector, an internal control for transfection efficiency) were co-transfected into neonatal rat cardiomyocytes and CHO cells with Lipofectamine 2000 (Invitrogen). Following transfection (48 h), the cells were lysed. Luciferase activity was measured on a Lumat LB9507 luminometer.

2.8. Mutation analysis of GATA elements

Three potential GATA4 binding sites (−294, −269 and −54) in PGL3-HKCNE1 (−311/+16) were mutated with the

![Fig. 1. 5′RACE results and proposed gene structure. Isoforms 1, 2 and 3 were identified by 5′RACE. Isoform 4 (BC046224) was identified by Blast search with GenBank. The positions and sizes of exons proposed in this figure are according to their location in GenBank segment NT_011512. There is an alternative splicing donor site within Exon 2 which gave rise to a 28 bp 5′ deletion (hatched box) of exon 2 in isoform 2. Two TSSs determined by 5′RACE are indicated by arrows.](image-url)
Primers listed in Table 1. GATA-F1, R1, F2 and R2 were used to mutate GATA-294 to TGCA; GATA-F1, R3, F3 and R2 were used to mutate GATA-269 to GTAG; GATA-F4, R4, F5 and R5 were used to mutate GATA-54 to AGCT. All primers were designed to avoid producing new transcription factor binding sites.

2.9. Silencing effects of GATA4 siRNA

GATA4 siRNA (50 nM, Santa Cruz biotechnology) and PGL3-HKCNE1 (−311/+16) with and without GATA mutations were co-transfected into neonatal rat cardiomyocytes with Lipofectamine 2000. GAPDH siRNA (Ambion) was used as a control.

2.10. Western blot

Neonatal rat cardiomyocytes (NRM) were transfected with 100 nM GATA4/GAPDH siRNA with Lipofectamine 2000. Forty-eight hours post-transfection, the cells transfected with siRNA GAPDH were lysed with RIPA buffer (solution dissolved in PBS containing 1.0% IGEPAL (Sigma), 0.5% Na+-deoxycholate, 0.1% SDS, 0.07% β-mercaptoethanol, pH 7.3) at 4 °C for 30 min. Following centrifugation (13,000 g, 30 min, 4 °C), the protein content of the supernatants was quantified. Nuclear extract from NRM transfected with GATA4 siRNA was prepared by adding a buffer (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol (DTT), 0.5 mM phenylmethylsulfonyl fluoride (PMSF))

Fig. 2. Human KCNE1 isoform quantification by real-time RT-QPCR. A. Absolute quantification of HKCNE1 isoforms 1 and 2, 3 and 4 expressions in different heart regions (n=3 per data point). Isoforms 1 and 2 were most abundant in all heart tissues. Because of their great similarity and shared promoter, isoforms 1 and 2 were quantified together. ***p<0.001 vs isoforms 1 and 2. B. Absolute quantification of HKCNE1 isoforms 1 and 2, 3 and 4 expression in various human tissues (n=3 per data point). Human GAPDH was used as an internal control.
fluoride (PMSF) and 10% IGEPAL) to the plate and incubating for 15 min (4 °C). The cells were collected with a scraper. The pellet was collected by centrifugation at 6000 rpm for 4 min and resuspended in 20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 10% Glycerol, 1 mM DTT and 1 mM PMSF. The tube was shaken at 4 °C for 2 h, centrifuged (14,000 rpm, 5 min, 4 °C), and supernatant containing nuclear extract collected. Whole-cell or nuclear extracts were fractionated on 12% SDS-PAGE and transferred electrophoretically to Immobilon-P polyvinylidene fluoride (PVDF) membranes (Millipore). Blots were probed with primary antibodies against GATA4 (Santa Cruz), GAPDH (Ambion), or actin (loading control, Santa Cruz) at room temperature for 18 h. Secondary antibodies were goat anti-mouse IgG or donkey anti-goat IgG conjugated to horseradish peroxidase (Santa Cruz) for 40 min.

2.11. Angiotensin-II stimulation

100 nM and 1 μM human synthetic angiotensin-II (Sigma) were used to stimulate NRMs transfected with PGL3-HKCNE1(−311/+16) and with PGL3-HKCNE1 (−311/+16, GATA (−269) mutated). Angiotensin-II was included in serum-free medium beginning 24 h after transfection.

**Fig. 3.**

A. Analysis of HKCNE1 isoform 1/2 promoter activity in NRM and CHO cells (n=6 for each cell type). A schematic representation of the 5′ deletion constructs of the HKCNE1 isoforms 1 and 2 promoter region is shown on the left. Nucleotides of fusion plasmids are numbered with respect to the TSS (+1) identified by 5′RACE. Firefly luciferase activities were divided by co-expressed Renilla luciferase activity and expressed as-fold activity of the promoter-less construct (PGL3-Basic). B. Comparison of HKCNE1 isoform 1/2 promoter activity in NRMs and in CHO cells. Core promoter (−311/+16) activity in NRMs was 4.9 times its activity in CHO cells, suggesting cardiomyocyte-selective transcription factor binding sites. C. Analysis of HKCNE1 isoform 3 promoter activity in NRMs and CHO cells (n=3 for each cell type) and comparison of activity in NRMs vs CHO cells.
2.12. Statistics

All experiments were performed at least three independent times. Results are mean ± SEM. Statistical analysis was with Student’s t-test (single comparisons only) and one-way ANOVA with Bonferroni’s multiple comparison post-test. Controls were performed concurrently for each set of experiments shown in each Figure to control for time- and technique-dependent variations.

3. Results

We identified three different HKCNE1 isoforms (1, 2 and 3) by 5′RACE (Fig. 1). Isoform 1 is similar to that reported by Splawski et al. [16] in 1998, but contains an additional 38 bp in the 5′ region. Isoform 2 is similar to isoform 1, except that the 5′ region of exon 2 in isoform 2 is 28 bp shorter than exon 2 in isoform 1. Isoform 3 has different nucleotide sequences upstream to exon 3, which we designate exon 2B. All 5′RACE product sequences match sequences in human chromosome 22 working draft sequence segment NT_011512. We found a forth isoform (BC046224) by BLAST search, which we designate isoform 4. This isoform consists of 4 exons: besides exon 2 and 3 shown in Figs. 1 and 2 other exons upstream to exon 1 are designated exon 0.5 and exon 1A. This forth isoform was demonstrated in human heart cDNA by quantitative RT-PCR and confirmed by sequencing the RT-PCR product. The sequences at all the exon–intron boundaries are consistent with the consensus sequence of the splice junction.

The four isoforms have identical coding regions but different 5′UTRs and alternative TSSs suggesting multiple promoters. Since isoforms 1 and 2 share the same exon-1 and differ only to a minor extent in exon-2, they likely share the same promoter. We therefore quantified the human cardiac expression of isoform 1 and 2 together and compared this to the expression of isoforms 3 and 4, with quite different 5′-upstream regions. Absolute quantification of HKCNE1 in human hearts (Fig. 2) showed that isoforms 1 and 2 are strongly expressed, contributing ~80% of HKCNE1 transcripts in the right ventricle, 85% in the left ventricle and 72% in the atrium. The isoform 3 expression level is 10 times smaller in right and left ventricles and 5 times smaller in the atrium. Isoform 4 is least-expressed. To assess the expression of various isoforms across a range of tissues, we repeated RT-QPCR with commercial human tissue-bank samples (Fig. 2B). Isoforms 1 and 2 are strongly expressed in heart, kidney and testis, with 4.5-fold greater concentration in heart vs isoform 3- and 9-fold greater vs isoform 4. Isoform 4 is particularly expressed in lung and trachea.

Based on these data indicating isoforms 1 and 2 dominance in the heart, we concentrated on characterizing the common isoform 1/2 promoter. The putative HKCNE1 isoforms 1 and 2 promoter immediately upstream of HKCNE1 exon 1 has no apparent TATA box, but contains two putative CCAAT boxes and is GC-rich. TRANSFAC predicts 4 GC-boxes that could serve as binding sites for Sp1 transcription factors. The high content of consensus GC elements, lack of consensus TATA boxes and multiple TSSs are characteristic of housekeeping promoters.

Varying lengths of the isoform 1/2 promoter were transfected into NRMs and CHO cells (Fig. 3). The promoter activity of the longest construct, PGL3(-1609/+16) was 31-fold that of PGL3-Basic in NRMs, and 16-fold in CHO cells. The second highest promoter activity in NRMs was associated with the shortest construct, the core promoter PGL3(-311/+16), 21–29-fold PGL3-Basic (Fig. 3A). Elements responsible for cardiomyocyte-selective promoter activity appear to be located in the core promoter, because PGL3(-311/+16) has a NRM/CHO expression ratio (NRM/CHO) of ~4.9 vs ~2.0 for PGL3(-1609/+16) (Fig. 3B). To assess the basis for the cardiac predominance of isoforms 1 and 2 compared to isoform 3, we cloned the potential 5′-promoter elements of isoform 3. The full-length putative isoform 3 promoter region (-1620/+21) produced reporter activity ~5-fold PGL3-Basic in NRM, 6 times weaker than the corresponding Isoform 1/2 construct (-1609/+16, Fig. 3C). The Isoform-3 full-length promoter showed a NRM/CHO expression ratio of ~1.0, vs ~2.0 for the corresponding isoform 1/2 construct.

Fig. 4. GATA element mutation analysis. The GATA elements, GATC(-294), GATC(-269), CATC(-54), in HKCNE1 isoform 1/2 core promoter PGL3(-311/+16) were mutated to TGCA, GTAG, AGCT respectively and the effects of these mutations on promoter activity measured (**p<0.01 vs non-mutated core promoter; n=6 for each data point).
To investigate the potential mechanisms of the cardio-myocyte-selective activity of PGL3-HKCNE1 (−311/+16), TRANSFAC was used to screen potential cardio-selective expression elements. No Nkx2.5 or MEF binding sites were found, but three potential GATA binding sites were found: GATA (−294), GATA (−269) and GATA (−54). Mutation of the first (−294) or third (−54) GATA element decreased promoter activity by ~36% in NRM (Fig. 4). Mutation of the second (−269) GATA element decreased promoter activity 55% (to 12.9±0.9-fold PGL3-Basic vs 29.0±1.9 without mutation) and reduced cardio-selective activity from NRM/CHO ~4.6 to 2.5. Mutation of all three GATA elements decreased promoter activity by 58% (to 12.2±0.6), no more than the 2nd GATA element mutation alone. None

Fig. 5. Effects of GATA4 siRNA on promoter activity and cardiomyocyte-selective expression. A. Co-transfection of GATA4 siRNA (50 nM) with the HKCNE1 isoform 1/2 core promoter decreased promoter activity. B. Co-transfection of GATA4 siRNA with the 3-GATA mutated core promoter construct did not affect promoter activity. Co-transfection with 50 nM GAPDH siRNA was used as a control (***p<0.001 vs result without co-transfection of siRNA). C. GATA4 and GAPDH siRNA knockdown efficiency analyzed by Western blot in the absence (control) or presence of siRNA (+siRNA). Actin served as a loading control (**p<0.01, ***p<0.001 vs result without siRNA co-transfection; n=5 for each data point).
of the GATA mutations significantly affected promoter activity in CHO cells. To confirm the functional importance of the GATA elements, siRNA silencing experiments were performed (Fig. 5). Co-transfection of PGL3(--311/+16) with 50 nM GATA4 siRNA decreased promoter activity by 43% (28.2 ± 0.7 vs 16.2 ± 0.5-fold, *p < 0.001; Fig. 5A), but co-transfection of GATA4 siRNA with the 3 GATA-mutated core promoter caused no significant change in promoter activity (Fig. 5B). There was no significant decrease in promoter activity when 50 nM GAPDH siRNA (control for gene-specific silencing effects) was co-transfected. Western blots (Fig. 5C) confirmed effective knockdown by siRNA, with ~75% GAPDH protein-expression reduction for

![Fig. 6. Effect of angiotensin-II (Ang II) on HKCNE1 promoter activity. NRMs transfected with HKCNE1 isoform 1/2 core promoter PGL3(--311/+16) and the GATA(--269)-mutated construct were incubated with 100 nM and 1 μM angiotensin-II (*p < 0.05, **p < 0.01 vs core promoter activity in the absence of angiotensin-II; n = 5 for each data point).](image)

![Fig. 7. Sequence alignments of mouse (M) KCNE1 core promoter with its human (H) counterpart (isoform 1/2 core promoter). Conserved regions are underlined. GATA-boxes are identified with solid lines and GC-boxes with dotted lines. The TSS in HKCNE1 is indicated by an arrow.](image)

![Fig. 8. Comparison of human (isoform 1/2) and mouse core promoter activity. A. Human and mouse core promoter activities (**p < 0.001 for human vs mouse; n = 5 for each data point). B. Human and mouse KCNE1 core promoter activity in NRMs divided by activity in CHO cells. Cardiomyocyte-selectivity was lower for the mouse promoter.](image)
GAPDH siRNA and ~70% decrease in GATA4 expression with GATA4 siRNA.

GATA4 is important in inducible gene expression evoked by a variety of hypertrophic stimuli [26]. We therefore tested the importance of GATA binding sites in mediating effects of angiotensin-II on HKCNE1 promoter activity. Angiotensin-II significantly increased luciferase activity of the HKCNE1 core promoter in transfected NRMs (Fig. 6), but failed to increase promoter activity when the core promoter lacked a functional 2nd GATA element (Fig. 6).

HKCNE1 appears important for species-specific $I_{Ks}$ expression, with low minK levels in mouse and rabbit associated with very small $I_{Ks}$ [17,18,20,21]. Alignment of human isoforms 1 and 2 and mouse HKCNE1 3'-regulatory regions revealed ~50% sequence identity, with none of the 3 GATA elements in the HKCNE1 core promoter conserved in the corresponding region of MKCNE1, although MKCNE1 has one GATA element not found in HKCNE1 (Fig. 7). Fig. 8 shows that the mouse core promoter has less activity in NRMs compared to its human counterpart (11.7±0.7 vs 29.0±1.9-fold PGL3-Basic, $p<0.001$) and less cardiomyocyte-selectivity.

4. Discussion

In this study, we identified several HKCNE1 isoforms and studied the 3'-regulatory elements determining the expression of the most strongly-expressed cardiac isoforms. We found that the core promoter significantly drives transcriptional activity and cardiomyocyte-selective expression, with a single GATA element of particular importance, and obtained evidence suggesting that core-promoter sequence differences contribute to species variations in KCNE1 expression.

The regulation of cardiac ion-channel subunit expression is an important area in rapid development [27]. Cardiac $K^+$-channel promoters typically lack TATA boxes, are GC-rich and contain multiple Sp1 binding sites [28,29], features we observed for the putative HKCNE1 isoform 1/2 promoter. Factors that control cardiac $K^+$-channel expression include cyclic AMP (cAMP) interactions with a cAMP response element in Kv1.5 [28] and GATA interacting with FOG2 for Kv4.2, despite the absence of consensus GATA binding sites in the Kv4.2 core promoter [30]. In the present study, GATA played a significant role in regulating HKCNE1 isoforms 1 and 2 expression via interactions requiring consensus GATA binding sequences in the core promoter.

The KCNE1 gene product minK is required for optimal function of the important cardiac repolarizing current $I_{Ks}$ [6,7,10,11] and alterations in minK expression contribute to disease-induced remodeling of cardiac electrical function [12–15]. Here, we cloned and characterized the putative promoter of the most commonly-expressed HKCNE1 isoforms. Our isoforms 2 and 3 correspond to the isoforms 1a and 1b recently reported by Lundquist et al. [15,31]. We also identified the expression of a 4th isoform in human hearts. Our isoforms 1 and 2, with the same DNA sequence 5' to the TSS, are much more strongly expressed in the heart than isoforms 3 and 4, with this cardiac predominance related to strong promoter activity in cardiomyocytes.

GATA4 (and/or GATA6) in the heart binds to GATA-binding elements and thereby regulates DNA transcription. Stretch induces release of norepinephrine, angiotensin-II, and endothelin-1 from cardiomyocytes and is associated with increased GATA4 binding to DNA; over-expression of GATA4 promotes cardiac hypertrophy [32]. GATA4 regulates the expression of numerous genes involved in cardiac hypertrophy, including genes encoding myosin heavy chains, natriuretic proteins (ANP, BNP), sodium/calcium exchanger, troponin-I, type-1 angiotensin receptors, M2-muscarinic receptors and A1-adenosine receptors [26,32]. Human KCNE1 expression increases in cardiomyopathic tissue [33]. This may be related to the enhancing effect of angiotensin-II on HKCNE1 promoter activity that we observed. This effect was abolished by mutating the 2nd GATA-binding element, and therefore requires this GATA binding site. Mutation of the three GATA binding sites did not completely abolish cardiomyocyte-selective expression of the human core promoter, suggesting that other local elements may contribute to transcriptional control.

The functional expression of $I_{Ks}$ varies widely among species [21,22,34], and there is evidence that differences in minK expression may be important in determining species-dependent $I_{Ks}$ expression differences [20]. $I_{Ks}$ density is known to be very small in the mouse [21], paralleling low-level KCNE1 gene expression [19]. We found differences in core-promoter sequences and activities between mouse and human KCNE1 that explain, at least in part, lower-level expression in the mouse heart.

In summary, we have identified 4 HKCNE1 isoforms expressed in human hearts and characterized the promoter driving expression of the predominant isoforms. GATA elements are important for activity of this promoter, as well as for cardiomyocyte-selective and species-selective expression. Our results may have important implications for understanding the transcriptional regulation of KCNE1/I$_{Ks}$ in normal conditions and in the presence of cardiac disease.

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