Published in final edited form as:

Hum Genet. 2009 December ; 126(6): 779–789. doi:10.1007/s00439-009-0731-9.

K_{ATP} channel Kir6.2 E23K variant overrepresented in human heart failure is associated with impaired exercise stress response

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Abstract

ATP-sensitive K^+ (K_{ATP}) channels maintain cardiac homeostasis under stress, as revealed by murine gene knockout models of the *KCNJ11*-encoded Kir6.2 pore. However, the translational significance of K_{ATP} channels in human cardiac physiology remains largely unknown. Here, the frequency of the minor K23 allele of the common functional Kir6.2 E23K polymorphism was found overrepresented in 115 subjects with congestive heart failure compared to 2,031 community-based controls (69 vs. 56%, $P < 0.001$). Moreover, the KK genotype, present in 18% of heart failure patients, was associated with abnormal cardiopulmonary exercise stress testing. In spite of similar baseline heart rates at rest among genotypic subgroups (EE: 72.2 ± 2.3 , EK: 75.0 ± 1.8 and KK: 77.1 ± 3.0 bpm), subjects with the KK genotype had a significantly reduced heart rate increase at matched workload (EE: $32.8 \pm 2.7\%$, EK: $28.8 \pm 2.1\%$, KK: $21.7 \pm 2.6\%$, $P < 0.05$), at 75% of maximum oxygen consumption (EE: $53.9 \pm 3.9\%$, EK: $49.9 \pm 3.1\%$, KK: $36.8 \pm 5.3\%$, $P < 0.05$), and at peak $\dot{V}O_2$ (EE: $82.8 \pm 6.0\%$, EK: $80.5 \pm 4.7\%$, KK: $59.7 \pm 8.1\%$, $P < 0.05$). Molecular modeling of the tetrameric Kir6.2 pore structure revealed the E23 residue within the functionally relevant intracellular slide helix region. Substitution of the wild-type E residue with an oppositely charged, bulkier K residue would potentially result in a significant structural rearrangement and disrupted interactions with neighboring Kir6.2 subunits, providing a basis for altered high-fidelity K_{ATP} channel gating, particularly in the homozygous state. Blunted heart rate response during exercise is a risk factor for mortality in patients with heart failure, establishing the clinical relevance of Kir6.2 E23K as a biomarker for impaired stress performance and underscoring the essential role of K_{ATP} channels in human cardiac physiology.

Introduction

ATP-sensitive potassium (K_{ATP}) channels are fine-tuned molecular biosensors capable of translating changes of intracellular metabolism into membrane excitability responses aimed at maintaining energy homeostasis (Alekseev et al. 2005; Nichols 2006; Sattiraju et al. 2008; Zingman et al. 2007). Widely expressed in metabolically active tissues and present in a variety of mammalian and non-mammalian species, functional K_{ATP} channels are formed through multimerization of SURx/Kir6.x subunits generating a hetero-octameric structure (Inagaki et al. 1995; Aguilar-Bryan et al. 1998; Miki and Seino 2005; Ashcroft 2006; Nichols 2006). Specifically in the heart, association of four pore-forming Kir6.2 (*KCNJ11*) and four regulatory SUR2A (*ABCC9*) subunits gives rise to inwardly rectifying potassium channels responsive to adenine nucleotides (Lorenz and Terzic 1999; Alekseev et al. 2005; Karger et al. 2008; Park et al. 2008). Through tight integration with cellular metabolic pathways, cardiac K_{ATP} channels have been increasingly recognized as molecular

safeguards of cardiac performance under stress (Carrasco et al. 2001; Abraham et al. 2002; Hodgson et al. 2003; Zingman et al. 2003; Selivanov et al. 2004; Kane et al. 2005).

Cardioprotective properties of K_{ATP} channels are underscored by *Kcnj11* deletion in mice, which renders the heart susceptible to maladaptation and predisposes to heart failure (Kane et al. 2006a; Yamada et al. 2008). Specifically, disruption of *Kcnj11* results in impaired tolerance to sympathetic surge (Zingman et al. 2002; Liu et al. 2004; Reyes et al. 2007), endurance challenge (Kane et al. 2004) and hemodynamic load (Kane et al. 2006b; Yamada et al. 2006), while compromising the protective benefits of ischemic preconditioning (Suzuki et al. 2002; Gumina et al. 2003, 2007). Furthermore, ablation of K_{ATP} channel function leads to increased susceptibility to calcium-dependent pathological remodeling, progressing to organ failure and death (Kane et al. 2006a; Zlatkovic et al. 2009), indicating that intact channels are required for the cardiac adaptive response under acute or chronic challenge (Kane et al. 2005).

Human investigations further reveal vulnerability to cardiac stressors mediated by genetic variation in the pore-forming and/or regulatory subunits of K_{ATP} channels. Mutations in *ABCC9* that disrupt channel gating have been linked with susceptibility to dilated cardiomyopathy and atrial fibrillation, implicating defective K_{ATP} channels in cardiac mechanical dysfunction and electrical instability (Bienengraeber et al. 2004; Olson et al. 2007). Moreover, a number of polymorphisms in *KCNJ11* have been identified (Riedel et al. 2005). In particular, human Kir6.2 contains a functional E23K amino acid substitution stemming from a c.67G → A single nucleotide polymorphism, and resulting in abnormal K_{ATP} channel gating (Riedel et al. 2005). Specifically, an altered sensitivity to ligands such as adenine nucleotides, long-chain acyl CoA esters and protons leads to aberrant channel activation and inhibition profiles in vitro (Li et al. 2005; Riedel et al. 2005; Villareal et al. 2009). In spite of extensive reports on the clinical significance of the E23K variant in type II diabetes (Riedel et al. 2005; Ashcroft 2006), its impact on heart physiology remains largely unknown. In a recent large community-based study, the E23K polymorphism was implicated as a risk factor for subclinical maladaptive cardiac remodeling among individuals with increased stress load due to hypertension (Reyes et al. 2008); however, its significance in a disease-specific cohort of heart failure subjects remains unstudied. Furthermore, lack of an atomic model for K_{ATP} channel subunits has limited understanding of the molecular mechanism for disease susceptibility imparted by this polymorphism.

Here, we investigated the clinical significance of the common E23K polymorphism in the *KCNJ11*-encoded Kir6.2 K_{ATP} channel subunit in heart failure patients. The frequency of the minor K23 allele in the heart failure cohort was overrepresented compared to the community at large. Furthermore, patients with the homozygous KK genotype had an abnormal response to exercise as demonstrated by a blunted heart rate elevation during cardiorespiratory treadmill stress testing. In silico modeling of full-length Kir6.2 suggested that the E23K amino acid substitution would disrupt the structural integrity of the protein complex, affecting channel gating. K_{ATP} channels harboring the K23 polymorphism thereby emerge as predictors of compromised heart rate stress response in human heart failure.

Methods

Study group

The study was approved by the Mayo Clinic Institutional Review Board. All participants provided written informed consent before enrollment, and all aspects of the study were performed according to the Declaration of Helsinki. Individuals from Olmsted County, Minnesota (98% Caucasian, 2% Hispanic) with a history of ischemic and non-ischemic dilated cardiomyopathy were recruited consecutively from the heart failure and

cardiovascular health clinics at Mayo Clinic ($n = 115$). Exclusion criteria included obesity, ejection fraction (EF) greater than 40%, age younger than 40 years, history of smoking, atrial fibrillation, valvular heart disease, and exercise capacity limited by peripheral vascular disease, chest pain, arrhythmia, musculoskeletal diseases or anxiety. All participants underwent resting echocardiography, neurohormonal assays, and a cardiopulmonary exercise test to exhaustion (Snyder et al. 2006; Wolk et al. 2007). Measurements were completed in a double-blind manner, with genotyping performed after testing had been completed. Characteristics of the cross-sectional community-based cohort ($n = 2,031$), used as a racially matched control group, have been previously reported (Reyes et al. 2008).

Genotyping

Genomic DNA was extracted from peripheral blood white cells (DNA isolation kit, Gentra Puregene, Minneapolis, MN). Forward (5'-CCACGTCCGAGGGGTGC-3') and reverse (5'-AGGAGTGGATGCTGGTGACACA-3') primers were utilized to PCR-amplify a 407 bp fragment comprising the c.67G variant of *KCNJ11*, resulting in the E23K amino acid substitution. The amplified fragment was digested with the restriction enzyme *BanII* (New England Biolabs, Ipswich, MA) and resultant fragments, varying in size based on presence (c.67G) or absence (c.67A) of a *BanII* recognition site, were resolved on 2% agarose gels to assign genotypes.

Echocardiography and cardiopulmonary exercise stress test

Left ventricular (LV) EF and LV dimensions were measured at rest by two-dimensional echocardiography, according to recommendations of the American Society of Echocardiography (Schiller et al. 1989). Heart rate (HR), gas exchange, and oxygen saturation were measured during graded treadmill testing to volitional fatigue. An initial treadmill speed and grade of 2.0 mph and 0%, respectively, were adjusted every 2 min to yield approximately 2-metabolic equivalent increases per work level until subjects could no longer exercise because of exhaustion, cardiac symptoms or significant electrocardiographic or blood pressure changes. Participants wore electrodes for the electrocardiogram (heart rate and monitoring), a blood pressure cuff, and a nose clip and standard mouthpiece attached to a PreVent Pneumotach (Medical Graphics, St. Paul, MN) throughout the testing procedure, including 3-min of recovery. Gas exchange measures (oxygen consumption, $\dot{V}O_2$; carbon dioxide production, $\dot{V}CO_2$; minute ventilation, VE), as well as heart rate and systolic (SBP) and diastolic (DBP) blood pressure measurements, were obtained continuously using a metabolic cart (Medical Graphics, St. Paul, MN), which has been validated with classic gas collection techniques, and averaged over 30-s intervals (Snyder et al. 2006; Wolk et al. 2007). The ventilatory equivalent ratio for carbon dioxide was calculated as $VE/\dot{V}CO_2$, and oxygen pulse (O_2 pulse) was calculated as $\dot{V}O_2/HR$. The rate pressure product was calculated as $HR \cdot SBP$. After the exercise stress test was completed, variables were analyzed at initial matched workload level, at the maximum rate of oxygen consumption (peak $\dot{V}O_2$), and at 75% of peak $\dot{V}O_2$. The oxygen uptake efficiency slope (OUES), an index of cardiovascular functional impairment in heart failure indicative of ventilation efficiency and exercise capacity, was calculated through a single-segment logarithmic curve-fitting model using the following equation: $\dot{V}O_2 = OUES \cdot \log VE + b$, in which the constant OUES represents the rate of increase in $\dot{V}O_2$ in response to an increase in VE.

Molecular modeling

Full-length Kir6.2 was modeled using the I-TASSER platform, a hierarchical approach to protein structure prediction based on an enhanced sequence Profile–Profile Alignment (PPA) algorithm combined with secondary structure matches and the iterative implementation of the Threading ASSEMBLY Refinement (TASSER) program. TASSER consists of template identification by threading, followed by tertiary structure assembly via

the rearrangement of continuous template fragments guided by an optimized C α , and side-chain-based potential driven by threading-based, predicted tertiary restraints (Zhang and Skolnick 2004; Wu et al. 2007; Zhang 2008). This computational program covers comparative modeling to ab initio folding. The Kir6.2 model contained abridged regions at N-terminal (1–31) and C-terminal (359–390) residues, which were unavailable in previous homology models (Antcliff et al. 2005). Assembly of Kir6.2 tetramer was constructed using the geometry-based docking program Symmdock, and the best model was selected based on geometric shape complementarity score and visual inspection of built tetra-molecules. The quality and stereo-chemical properties of Kir6.2 model were assessed using PROCHECK V3.4.5 (Morris et al. 1992).

Statistical analysis

Data are presented as mean $\pm$ SEM or SD. Genotype frequencies were compared using Fisher's exact test. New York Heart Association classifications and proportions of patients treated with α -adrenergic receptor antagonists or having ischemic heart disease were compared using Pearson's chi-square test. Other continuous variables were compared using ANOVA and Tukey's post hoc test. $P < 0.05$ was set to indicate significant differences. Statistical power for the sample size (115 cases vs. 2,031 controls) was calculated using a Web-based software package (http://www.dssresearch.com/toolkit/spcalc/power_p2.asp), revealing 82.5 and 93.8% values according to the K23 allele and the KK genotype frequencies, respectively.

Results

E23K is overrepresented in a heart failure cohort

The common single nucleotide polymorphism c.67G $\rightarrow$ A causing the E23K amino acid substitution in Kir6.2 results in disruption of a *Ban*II restriction enzyme recognition site by changing the DNA sequence from $_{67}\text{GAGCCC}_{72}$ in the E allele to $_{67}\text{AAGCCC}_{72}$ in the K allele (Fig. 1a). PCR-amplified fragments comprising the E23 region thus harbored three versus two *Ban*II restriction sites in the E or K allele, respectively, thereby producing different DNA fragment sizes after *Ban*II digestion (Fig. 1a). In contrast to EE or KK homozygous alleles, EK heterozygous alleles yielded a mixture of restriction enzyme digested DNA fragment sizes, both from E and K alleles, distinctively resolved on agarose gel electrophoresis (Fig. 1a).

Genotypes were determined for a cohort of 115 consecutive Mayo Clinic patients with heart failure, and found to be in Hardy–Weinberg equilibrium (Fig. 1b). The K23 allele was overrepresented in the heart failure cohort when compared to the cross-sectional community cohort (Odds ratio = 1.68, 95% confidence interval 1.13–2.51; $P = 0.011$). While the EK genotype was equally present ($P = 0.565$), the frequency of the KK genotype was greater in the heart failure cohort (EE = 31%, EK = 51%, KK = 18%) versus the population at large (EE = 44%, EK = 47%, KK = 9%; Odds ratio = 2.32, 95% confidence interval 1.40–3.85, $P = 0.002$; Fig. 1b). The racial makeup of each cohort was comparable; among individuals with EE or EK genotypes, 98% were Caucasian and 2% were Hispanic; all KK subjects were Caucasian. There were no significant statistical differences among the three genotype groups within the heart failure cohort with respect to baseline variables such as age, weight, body mass index (BMI), gender or blood pressure (Fig. 2). Furthermore, there were no significant differences in the proportion of subjects with ischemic heart disease or under α -adrenergic blocker treatment, resting heart rates were comparable, and heart failure patients within all genotype groups had similar degrees of left ventricular dysfunction and remodeling, as well as equivalent New York Heart Association classification (Fig. 2). Moreover, circulating levels of the neurohormones norepinephrine and B-type natriuretic

peptide (BNP) were indistinguishable in the EE, EK and KK groups. Thus, despite an increased incidence of KK genotype in heart failure patients, no significant differences were observed among genotype groups at baseline in the study cohort, indicating comparable states of cardiac disease.

KK genotype is associated with impaired cardiac response to exercise stress

At rest, cardio-respiratory variables were equivalent among the studied heart failure patients when stratified by E23K genotype. Accordingly, all heart failure subjects underwent an incremental exercise test with on-line measurement of matching variables (Fig. 3). The 2-min-long increments in workload followed an initial warm-up period at matched workload, until maximum $\dot{V}O_2$ (peak) was obtained prior to fatigue and termination of exercise. Measured variables at rest, matched workload, peak $\dot{V}O_2$ and 75% of maximum oxygen consumption (see “Methods”) were analyzed and compared off-line, revealing equivalent exercise stress. In particular, there were no significant differences in respiratory exchange ratio (RER), oxygen pulse (O_2 pulse), rate pressure product, and ventilatory equivalent ratio for carbon dioxide ($VE/\dot{V}CO_2$) levels among genotype groups throughout the exercise protocol (Fig. 4). Moreover, all genotype groups were similar with respect to potentially confounding variables, such as tolerated workload (Work), time of exercise at each level, and the slope of oxygen uptake efficiency (OUES; EE: 1.72 ± 0.10 ; EK: 1.84 ± 0.08 ; KK: 1.60 ± 0.13 , $P = 0.24$; Fig. 4). However, despite comparable heart rates at rest (EE: 72.2 ± 2.3 , EK: 75.0 ± 1.8 and KK: 77.1 ± 3.0 beats per minute, $P = 0.39$), the physiological increase in heart rate with exercise observed in individuals with at least one E23 allele (EE and EK genotypes) was blunted in patients with the KK genotype at all stages of the treadmill stress test (Fig. 3). Specifically, the percentage increase in heart rate of KK subjects was $21.7 \pm 2.6\%$ at the initial level of matched workload, $36.8 \pm 5.3\%$ at 75% of maximum oxygen consumption, and $59.7 \pm 8.1\%$ at peak $\dot{V}O_2$, significantly reduced compared to the response of EE and EK heart failure patients who had heart rate increases of 32.8 ± 2.7 and $28.8 \pm 2.1\%$ at matched workload, 53.9 ± 3.9 and $49.9 \pm 3.1\%$ at 75% of peak $\dot{V}O_2$, and 82.8 ± 6.0 and $80.5 \pm 4.7\%$ at peak $\dot{V}O_2$, respectively. These data indicate that altered K_{ATP} channel function caused by homozygosity for the minor K23 allele is associated with blunted adaptive heart rate response during exercise in patients with heart failure.

Structural implication of Kir6.2 E23K polymorphism

Based on secondary structure prediction, the E23 residue is located in a disordered N-terminal region where the structural templates were previously unavailable for protein homology modeling (Antcliff et al. 2005). In fact, protein sequence alignment of Kir6.2 orthologs demonstrated a highly conserved N-terminal intracellular domain among mammalian species, implicating the functional conservation of this region throughout evolution (Fig. 5a). The high-resolution molecular model of the Kir6.2 channel, constructed here with a combination of ab initio and comparative modeling using the I-TASSER platform, resolved the structural implication of the highly conserved E23 residue. The tetrameric structure of the Kir6.2 channel displayed the intracellular (IC) and transmembrane (TM) domains with overall dimensions of ~ 115 Å in length and ~ 70 Å in width (Fig. 5b). The IC domain, harboring the ATP binding site, was composed of Kir6.2 N- and C-termini regions with extensive intra- and inter-protein–protein interactions among subunits. Furthermore, the IC domain forms a large cytoplasmic pore structure characteristic of the Kir channel family (Nishida and MacKinnon 2002). The TM domain of Kir6.2 contained two membrane-spanning α -helices (M1 and M2) linked with a short pore-forming helix and a selectivity filter loop responsible for the discriminating permeability to potassium ions. While the inner TM α -helix (M2) linked directly to the C-terminal domain, the outer TM α -helix (M1) connected with the N-terminal domain through a short α -helix

or “slide helix” at the interface between the TM and IC domains (Fig. 5b, c; Kuo et al. 2003). A cluster of conserved amphipathic residues in the slide helix created close contacts with the lipid head groups for stable lipid–protein interactions. The lateral movement of the slide helix has been implicated as part of a channel gating mechanism in Kir channels (Kuo et al. 2003; Haider et al. 2007).

The highly conserved negatively charged E23 mapped to the loop region of the N-terminal IC domain, preceded by the previously unresolved first α -helix and followed by the critical slide helix; E23 positioned planar to these two helices (Fig. 5b, c). As E23 appeared fully exposed to solvent and unengaged from direct interactions with adjacent residues, the K23 variant would not be directly involved in structural distortion. Rather, K23 would be predicted to disrupt the integrated interaction of Kir6.2 channel structure with membrane phospholipid head groups due to unbalancing charges in a cluster of charged residues at the first α -helix (R16/E19/D20) and the slide helix (R54/D58/D65/K67) (Fig. 5d). In addition, the bulkier and positively charged amino acid could destabilize a potential electrostatic interaction with R325 in the neighboring subunit (Fig. 5d). Indeed, this electrostatic interaction is highly conserved not only in mammals but also in non-mammalian species such as *Rana catesbeiana* (frog) and *Clonorchis sinensis* (liver fluke). The E23/R325 electrostatic interaction was not conserved in *Danio rerio* (zebrafish) and *Tetraodon nigroviridis* (green puffer), yet corresponding residues in these two species, Q/M and M/A, are both uncharged residues that prevent electrostatic crashes. The E23K polymorphism would thus be predicted to compromise the structural integrity at the TM/IC domain interface and perturb protein–protein interaction between subunits.

Discussion

It is increasingly recognized that physiological and disease phenotypes are modulated by genetic variation ranging from risk-conferring polymorphisms to disease-causing mutations. However, discerning the contribution of bi-allelic single nucleotide variants to human traits remains a challenge (Goldstein 2009). The biological effects of polymorphisms used in genome-wide association studies on gene expression and/or protein function may be unknown. Moreover, common variants typically have relatively small effect sizes. On the other hand, natural selection would predictably render alleles with greater risk-conferring effect too rare to study in vivo dose effects conferred by the homozygous state. By contrast, these limitations are largely mitigated by the Kir6.2 E23K polymorphism targeted in this study. K23 has been shown to alter K_{ATP} channel gating in vitro (Riedel et al. 2005) and genetic studies in humans and mice have established a link between K_{ATP} channel gene mutations, susceptibility to stress load, and heart disease (Bienengraeber et al. 2004; Kane et al. 2006a, b; Yamada et al. 2006; Olson et al. 2007). Investigating potential effects of Kir6.2 K23 on cardiac structure and function can exploit the physiological role of K_{ATP} channels in stress responsiveness. Namely, imposed stressors such as hypertension (Reyes et al. 2008) or exercise (the current study) can amplify the phenotypic effects of the K23 allele. The K23 allele is not rare and its persistence in the population could be explained by a relatively benign effect on K_{ATP} channel function in the heterozygous state, i.e., at least one wild-type subunit within the homo-tetrameric pore assembly may be sufficient to maintain proper channel gating. In addition, its persistence in the gene pool could represent a variation of the “thrifty gene” hypothesis. K_{ATP} channels harboring the K23 variant could confer improved substrate supply for all tissues and improved muscle performance during sustained exercise (Riedel et al. 2005). However, this advantage could become risk-conferring in the setting of imposed stress load due to impaired intracellular ion homeostasis from lack of tight regulation by K_{ATP} channels. Indeed, we observed an overrepresentation of the K23 allele in a heart failure cohort compared to the population.

Our previous community-based study indicated that the common K23 variant of Kir6.2 was associated with left ventricular size in hypertensive individuals, implicating E23K as a risk factor for heart disease in the presence of a concomitant stressor (Reyes et al. 2008). Yet, the impact of the E23K variant in heart failure had not been investigated until now. In this study, we found that the minor K23 allele was significantly more frequent in heart failure patients versus the general population within the same geographical area (Reyes et al. 2008). Together with previous genetic investigations of the regulatory SUR2A and pore-forming Kir6.2 channel subunits, this cohort-based finding lends further support to a role for K_{ATP} channel gene variants in risk for human heart disease.

Exercise results in increased systemic sympathetic stimulation in patients with heart failure (Colucci et al. 1989), enabling the heart to increase performance and match metabolic demand. The significance of this adaptive response is emphasized by a well-established significant association between the inability to properly increase heart rate during exercise and mortality reported in independent study cohorts and validated by careful adjustment for possible confounders such as left ventricular function, severity of myocardial ischemia and coronary artery disease, anti-arrhythmic medication use, and exercise capacity (Dresing et al. 2000; Elhendy et al. 2003; Jouven et al. 2005; Diller et al. 2006; Savonen et al. 2006). A genetic basis for impaired heart response to exercise has been in fact previously implicated. The Gly16Arg polymorphism in the α_2 -adrenoceptor gene was demonstrated to affect heart rate response to isometric exercise (Eisenach et al. 2005). In addition, the common insertion/deletion polymorphism in the angiotensin converting enzyme gene (ACE I/D) results in abnormal heart rate dynamics during exposure to exercise heat stress (Heled et al. 2004). In this regard, the present study identifies a new molecular determinant of heart rate response to exercise.

K_{ATP} channels function as part of a feedback regulation system that allows for cardiac adaptation to stress imposed by the energetic challenge of exercise (Kane et al. 2004). While in healthy individuals E23K was not associated with exercise training-induced adaptation (Yi et al. 2008), in heart failure patients the Kir6.2 polymorphism was here linked to abnormal heart rate performance under cardiopulmonary treadmill stress. The equivalent demographic, anthropometric, and cardiovascular baseline characteristics across genotypes, including heart rates and oxygen uptake efficiency slope (OUES), enabled an accurate evaluation of the compensatory response of the heart to exercise. Homozygosity for the E23K amino acid substitution was demonstrated to impair the adjustive heart rate response to exercise stress, a phenomenon predictive of poor outcome in patients with heart failure (Myers et al. 2008). Thus, intact high-fidelity K_{ATP} channel function is underscored as essential for optimal cardiac performance in the setting of energetic deficit in heart failure and increased metabolic demand imposed by exercise in humans.

The pathophysiological basis for chronotropic incompetence in response to exercise is not fully understood, and is thought to be multifactorial (Elhendy et al. 2003). In this regard, it is notable that K_{ATP} channels have been identified not only in the myocardium per se, but also in the sinoatrial node where they contribute to the autonomically regulated pacemaker cell automaticity (Han et al. 1996; Marionneau et al. 2005; Fukuzaki et al. 2008). Moreover, we previously demonstrated that the impact of K_{ATP} channel mutation on electrical instability is amplified in the setting of adrenergic stimulation (Olson et al. 2007). Here, we show that the maladaptive phenotype resulting from the Kir6.2 polymorphism becomes evident within the heart failure cohort only during the sympathetic surge of exercise. Thus, the cellular mechanism for impaired heart rate response due to the K23 variant may involve an adverse synergistic gene–environment interaction precipitating suboptimal performance.

The E23K variant in Kir6.2 is the most studied K_{ATP} channel polymorphism, especially in the context of type II diabetes (Riedel et al. 2005), yet the precise structure–function impact of this amino acid substitution remains unclear. In our proposed model, E23 lies nearby the specific slide helix domain of Kir6.2, in plane with the plasma membrane. Of note, naturally occurring neonatal diabetes mutations affecting K_{ATP} channel open-state stability group along this slide helix (Ashcroft 2005; Nichols 2006), under-scoring the importance of this region in regulating channel gating. As E23 is distant from ATP binding sites, amino acid replacement with a lysine (K) residue may not directly affect ATP binding but would rather compromise structural intactness, and ultimately lead to improper K_{ATP} channel gating, as previously demonstrated by in vitro electrophysiological measurements (Schwanstecher et al. 2002; Riedel et al. 2003; Villareal et al. 2009). The K_{ATP} channel pore is composed of four Kir6.2 subunits and it is conceivable that a functional phenotype would only be evident in subjects with complete absence of wild-type subunits, i.e., with a KK genotype. While the K23 variant is predicted to alter the structural integrity of the Kir6.2 channel, the functional outcome could be attenuated in EK heterozygous patients. In principle, the binomial distribution of EK alleles ($E^4 + 4E^3K + 6E^2K^2 + 4EK^3 + K^4$) reveals that >90% of channels would incorporate at least one E-containing subunit (Kowles 2001), thus retaining characteristic gating properties. Indeed, recent measurements of ATP sensitivity have revealed that while K⁴ channels exhibit decreased ATP inhibition, cells transfected with a 1:1 mixture of E and K cDNAs, generating EK channel combinations, display an ATP sensitivity profile that was not statistically different from E⁴ channels (Villareal et al. 2009). This is consistent with our finding that impaired heart rate response in heart failure patients was only detected in the presence of the KK genotype and under exercise stress conditions.

In conclusion, we found an increased prevalence of the Kir6.2 E23K polymorphism in human heart failure. Moreover, this study uncovers a functional association between the K23 variant of the K_{ATP} channel pore and impaired heart rate response to exercise, a surrogate of poor outcome in patients with heart failure. Protein modeling predicts perturbed structural integrity at the transmembrane/ intracellular domain interface and protein–protein interactions between subunits imposed by the E23K amino acid substitution. While initially attributed to mutations in *ABCC9*, identification of E23K in *KCNJ11*-encoded Kir6.2 as a novel biomarker of stress-provoked maladaptation thereby expands the spectrum of human cardiac K_{ATP} channelopathies.

Acknowledgments

This work was supported by the National Institutes of Health (HL071225, HL071478, HL064822), Marriott Heart Disease Research Program, and Mayo Graduate School.

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Fig. 1.

K23 variant of Kir6.2 is overrepresented in a heart failure cohort. Restriction enzyme digestion of PCR-amplified genomic DNA allowed determination of Kir6.2 E23K genotypes. **a** The c.67G → A single nucleotide polymorphism within codon 23, coding for the K allele, resulted in abolition of a *Ban*II restriction site and discrete fragment sizes for homo- (*EE* and *KK*) and hetero-zygous (*EK*) DNA samples. **b** Compared to frequencies of *EE*, *KK* and *EK* genotypes in the ethnically matched population of Olmsted County (Rochester, MN), the presence of the K23 allele is overrepresented in a cohort of patients with heart failure

Genotype Phenotype	EE (n = 36)	EK (n = 59)	KK (n = 20)	P value
Age (years)	59.4 ± 15.6	53.8 ± 11.8	58.1 ± 11.8	0.17
Male gender (%)	71.4	64.5	66.7	0.84
Weight (kg)	83.5 ± 20.5	82.3 ± 18.1	79.5 ± 11.6	0.43
Body mass index (kg/m ²)	29.0 ± 5.0	28.0 ± 4.6	27.7 ± 4.3	0.49
Systolic BP (mmHg)	112.4 ± 21.0	105.3 ± 18.4	104.9 ± 15.8	0.15
Diastolic BP (mmHg)	71.2 ± 12.5	67.3 ± 12.0	65.5 ± 8.6	0.14
Mean BP (mmHg)	84.9 ± 13.8	79.9 ± 13.1	78.7 ± 9.5	0.10
LV diastolic dimension (cm/m ²)	3.4 ± 0.7	3.3 ± 0.6	3.3 ± 0.5	0.94
LV systolic dimension (cm/m ²)	2.8 ± 0.6	2.9 ± 0.8	2.6 ± 0.9	0.55
LV systolic volume (ml/m ²)	38.6 ± 12.1	38.5 ± 13.3	39.7 ± 9.5	0.93
LV mass (g/m ²)	151.9 ± 52.4	144.4 ± 46.7	121.3 ± 27.7	0.12
NYHA class	I:0.39, II:0.39, III:0.19, IV:0.03	I:0.25, II:0.34, III:0.30, IV:0.10	I:0.40, II:0.20, III:0.25, IV:0.15	0.35
LV ejection fraction (%)	28.8 ± 12.6	31.1 ± 12.3	26.8 ± 8.6	0.17
Ischemic heart failure (%)	25.0	32.2	50.0	0.16
Norepinephrine (pg/ml)	361.8 ± 52.4	400.8 ± 43.6	437.3 ± 72.1	0.68
BNP (pg/ml)	200.2 ± 40.1	212.5 ± 39.1	354.4 ± 75.9	0.13
β-blocker treatment (%)	65.7	86.2	80.0	0.07

Fig. 2.

Demographic information and left ventricular structure and function. Data are shown as mean ± SD, or count (%) where appropriate. No significant statistical differences among the three genotypes were found in the study cohort at baseline. Weight and body mass index (BMI), blood pressure (BP), left ventricular (LV) morphology and contractile function, as well as blood levels of catecholamines (norepinephrine) and B-type natriuretic peptide (BNP), were similar among groups. The proportion of subjects presenting with ischemic heart disease or treated with β-blockers was equivalent across genotype groups.

Fig. 3.

KK genotype is associated with abnormal response to exercise stress. Heart failure subjects underwent a progressive exercise stress test, starting at a warm-up matched workload level followed by increasing workloads at 2-min intervals until subjects could no longer exercise. Variables were analyzed post hoc at the initial matched work-load level, at peak VO_2 and at an intermediate level 75% of peak. **a** In spite of comparable heart rates at rest (EE: 72.2 ± 2.3 , EK: 75.0 ± 1.8 and KK: 77.1 ± 3.0 beats per minute; $P = 0.39$), **b** individuals with the KK genotype demonstrated an abnormal heart rate response at all levels of the cardiopulmonary exercise stress test (EE: $32.8 \pm 2.7\%$, EK: $28.8 \pm 2.1\%$, KK: $21.7 \pm 2.6\%$ at the initial matched workload level, EE: $53.9 \pm 3.9\%$, EK: $49.9 \pm 3.1\%$, KK: $36.8 \pm 5.3\%$ at 75% of peak VO_2 , and EE: $82.8 \pm 6.0\%$, EK: $80.5 \pm 4.7\%$, KK: $59.7 \pm 8.1\%$ at peak VO_2 , * $P < 0.05$)

Genotype Phenotype	EE (n = 36)	EK (n = 59)	KK (n = 20)	P value
Rest				
RER	0.91 ± 0.02	0.89 ± 0.01	0.87 ± 0.03	0.57
O ₂ pulse (L/min ²)	4.3 ± 0.3	4.2 ± 0.2	4.2 ± 0.3	0.92
VE/VCO ₂	41.4 ± 1.3	41.0 ± 0.7	44.5 ± 6.8	0.11
Rate-pressure (mmHg·beats/min)	8086.3 ± 335.7	7858.2 ± 258.6	8183.8 ± 444.1	0.77
Matched workload				
RER	0.85 ± 0.02	0.82 ± 0.01	0.82 ± 0.02	0.37
O ₂ pulse (L/min ²)	9.5 ± 0.5	9.7 ± 0.4	9.5 ± 0.7	0.96
VE/VCO ₂	35.2 ± 1.2	34.3 ± 6.0	36.4 ± 6.5	0.46
Rate-pressure (mmHg·beats/min)	11406.2 ± 533.5	10951.6 ± 414.4	10535.5 ± 705.8	0.60
75% of peak VO₂				
RER	0.94 ± 0.01	0.91 ± 0.01	0.90 ± 0.02	0.20
O ₂ pulse (L/min ²)	11.3 ± 0.7	11.9 ± 0.5	11.2 ± 0.9	0.74
VE/VCO ₂	32.6 ± 1.1	32.1 ± 0.8	35.7 ± 1.2	0.08
Rate-pressure (mmHg·beats/min)	14178.2 ± 723.1	13821.8 ± 561.7	12570.3 ± 956.6	0.39
Work (W)	125.0 ± 7.3	129.4 ± 5.7	121.7 ± 9.8	0.76
Exercise time (min)	4.4 ± 0.3	4.3 ± 0.2	4.2 ± 0.4	0.90
Peak VO₂				
RER	1.15 ± 0.02	1.14 ± 0.02	1.09 ± 0.03	0.23
O ₂ pulse (L/min ²)	12.4 ± 0.7	12.9 ± 0.6	12.5 ± 1.0	0.83
VE/VCO ₂	33.6 ± 1.3	34.2 ± 0.9	36.3 ± 1.5	0.38
Rate-pressure (mmHg·beats/min)	17750.5 ± 1059.9	17919.0 ± 816.4	15039.2 ± 1402.2	0.19
Work (W)	162.8 ± 10.5	174.0 ± 8.2	152.9 ± 14.0	0.39
Exercise time (min)	7.3 ± 0.4	7.5 ± 0.3	6.8 ± 0.6	0.62
OUES	1.72 ± 0.10	1.84 ± 0.08	1.60 ± 0.13	0.24

Fig. 4.

Cardiac function during cardiopulmonary exercise stress test. Parameters of cardiovascular and respiratory function were obtained at rest, at a first stage of matched workload for all individuals (Matched), at 75% of peak oxygen consumption (75%), and at peak oxygen consumption (peak) on a treadmill exercise stress test. Amount of work and duration of exercise were equivalent in all groups throughout the exercise stress test, along with similar respiratory exchange ratio (RER), oxygen pulse (O₂ pulse), ventilatory equivalent ratio for carbon dioxide (VE/ VCO₂), rate pressure product and oxygen uptake efficiency slope (OUES)

Fig. 5. Molecular model of Kir6.2 channel pore. **a** Sequence alignment of mammalian *KCNJ11*-encoded Kir6.2 shows highly conserved amino acid residues in N-terminus. The predicted secondary structure below the alignment marks was obtained from PredictProtein (<http://www.predictprotein.org>). **b** Ribbon illustration of Kir6.2 representing individual subunits. A slide helix, involved in lateral movement for the gating mechanism, lies between the transmembrane (TM) and intracellular (IC) domains. The dimensions of TM and IC domains are ~44 and ~72 Å long, respectively. E23 is denoted. **c** A tetramer of Kir6.2 viewed from above with a partial removal of TM and IC domains to clarify the location of the slide helix, E23, and the cytosolic pore which pervades the IC domains. **d** Structure of two Kir6.2 subunits with TM α -helices, slide helix, and the previously unrecognized first α -helix region. The E23 residue is located at the inference between TM and IC domains, where a cluster of charged residues (negative E19/D20/D58/D65 and positive R16/R54/K67)

interacts with phospholipid head groups. Substitution of K23 could be involved in electrostatic repulsion with R325 from an adjacent subunit