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Elevated myocardial and lymphocyte GRK2 expression and activity in human heart failure

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Aims The G protein-coupled receptor kinase-2 (GRK2 or β-ARK1) regulates β-adrenergic receptors (β-ARs) in the heart, and its cardiac expression is elevated in human heart failure (HF). We sought to determine whether myocardial levels and activity of GRK2 could be monitored using white blood cells, which have been used to study cardiac β-ARs. Moreover, we were interested in determining whether GRK2 levels in myocardium and lymphocytes may be associated with β-AR dysfunction and HF severity.

Methods and results In myocardial biopsies from explanted failing human hearts, GRK activity was inversely correlated with β-AR-mediated cAMP production ($R^2 = -0.215$, $P < 0.05$, $n = 24$). Multiple regression analysis confirmed that GRK activity participates with β-AR density to regulate catecholamine-sensitive cAMP responses. Importantly, there was a direct correlation between myocardial and lymphocytes GRK2 activity ($R^2 = 0.5686$, $P < 0.05$, $n = 10$). Lymphocyte GRK activity was assessed in HF patients with various ejection fractions (EFs) ($n = 33$), and kinase activity was significantly higher in patients with lower EFs and was higher with increasing NYHA class ($P < 0.001$).

Conclusion Myocardial GRK2 expression and activity are mirrored by lymphocyte levels of this kinase, and its elevation in HF is associated with the loss of β-AR responsiveness and appears to increase with disease severity. Therefore, lymphocytes may provide a surrogate for monitoring cardiac GRK2 in human HF.

Introduction

β-Adrenergic receptors (β-ARs) represent pivotal molecules in the control of cardiac function through sympathetic nervous system control of inotropy and chronotropy. Adult cardiac myocytes express primarily β_1- and β_2-ARs, with the β_1-AR being the most abundant subtype (>75%).[1] After agonist binding, both subtypes primarily lead to the activation of adenylyl cyclase and cAMP production in the cardiac myocyte.[2] In chronic human heart failure (HF), the deterioration of ventricular function is associated with alterations of cardiac β-AR signalling, which occurs both by a reduction of β_1-AR density and by the uncoupling of remaining β-ARs from G protein effector pathways.[3] This latter phenomenon is known as desensitization and is triggered by the phosphorylation of agonist-occupied β-ARs by a class of serine/threonine kinases, known as G protein-coupled receptor (GPCR) kinases (GRKs). Both β_1- and β_2-ARs can be phosphorylated by these kinases, particularly GRK2, also known as the β-AR kinase (β-ARK1), which is the most abundant GRK in the heart.[4] Desensitization of β-ARs as well as other GPCRs precedes receptor internalization, which is directed by arrestin binding to the phosphorylated receptor, and this can lead to either receptor resensitization or degradation and the loss of receptor density.[5]

GRK2 is a cytosolic enzyme that localizes to the plasma membrane through binding to the βγ subunits ($G_{\beta\gamma}$) of activated heterotrimeric G proteins.[3–5] As a kinase, it plays a major role in the control of cardiac β-AR signalling and functions as demonstrated in transgenic mice with cardiac overexpression of the kinase.[6] In mice with cardiac-targeted three- to four-fold GRK2 overexpression, there was a significant loss of β-AR-mediated inotropic reserve and cAMP production was crippled.[6] This apparent importance of GRK activity in the heart was supported by

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further studies in mice where cardiac GRK2 levels and/or activity was lowered.[6,7] Both transgenic cardiac expression of a peptide inhibitor of GRK2 activity (the β-ARKct)[6] and a loss of 50% of GRK2 expression in the heart present in heterozygous GRK2 gene knockout mice[7] resulted in a phenotype of enhanced cardiac function basally and a contractile supersensitivity to β-AR agonists. Thus, manipulation of GRK2 levels and activity in the heart has profound effects on cardiac function.

Adding to the importance of GRK2 in the heart is the finding that myocardial levels of GRK2 appear to be actively regulated, because in human HF as well as in animal models, there is a characteristic elevation of myocardial expression and activity of this kinase.[3,8–13] In human, this includes both ischaemic and idiopathic dilated cardiomyopathies.[8,9] This increase specifically in GRK2 (two- to three-fold) appears to be responsible for the enhanced β-AR desensitization seen in compromised myocardium.[14,15] Much data have recently accumulated in experimental models, suggesting that increased levels of GRK2 in failing myocardium can contribute to the pathogenesis of HF.[3]

The relevance of the molecular abnormalities of β-AR signalling to the natural history and pathogenesis of human HF, and perhaps more importantly to HF outcome, is not completely understood. An important aspect of β-AR signalling is that properties of the system in circulating white blood cells appear to mirror those observed in solid tissues. This was first observed in heart in 1986,[16] and since then, many other reports have used the lymphocyte system to study β-AR signalling and to make extrapolations to the cardiac β-AR system.[17–20] Thus, lymphocytes represent a valuable and reliable marker of the functional state of cardiac β-AR signalling, which may also extend to GRK2 regulation.

In the present study, we investigated whether blood and cardiac (right atrium) GRK2 levels and activity correlate in a direct fashion, so that lymphocyte GRK2 content might serve as an easily accessible means of monitoring cardiac GRK2 levels and provide a useful gauge of myocardial β-AR and GPCR signalling. Moreover, we were interested in learning whether blood GRK activity may be a potential biomarker that associates with varying severity of cardiac dysfunction in HF.

Methods

Study population

We studied samples obtained from two groups of patients. The first group consisted of 24 patients undergoing cardiac transplant due to severe deterioration of cardiac function. Patients were enrolled consecutively over a period of 8 weeks at the Cardiac Surgery of Temple University, PA, USA. Patients who had endstage HF, with severely compromised cardiac function, were enrolled in the Institutional heart transplant list. Those patients who presented with a concomitant oncological disease or were affected by chronic infective disease, which could pose threat to the health of the personal involved in lab assessment on samples, were excluded from the enrolment. All patients included originally in the study gave permission to sample left ventricular (LV) specimens of the explanted hearts. These patients presented with the clinical characteristics shown in *Table 1* (transplant group). Another group included 58 patients that were consecutively admitted and met the inclusion criteria over a period of 4 weeks into the intensive care coronary unit (ICU) at the Federico II University of Naples, with varying degrees of ventricular dysfunction. Inclusion criteria were the presence of a cardiovascular condition posing threat to survival and the absence of signs, symptoms, or history of asthma or other respiratory, gastrointestinal, hepatic or renal disease, anaemia, electrolyte or endocrine impairments, or concomitant oncological condition. We obtained permission from 55 patients to sample blood and lymphocytes for research purposes (ICU group). Among the patients of the second group, 10 underwent elective cardiac surgery, and we obtained permission to sample the right atrium (ICU + surgery group). The clinical characteristics of this study population are found in *Table 1*, including drug treatment regimens. All procedures were performed in compliance to institutional guidelines for human research.

Table 1 Clinical characteristics of patients analysed in this study

	Transplant	ICU	
		Whole group (ICU)	Surgery subgroup (ICU + surgery)
n	24	55	10
NYHA class	3–4	1–4	1–3
Age (years)	60 ± 10	65 ± 15	71 ± 6
Gender (%M/%F)	70/30	65/35	86/14
% Ischaemic/% dilated cardiomyopathy	50/50	n.a.	n.a.
Beta blockade (%)	8	22	20
ACE inhibition (%)	50	58	50
AR blockade (%)	58	8	0
Diuretics (%)	42	19	40
Calcium antagonists (%)	25	31	90
Nitrates (%)	42	69	70
Digoxin (%)	25	50	20

Mean ± SD; percentage of patients presenting that characteristic or treated with the corresponding drug.

Myocardial samples

Following *in situ* blood-buffered cardioplegia, transmural LV tissue (≈2 g) specimens from failing hearts were obtained during cardiac transplantation from 24 patients with HF due to ischaemic or dilated cardiomyopathy. Right atrial appendages (≈200 mg) were also obtained from 10 patients undergoing elective cardiac surgery (aortocoronary bypass grafting or valvular replacement). Immediately after removal, all specimens were placed in ice-cold saline, rinsed, frozen in liquid nitrogen, and stored at −80°C.

Peripheral lymphocyte samples

Blood was collected and anticoagulated with EDTA. In ICU + surgery patients, blood was collected on the day before surgical treatment for valvular replacement or coronary bypass surgery. Lymphocytes were isolated by ficoll gradient using HISTOPAQUE-1077 (Sigma), frozen, and stored at −80°C until the day of the assay. All procedures were performed in compliance to institutional guidelines for human research.

β-AR density and membrane adenylyl cyclase activity assays

Crude myocardial membranes were prepared from myocardial biopsies or lymphocytes as previously described.[21,22] β-AR density was determined by radioligand binding with the non-selective β-AR ligand [^{125}I]-CYP and membrane adenylyl cyclase activity

under basal conditions or in the presence of either 10 μmol/L isoproterenol or 10 mmol/L NaF and cAMP was quantified using standard methods.[21,22] All β-AR signalling results were normalized to the amount of protein added during the experiments. For example, all cAMP data were normalized to milligram of membrane protein.

Protein immunoblotting

Immunodetection of myocardial levels of GRK2 was performed using detergent-solubilized cardiac extracts after immunoprecipitation (IP) as previously described.[21,22] IPs were done using a monoclonal anti-GRK2/3 antibody (C5/1, Upstate) followed by western blotting with a GRK2 polyclonal antibody (C-20, Santa Cruz Biotechnology) as described earlier.[21–24] All IPs were done in protein lysates of the same quantity (i.e. same starting amount in micrograms of protein). Post-IP lysates have been blotted for residual GRK2 amounts, and typically none has been found as in our previous studies,[21,22,24] demonstrating the quantitative nature of these experiments. The 80 kDa GRK2 protein was visualized using standard enhanced chemiluminescence (ECL Kit, Amersham). Quantitation of immunoreactive GRK2 was done by scanning the autoradiography film and using ImageQuant software (Molecular Dynamics).

GRK activity assays

Extracts were prepared through homogenization of cardiac tissue or lymphocytes in 2 mL of ice-cold detergent-free lysis buffer. Cytosolic fractions and membrane fractions were separated by centrifugation, and soluble GRK activity was assessed in cytosolic fractions (100–150 μg of protein) by light-dependent phosphorylation of rhodopsin-enriched rod outer segment membranes using [γ-^{32}P]-ATP as described.[21,22,24] Soluble GRK activity represents primarily GRK2 (β-ARK1) activity, and changes in GRK2 expression correlate with altered β-AR signalling.[25] GRK activity from membrane fractions was not assessed. Phosphorylated rhodopsin was visualized by autoradiography of dried gels, and the amount of [γ-^{32}P]-ATP incorporated was quantified using a Molecular Dynamics PhosphorImager and a standard curve of labelled cocktail.[21,22,24]

Statistical analysis

Statistical analysis was performed using SPSS 11.5 software for Windows. Values are given as the mean ± SD. The study was originally ideated as an observational study. We planned to measure β-ARK activity in a sample large enough to define the mean value with a precision of 5 fmol/mg/min. Our sample size calculation showed that the least *n* had to be equal 12, considering an SD of 15 fmol/mg/min,[25] according to the formula $n = 4 \times (\text{SD}/\text{precision})$.[2,26] To compare groups, we used a Student's unpaired *t*-test. We studied correlations between variables using linear regression analysis. All linear regression analyses were aimed to verify, in our model, the relationship between variables that were previously demonstrated to interact in animal models but never before in humans. All analyses were performed using a two-sided model. The *P*-value less than 0.05 was considered statistically significant. We also calculated the relative effect of receptor density and GRK activity on adenylyl cyclase activity using a stepwise multiple regression analysis. Both β-AR density and β-ARK activity have been demonstrated in animal models to affect adenylyl cyclase response,[6,27] but this was never before tested in human left ventricles. First, by linear regression analysis, we verified that β-ARK activity and β-AR density affect adenylyl cyclase response in human ventricles. On the basis of this notion, we then verify that adding in the model the two predictors resulted in a significant increase in the model R^2 value. For this reason, we used a multivariate analysis as a forward stepwise approach. The model building was validated using a let-one-out and a k=2 partitioning approach. The relative importance of each variable in the model was indicated by *t* statistics. Statistical significance was considered when $P < 0.05$, and where applicable, tests were two-sided.

Results

β-Adrenergic signalling in failing human myocardium

We first assessed GRK2 (also known as β-ARK1) expression and activity in cytosolic extracts from these failing heart samples and found that there was a direct correlation between immunoblotted GRK2 protein and *in vitro* GRK activity ($R^2 = 0.359$, $P = 0.002$, $n = 24$) (*Figure 1A*). Because experimental studies in animals have shown that levels of myocardial β-ARK1 can greatly influence β-AR signalling in the heart,[6,7] we evaluated the relationship

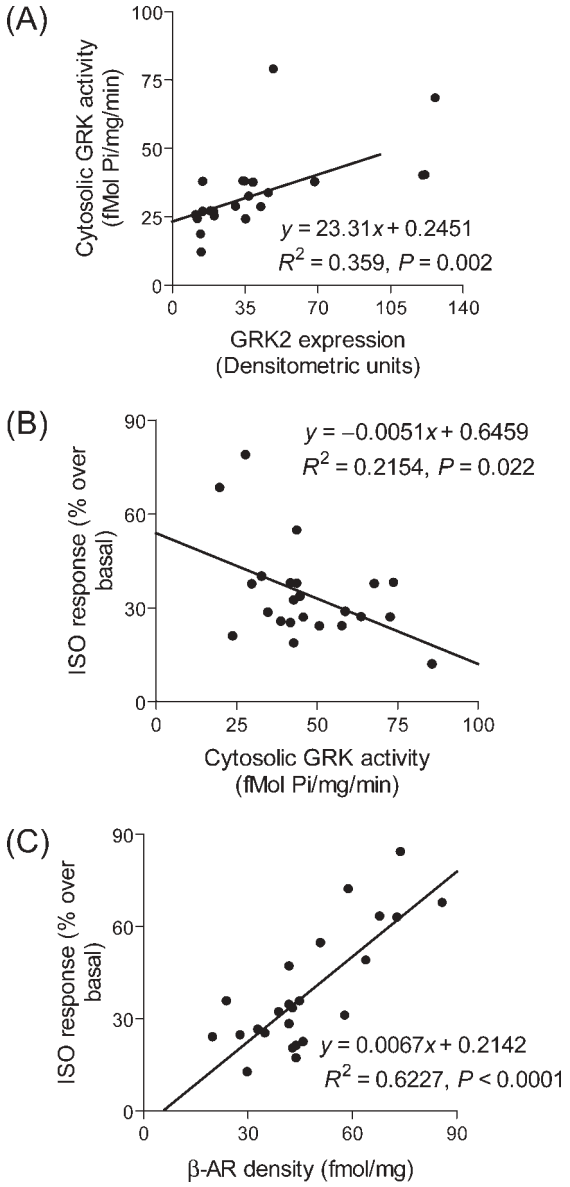

Figure 1 (*A*) Graph showing the direct correlation between soluble GRK activity measured *in vitro* phosphorylation of rhodopsin (see Methods) and GRK2 expression detected by western blot with a selective antibody (*B*) Graph showing an inverse correlation between soluble GRK activity and isoproterenol (ISO) stimulation of adenylyl cyclase activity in cardiac membranes from LV biopsies explanted failing human hearts. Adenylyl cyclase activity is plotted by the %ISO response over basal stimulation ($n = 24$, $P < 0.05$). (*C*) Using a similar approach in the same samples, we observed a direct correlation between β-AR density and β-AR signalling (ISO-stimulated adenylyl cyclase activity over basal stimulation, $n = 24$, $P < 0.0001$).

between β-AR-mediated adenylyl cyclase activity in cardiac membranes and cytosolic GRK activity. We also assessed the relationship between β-AR density and cAMP production in the same failing heart biopsies. First, we found a significant inverse correlation between GRK2 activity and β-AR responsiveness. As *Figure 1B* shows, when GRK2 activity is greater, β-AR signalling, as measured by isoproterenol-stimulated adenylyl cyclase activity, is depressed. Thus, for the first time in humans, myocardial GRK2 has been shown to negatively affect β-AR signalling. In addition, as would be expected, there was a positive correlation found between isoproterenol-mediated cAMP production and the density of myocardial β-ARs (*Figure 1C*). It is known that both β-AR density and GRK2 can affect β-AR induced adenylyl cyclase activity. Using a multivariable model for the analysis of the linear regression, we confirmed that indeed in LV samples, both determinants can affect cAMP production, (F: 31.861, $P < 0.001$; β-AR density = T:6.285, $P < 0.001$; GRK activity = T: − 3.311, $P < 0.005$).

β-Adrenergic signalling in peripheral lymphocytes in HF

A hypothesis that we wanted to test was whether the β-AR system, particularly GRK2, in white blood cells could be used as a surrogate for what is seen in failing myocardium. To verify any correlation between cardiac and peripheral lymphocytes in terms of GRK activity, we measured GRK2 expression in atrial appendages (biopsies) and lymphocytes from patients with cardiovascular disease undergoing cardiac surgery. These patients underwent surgery for coronary artery disease or valvular replacement and were generally in NYHA HF class 1–3. Their clinical characteristics are described in *Table 1* (ICU + surgery group). As shown in *Figure 2A*, we found a direct correlation between myocardial and lymphocyte GRK2 expression, indicating that lymphocyte levels of this GRK mirrors cardiac expression. Soluble GRK activity gave identical results (data not shown) showing that we can measure lymphocyte GRK2 either by protein immunoblotting or by soluble GRK activity. Importantly, we have found that when GRK2 expression is elevated in the myocardium, enhanced levels and activity are also apparent in lymphocyte extracts. An example of this is shown in *Figure 2B* in two HF patients with different disease severity.

On the basis of this observation, we extended lymphocyte GRK2 expression and activity analysis to a larger number of patients, with different degrees of cardiac function, ranging from normal to significantly depressed cardiac function, as assessed by clinical (NYHA class) and instrumental (echocardiography) evaluation of their HF. The characteristics of these patients (ICU group) are listed in *Table 1*. As shown in *Figure 3A*, when patients were divided into two groups using a functional cutoff of 45% left ventricular ejection fraction (LVEF) at echocardiography, soluble, cytosolic GRK activity is significantly higher in the blood from patients with poorer LV function. Furthermore, cytosolic GRK correlates significantly to echocardiographic LVEF ($R^2 = 0.193$, $P = 0.01$). Similarly, we observed a stepwise increase in GRK activity with the NYHA functional HF class (*Figure 3C*). Therefore, our results appear to indicate that in patients with lower ventricular function or higher

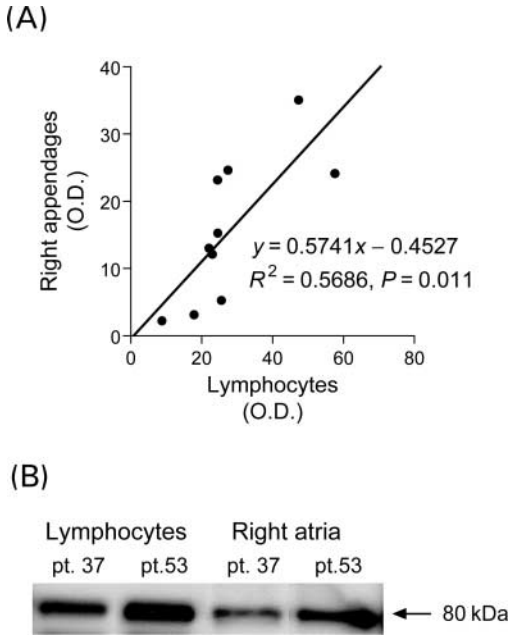

Figure 2 (*A*) Graph showing the direct correlation between GRK2 expression in the heart and lymphocytes of HF patients. GRK2 expression was assessed by protein immunoblotting and the data is expressed as arbitrary densitometry units of scanned chemiluminescent autoradiograms. (*B*) Representative autoradiograph from a protein immunoblot showing GRK2 expression in lymphocyte extracts and in extracts from right atrial appendages from the same sets (pt.37 and pt.53) of human HF patients undergoing cardiac surgery.

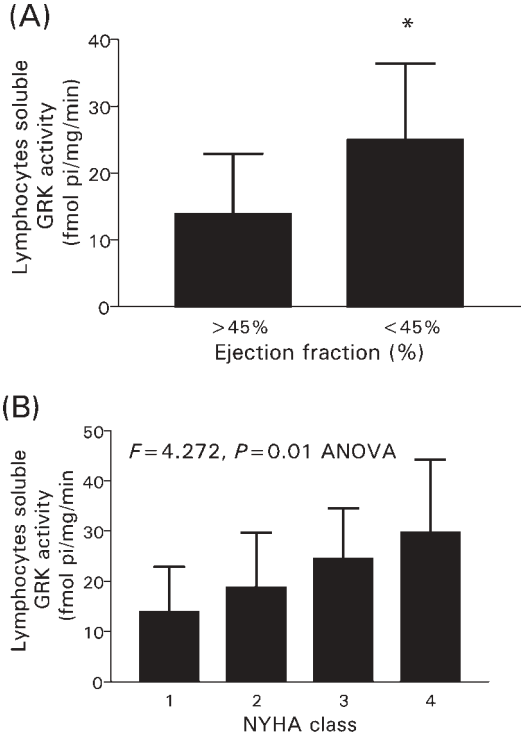

Figure 3 (*A*) Using a cutoff of 45% LVEF, we divided the 55 HF patients into two groups. Those showing reduced cardiac function also had higher lymphocytes GRK2 activity. *$P = 0.02$ (unpaired Student's t-test). (*B*) When patients were stratified according to their NYHA functional HF class, there was a stepwise significant increase in lymphocyte soluble GRK activity.

classes of clinical HF, there are higher levels of cardiac GRK2 that can be measured in peripheral lymphocytes.

Discussion

This paper represents the first extended study focused on the GRK2 (or β-ARK1) in different groups of patients with varying degrees of cardiac dysfunction and HF. In animal models, a series of experiments have provided a thorough analysis of the mechanisms by which GRK2 and its activity participates in the worsening of β-AR signalling and the onset of HF.[3,28] In contrast, only two studies have described increased levels of GRK2 in autopsy specimens from failing human hearts at the time of explantation.[8,9] GRK2 appears to be the primary β-AR regulatory molecule altered in human HF and other GRKs expressed in the heart (i.e. GRK3 and GRK5) or the arrestins do not appear altered in failing human hearts.[8,9] Assessing GRK2 and β-AR signalling from similar LV biopsies taken at explantation, we found an inverse correlation between GRK2 activity and β-AR signalling. This is an important information to go along with existing knowledge that there is a direct correlation between myocardial β-AR density and cardiac cAMP production in response to β-AR stimulation. These data point towards a critical relevance of GRK2 in the setting of β-AR dysfunction in the human heart.

Key regulatory processes involved in β-AR signalling are receptor desensitization and internalization, and importantly, these are both triggered by β-AR phosphorylation by GRK2 or other GRKs.[3–5] Other signal transduction partners may contribute to β-AR dysfunction in human HF such as Gαi up-regulation and altered expression of adenylyl cyclase isoforms[29] as well as genetically determined variants of AR genes.[30,31] The fact that we found a significant inverse correlation between β-AR responsiveness and GRK2 activity in the failing heart demonstrates that this kinase plays a critical role in human myocardial β-AR regulation and function. Although this has been extensively shown in genetically engineered mice and larger animal models,[6,7,14,28,29] this is the first demonstration of the importance of GRK2 in β-AR signalling in human HF.

Discussion concerning the role of cardiac β-AR desensitization through enhanced GRK2 activity in HF is somewhat complex. To combat the bombardment of catecholamines present during enhanced sympathetic nervous system activity in HF, the dampening of β-AR signalling appears to be adaptive and protective as the heart is compromised. However, this is also harmful and maladaptive to the heart because it perpetuates a dysfunctional myocardium. Interestingly, GRK2 appears to be the central molecule in this maladaptation as prevention of β-AR desensitization and down-regulation protects the infarcted heart against the development of HF.[6,7,14,28,29] Thus, cardiac levels of GRK2 appear to be an important determinant of cardiac signalling and function that we have shown can be monitored through blood sampling.

Indeed, a key finding in this study is the demonstration that there is a direct correlation between lymphocyte and cardiac (right atrial appendages) GRK2 expression and activity. Thus, measuring GRK2 in blood samples, either by immunoblotting or GRK activity, can be used to monitor relative levels of this GRK in myocardium. The possibility to use lymphocytes for monitoring drug- or disease-induced β-AR changes in the heart, which is not easily accessible in humans, was first hypothesized by Brodde *et al.*[16] and further realized by others.[32] Importantly, in this study, we add to this scenario with the novel finding that this system can be used to study other key β-AR associated molecules such as GRK2. We did find a statistically significant relationship between lymphocyte GRK2 and cardiac β-ARs, suggesting a close relationship between these two systems. These data are correlative in nature and the overall prediction of lymphocyte GRK2 expression and cardiac β-AR function may be variable. However, the importance is that blood GRK2 mirrors cardiac levels of this kinase and previous studies in animal models have demonstrated the critical role of cardiac GRK2 on myocardial β-AR signalling.[3] Thus, if GRK2 is elevated in the heart as indicated by the biomarker levels measured in lymphocytes, β-AR signalling consequences should be expected. Of further interest is the fact that GRK2 in lymphocytes has been found to be elevated in human hypertensive patients,[33] adding further support for using blood GRK2 levels for monitoring of patients with cardiovascular disease.

The mechanism responsible for similar alterations in the β-AR system of lymphocytes and myocardium is uncertain. Recent data from the Brodde and colleagues[34] show that β-AR blockade in HF also blocks catecholamine responses in lymphocytes. These data support the concept that the GRK system in lymphocytes and heart is regulated in a similar manner. It is known that chronic catecholamine exposure induces β-AR signalling abnormalities such as β-AR down-regulation and that HF is associated with increased circulating norepinephrine.[29,35] As myocardial GRK2 is up-regulated in response to chronic adrenergic activation[21,22,24,36], one possibility is that the increased circulating catecholamines, i.e. norepinephrine and epinephrine, can trigger an increase in GRK2 expression both in the lymphocyte and in the heart by β-ARs. However, this hypothesis needs to be explored further, perhaps in HF patients who have been treated with β-AR antagonists, to determine whether blockade of chronic catecholamine activation of β-ARs in the heart and circulating white blood cells could affect GRK2 expression. Interestingly, this has been shown to be the case in hearts of mice chronically exposed to carvedilol and atenolol[21] and in pigs treated with bisoprolol[37] as chronic β-AR blockade reduced cardiac GRK2 levels and increased β-AR signalling. Thus, measuring GRK2 levels in peripheral blood in HF patients treated with β-AR blockers, as we have shown for feasibility in this study, may have a role in monitoring treatment by these agents. Further clinical research is needed to test this hypothesis.

Indeed, our current data provide the background for a larger study to evaluate any potential predictive or biomarker role of GRK2 in human HF. Certainly, in animal models of HF, cardiac GRK activity up-regulation is frequently observed.[10–14,24,38–41] However, there are some cardiomyopathic models where it is not elevated.[42] This observation might suggest a differential role of this kinase in HF. In this study, nevertheless, we observed that decreased cardiac performance (i.e. EF) associated with increased GRK2 levels found in peripheral white blood cells. Although there might be a certain level of variability among failing patients depending on the treatment, the aetiology, and the clinical conditions of patients, it is possible to hypothesize that patients that have higher level of

GRK2 may have more negative outcomes in HF. This hypothesis is supported by a recent study in transgenic mice, in which increased GRK2 expression and activity was associated with severe cardiomyopathy and early mortality.[24] In this study, transgenic mice with myocardial-targeted overexpression of the α_{1b}-AR were chronically treated with phenylephrine and this caused a rapid worsening of cardiac performance, with increased GRK2 expression and activity and increased mortality.[24] In wild-type mice, chronic phenylephrine treatment does not lead to increased GRK2 expression or activity in the heart.[22,24]

Interestingly, data presented in this study demonstrate that GRK2 in lymphocytes have an association with LVEF and NYHA HF class. These clinical endpoints are multi-factorial in nature and not solely dependent on cardiac β-AR status. Importantly, it should be noted that GRK2 targets many other GPCRs in the heart and thus can affect other systems besides β-ARs that could have key associations with heart function determination.[3] GRK2 activity has been shown in several animal studies to have a profound effect on cardiac function, which no doubt has non-β-AR mechanistic components.[3] In addition to the potential of cardiac non-β-AR mechanisms to account for our important clinical associations found between lymphocyte GRK2 and cardiac function, elevated GRK2 may correlate with non-cardiac β-AR functions such as β-ARs found in the coronary vasculature.[43]

In summary, this study provides three major novel observations: (i) the demonstration that increased cardiac GRK2 levels correlate with decreased β-AR signalling in failing human hearts; (ii) the direct demonstration that cardiac GRK2 levels can be monitored using peripheral lymphocytes; and (iii) the suggestion that increased GRK2 levels may be associated with more severe cardiac function or clinical signs of HF. Further clinical studies will be required to determine the biomarker activity of lymphocyte GRK2 in HF or the changes in GRK2 levels can be used to monitor the effects of adrenergic blocking agents in HF, which may better define the relationship between lymphocyte GRK2 activity and myocardial adrenergic responsiveness.

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