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Cardiac Adenylyl Cyclase and Phosphodiesterase Expression Profiles Vary by Age, Disease, and Chronic Phosphodiesterase Inhibitor Treatment

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Abstract

Background—Pediatric heart failure (HF) patients have a suboptimal response to traditional HF medications, although phosphodiesterase-3 inhibition (PDE3i) has been used with greater success than in the adult HF population. We hypothesized that molecular alterations specific to children with HF and HF etiology may affect response to treatment.

Methods and Results—Adenylyl cyclase (AC) and phosphodiesterase (PDE) isoforms were quantified by means of quantitative real-time polymerase chain reaction in explanted myocardium from adults with dilated cardiomyopathy (DCM), children with DCM, and children with single-ventricle congenital heart disease of right ventricular morphology (SRV). AC and PDE expression profiles were uniquely regulated in each subject group and demonstratde distinct changes in response to chronic PDE3i. There was unique up-regulation of AC5 in adult DCM with PDE3i

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Supplementary Data

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(fold change 2.415; $P = .043$), AC2 in pediatric DCM (fold change 2.396; $P = .0067$), and PDE1C in pediatric SRV (fold change 1.836; $P = .032$). Remarkably, PDE5A expression was consistently increased across all age and disease groups.

Conclusions—Unique regulation of AC and PDE isoforms supports a differential molecular adaptation to HF in children compared with adults, and may help identify mechanisms specific to the pathogenesis of pediatric HF. Greater understanding of these differences will help optimize medical therapies based on age and disease process.

Keywords

Adenylyl cyclase; phosphodiesterase; single ventricle congenital heart disease; dilated cardiomyopathy

Despite clear improvement in adult heart failure (HF) outcomes over the past several decades, pediatric HF outcomes remain comparatively poor. Treatments for children with HF, largely regardless of etiology, are based on clinical trials performed in adults with systolic HF, but a growing body of clinical evidence suggests that established adult HF medications, such as β-adrenergic receptor blockers and angiotensin-converting enzyme inhibitors, may not provide the same benefit to children with HF.[1–4] Additionally, there is differential adaptation of β-adrenergic receptors and adrenergic signaling pathways in children with HF secondary to dilated cardiomyopathy (DCM) as well as single right ventricle heart disease (SRV), suggesting that both age and disease etiology may influence response to therapy.[5,6] Certainly, the treatment of pediatric HF patients comprises unique challenges, including (1) diverse etiologies of HF, (2) age-related changes, (3) treatment of a vulnerable population, and (4) a relatively low incidence of HF. Therefore, investigations into the differences between a spectrum of pediatric and adult HF populations may further our understanding of disparate mechanisms of disease as well as the variable therapeutic potential of certain medications.

The second messenger cyclic adenosine monophosphate (cAMP) is highly regulated, in terms of both concentration and location. A decrease in global myocardial cAMP level is a common feature across multiple HF populations and phenotypes, specifically adults and children with DCM[7] and children with SRV,[8] suggesting that alterations in cAMP generation via adenylyl cyclases (ACs) or cAMP hydrolysis via phosphodiesterases (PDEs) may be involved in HF. Both ACs and PDEs have numerous isoforms with differing degrees of expression and activity in the myocardium. Activation of ACs via β-adrenergic receptor stimulation leads to increased production of cAMP, which provides short-term improvement in cardiac contractility. Nevertheless, prolonged and excessive adrenergic stimulation, as in the setting of HF, leads to adverse cardiac remodeling as well as induction of cardiac myocyte apoptosis.[9,10] Inhibition of PDEs can also contribute to improved inotropy and lusitropy through decreasing cAMP and/or cyclic guanosine monophosphate (cGMP) hydrolysis in the appropriate subcellular compartments. Therefore, stimulation or inhibition of AC isoforms and inhibition of select PDEs have been investigated as potential HF therapies, with varying success and utility. Most notably, PDE3 inhibition (PDE3i) is commonly used on a chronic basis as a bridge to transplant or recovery in children with HF,[11,12] with a low incidence of sudden death, whereas a clinical trial of PDE3i in adults

with severe HF demonstrated a 34% increase in cardiovascular mortality,[13] tempering its long-term use in adult HF. Specifically, our previous work demonstrated that only pediatric DCM patients treated with PDE3i benefit from augmentation of myocardial cAMP and phospholamban phosphorylation, which may contribute to sustained hemodynamic benefits; these changes were not demonstrated in myocardium from adults with DCM[7] or children with SRV.[8] The differential response to PDE3i, both clinically and molecularly, is suggestive of fundamental differences in molecular adaptation to HF secondary to age and disease etiology. AC and PDE isoforms can uniquely target cAMP or cGMP and be present in different compartments. The physiologic consequences of activation of these isoforms varies from increased contractility to relaxation (which is addressed in the Discussion section). Although multiple AC and PDE isoforms are expressed in human heart tissue, little is known about the isoform levels in response to HF or PDE3i. Therefore, our objective was to identify changes in isoform expression of myocardial AC and PDE to provide insight into which subcellular compartments may be altered by disease, age, and PDE3i treatment. These expression profiles can be used in conjunction with additional studies to ultimately (1) characterize molecular alterations that may contribute to HF pathogenesis, particularly in the pediatric population, (2) identify targets unique to pediatric DCM or SRV that have therapeutic potential, and (3) further elucidate the mechanism of the beneficial effects of PDE3i in the pediatric population and, conversely, the mechanism underlying adverse outcomes with PDE3i in adults.

Methods

Human Samples

All subjects gave informed consents and donated their hearts to the Institutional Review Board–approved Pediatric or Adult Cardiac Transplant Tissue Bank at the University of Colorado, Denver. Nonfailing tissues were from adult or pediatric organ donors without previous heart disease; nonfailing hearts were deemed to be suitable for transplantation but could not be placed owing to technical reasons (size or blood type mismatch). All adult patients with DCM had nonischemic cardiomyopathy without any definitive contributing comorbidity. Pediatric hearts from patients transplanted for SRV morphology were included in this study; patients with single left ventricle heart disease or indeterminate morphology were excluded. Subjects with HF (DCM or SRV) were divided into 2 groups based on whether they were on PDE3i at the time of explantation. At the time of cardiac transplantation or donation, the heart tissue is rapidly dissected, flash frozen and stored at −80°C until further use.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted from homogenized cardiac myocardium with the use of the mirVana kit (Ambion, Austin, Texas) and reverse transcribed into complementary DNA with the use of the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, California). Quantitative real-time polymerase chain reaction (qRT-PCR) was then performed with Power Sybr Green PCR Master Mix (Applied Biosystems/Life Technologies, Carlsbad, California) with the use of the ABI Step-One Plus system. Primer sequences are listed in Supplemental Table 1. Melt curve analysis was performed on each primer pair to confirm target specificity.

Data Analysis and Statistics

Statistical analyses were performed with the use of Graphpad Prism version 6.0c. Statistical significance was set a priori at $P < .05$. Normality of the data was not assumed, and Mann-Whitney tests were performed. Comparisons were made between nonfailing control and disease (treated and untreated combined) and between untreated disease and treated disease.

Results

Subject Characteristics

Adult and pediatric subject characteristics are listed in aggregate form in Table 1; individual subject characteristics are detailed in Supplemental Tables 2–4.

ACs Are Differentially Regulated by Age and Disease

Table 2 presents variable expression of the different AC types among adult and pediatric subjects with DCM and pediatric subjects with SRV; AC expression levels were also variably affected by PDE3i treatment (Table 3). In adult DCM myocardium, AC7 (fold change −2.250; $P = .0004$) was down-regulated compared with nonfailing adult LV myocardium and AC6 was up-regulated (fold change 2.857; $P = .045$). The adult DCM group that was chronically treated with PDE3i demonstrated AC levels similar to DCM patients not treated with PDE3i, except for an increase in AC5 (fold change 2.415; $P = .043$) and AC6 (fold change 1.981; $P = .035$) expression and lower AC7 levels (fold change 1.468; $P = .029$). In contrast, AC5 and AC6 were unchanged in pediatric DCM and there was a significant unique up-regulation of AC2 (fold change 2.396; $P = .0067$). AC levels between the PDE3i-treated and untreated pediatric DCM subjects were equivalent except for higher AC7 expression (fold change 1.764; $P = .013$). Conversely, the pediatric SRV group demonstrated higher AC5 levels (fold change 2.399; $P = .0018$) and lower AC7 levels (fold change −1.606; $P = .020$) compared with nonfailing pediatric right ventricular myocardium; all tested AC isoforms had similar levels of expression between the PDE3i-treated and untreated SRV groups.

Variable Changes in PDE Isoform Expression Based on Age, Disease, and PDE3i Treatment

Table 4 presents differential expression of various cardiac-relevant PDE isoforms between adult and pediatric DCM subjects and pediatric SRV subjects. In adult LV myocardium with DCM, there was decreased expression of select PDE4 isoforms, specifically PDE4A3 (fold change −2.240; $P = .038$) and PDE4D8 (fold change −2.064; $P = .0023$) compared with nonfailing adult LV myocardium. The down-regulation of PDE4 isoforms was not influenced by PDE3i treatment in adult DCM; all PDE4 isoforms expression levels were equivalent between adult DCM with and without PDE3i (Table 5). Pediatric LV myocardium with DCM also demonstrated decreased expression of select PDE4 isoforms, but this was limited specifically to PDE4D8 (fold change −2.880; $P = .0071$) and PDE4D9 (fold change −2.828; $P < .0001$) compared with nonfailing pediatric LV myocardium (Table 4). PDE3i treatment in pediatric DCM had no effect on PDE4D8 expression, although both PDE4D5 (fold change 1.594; $P = .046$) and PDE4D9 (fold change 1.773; $P = .042$) showed trends

toward being higher compared with pediatric DCM not treated with PDE3i (Table 5). There was unique up-regulation of PDE1C1 (fold change 1.836; $P = .032$), PDE3A1-1 (fold change 2.295; $P = .0042$), PDE3B (fold change 2.464; $P = .0014$), and PDE4D5 (fold change 2.296; $P = .0031$) in pediatric SRV myocardium compared with nonfailing right ventricular myocardium. Levels of all tested PDE isoforms were not influenced by PDE3i treatment in SV myocardium (Table 5).

Common Up-Regulation of PDE5A

As presented in Table 4, expression of the PDE5A isoform was universally higher in adult and pediatric LV myocardium with DCM (fold changes 4.131 [$P = .0007$] and 2.573 [$P = .0002$], respectively, above nonfailing levels), as well as in pediatric SRV (fold change of 2.797 [$P < .0001$] above nonfailing right ventricular levels). In all 3 groups, PDE5A expression was equivalent between failing patients who received PDE3i treatment and those who did not.

Discussion

The molecular bases of clinical disparities between pediatric and adult HF have, to this point, gone largely unresolved. Adaptation of β-adrenergic receptors is a hallmark of HF, but β-adrenergic receptor adaptation in children with DCM is distinct from that of adults with DCM. In children, both β_1- and β_2-adrenergic receptor subtypes are down-regulated, whereas only β_1-adrenergic receptors are down-regulated in adults.[5] Nevertheless, children with HF from SRV demonstrate selective down-regulation of β_1-adrenergic receptors with preserved β_2-adrenergic receptor expression.[6] We hypothesized that the pathogenesis and regulation of cAMP and cGMP in pediatric HF are dissimilar from those of adult HF and, furthermore, that the expression profile of pediatric HF is likely distinct based on disease etiology. The molecular alterations specific to each patient population would have important implications regarding putative responses to PDE3i and other medical therapies. Our findings were supportive of these hypotheses and provide stimulus for further pediatric-specific investigation.

Differential Regulation of AC5 and AC6

The unique composition of AC isoforms likely confers distinct β-adrenergic receptor signaling attributes to each tissue, with the dominant AC isoform playing the largest role.[14] AC5 and AC6 are the 2 predominant AC isoforms in cardiac myocytes and are responsible for the generation of cAMP in response to β-adrenergic receptor stimulation.[15]

AC5, in particular, has been shown to be the dominant isoform in the heart and possesses the highest enzyme catalytic activity among the AC isoforms.[16] Increased AC5 is associated with adverse myocardial remodeling, and transgenic mice overexpressing AC5 have elevated reactive oxygen species generation, oxidation of calcium/calmodulin–dependent protein kinase II, and phosphorylation of ryanodine receptor 2 which contribute to higher arrhythmic susceptibility.[15] Furthermore, selective inhibition of AC5 or AC5 knockout in mice significantly suppresses cAMP accumulation and cardiac apoptosis induced by β_1-adrenergic receptor stimulation, suggesting that the β_1-adrenergic receptor selectively

couples with AC5.[17] Therefore, increased AC5 in adult DCM subjects treated with PDE3i may counter the effects of β_1-adrenergic receptor desensitization and contribute to the adverse clinical outcomes with the use of PDE3i documented in this population,[13] namely, progression of underlying disease or development of life-threatening ventricular arrhythmias. Interestingly, this finding is isolated to the adult myocardium. Pediatric DCM patients chronically treated with PDE3i do not succumb to the same increase in cardiovascular mortality,[11,12] and no increase in AC5 expression in PDE3i-treated pediatric myocardium was demonstrated in the present study. Nevertheless, AC5 is higher in the pediatric SRV group when compared to nonfailing right ventricular control subjects. Of particular relevance to SRV disease, AC5 has been shown to increase in response to pressure overload hypertrophy, paralleling the fetal gene program.[18] The myocardium in SRV disease is under an abnormally high (systemic) pressure load, which may contribute to elevated AC5 in this population. Nevertheless, although increased AC5 may contribute to disease pathogenesis in pediatric SRV, it may also reflect a normal developmental phenomenon. In mice, rats, and pigs, AC5 was shown to be highest at birth and then gradually decline with maturation,[18] and in the present study, the SRV group was significantly younger (median age 2.6 years) than our nonfailing right ventricular control group (median age 7.5 years). In contrast to adult DCM and consistently with a low incidence of arrhythmogenic death, PDE3i treatment in the SRV group did not yield any additional alterations in AC5 expression.

In adult DCM, AC6 is uniquely up-regulated and is not significantly altered by PDE3i therapy. AC6 is known to complex with A-kinase anchoring protein 5 and caveolin-3 to mediate β-adrenergic stimulation of calcium transients in adult murine cardiac myocytes,[19] and disruption of AC6 promotes development of myocyte apoptosis and HF in response to chronic catecholamine or pressure-overload stress.[20–22] It has been suggested that β_2-adrenergic receptors may preferentially couple with AC6 at surface sarcolemma to activate cell survival pathways through activation of phosphatidylinositol 3-kinase/Akt signaling in the heart.[17,23–26] Therefore, the increase in AC6 may be a compensatory and possibly protective mechanism in adult DCM induced by chronic PDE3i treatment, or it could reflect a clinical bias toward PDE3i treatment being initiated in patients with more severe HF (despite DCM subjects treated or not treated with PDE3i having equivalent ejection fractions and all DCM subjects having end-stage HF). Compared with AC5, AC6 is more highly expressed in the neonatal rat,[27,28] and an increase in AC6 in adult DCM + PDE3i may simply represent a reversion to a more immature gene expression profile associated with HF, similar to the reexpression of the fetal gene program. Additionally, AC6 is highly expressed in adult cardiac fibroblasts,[29] so the elevated AC6 expression in adult DCM + PDE3i may be indicative of an overall increase in fibrosis burden in adult DCM, which is not seen in pediatric DCM (manuscript submitted to *Journal of Cardiac Failure*) or SRV (manuscript in preparation). The up-regulation of AC6 is not observed in pediatric DCM, however, which may be secondary to the distinctive down-regulation of β_2-adrenergic receptors in this population.[5] In pediatric SRV, β_2-adrenergic receptor expression is preserved and AC6 expression remains unchanged.

Contribution of Other ACs

The cardiac contribution of AC7 is largely unknown, but it appears to be differentially regulated by age, disease, and PDE3i treatment. The direction of AC7 expression changes suggests that a decrease in AC7 is detrimental, and PDE3i treatment has variable effects on AC7 expression based on age and disease. AC2 expression has been reported in cardiac myocytes and can associate with KCNQ1 and Yotiao (important in modulating the slow delayed rectifier current contributing to the late-phase repolarization of the cardiac action potential),[30] but its specific physiologic role has yet to be determined. Interestingly, AC2 expression is elevated exclusively in pediatric DCM myocardium, which may imply a specific role for AC2 in disease pathogenesis in this population.

Unique Up-Regulation of PDE1C in SRV

Although PDE1 isoforms are highly expressed[31] and have high activity levels[32] in human cardiac myocytes, the physiologic and pathologic roles of PDE1 in the heart have yet to be elucidated. PDE1C is localized to the Z- and M-lines within the cardiac myocyte,[31] implying that it is associated with the sarcomere and may be involved in contractile responses. Cell culture studies suggest that PDE1C may not be active under basal conditions but becomes active in regulating cAMP signaling in conditions of enhanced intracellular Ca^{2+} concentration.[33] Because PDE1C hydrolyzes both cAMP and cGMP with equally high affinity[34] and is modulated by Ca^{2+}/calmodulin, it is likely that PDE1C is important in the integration of these signaling pathways.[31] Therefore, the increase in PDE1C expression in pediatric SRV may represent unique adaptation of the right ventricular myocardium to pressure and/or volume overload.

PDE5A Up-Regulation Is a Common Hallmark of HF

Of the AC and PDE isoforms evaluated in our study, PDE5A is the only gene consistently up-regulated with disease across adult and pediatric DCM as well as pediatric SRV. PDE5 selectively hydrolyzes cGMP, which is a second messenger in nitric oxide signaling and regulates a wide variety of cellular functions, including hypertrophy and remodeling of the ventricular myocardium. The physiologic effects of cGMP are primarily mediated through activation of protein kinase G (PKG, principally PKG1α in cardiac myocytes), resulting in increased phosphorylation of intracellular targets.[35] cGMP levels are enhanced by nitric oxide and natriuretic peptides or by inhibition of cGMP-hydrolyzing PDEs, such as PDE5. Although PDE5 is minimally expressed in normal adult myocardium,[36] PDE5 expression is increased in pressure-overload hypertrophy in mice[37] and in hypertrophied and failing adult human myocardium.[38–40] This up-regulation of myocyte PDE5 has been associated with decreased cGMP and subsequent decreased PKG activity, supporting the critical role of PKG in modulating pathologic remodeling during the development of HF.[41–43] Given the numerous differences between pediatric and adult HF, it is noteworthy that this increase in myocardial PDE5 is common to adult and pediatric left ventricular myocardium with DCM as well as pediatric right ventricular myocardium with SRV. Furthermore, all subjects treated with PDE3i did not have any significant change in PDE5A expression compared with failing subjects not treated with PDE3i. Therefore, it is likely that any beneficial effects of PDE3i therapy are mediated via PDE5-independent pathways. Overall, enhancement of the

myocardial cGMP signaling cascade through decreasing PDE5 expression or PDE5 inhibition could exert antihypertrophic effects[44] that may be useful in the treatment of HF regardless of age or disease etiology.

Clinical trials of PDE5 inhibition in adult systolic HF have demonstrated improvements in exercise capacity and echocardiographic measures of function,[45,46] although this is in contrast to the lack of improvement demonstrated in adults with HF with preserved ejection fraction.[47] In pediatric and young adult patients status after Fontan palliation for single-ventricle heart disease, PDE5 inhibition has also been shown to improve exercise capacity and echocardiographic indices of myocardial performance,[48,49] and larger clinical trials with PDE5 inhibition in this population are underway. Nevertheless, the ability to isolate the myocardial effects of PDE5 inhibition in vivo is complicated by the accompanying pulmonary vasodilatory effects (of particular importance in patients with a univentricular circulation and passive pulmonary blood flow), emphasizing the importance of such in vitro and ex vivo studies.

Down-Regulation of PDE4 in DCM

Isoforms of the PDE4 family are decreased in DCM, although there are differences in specific PDE4 isoform down-regulation based on age. Notably, PDE3i treatment does not significantly alter PDE4 expression in adult DCM but can increase expression of select PDE4 isoforms in pediatric DCM. Both PDE4D9 and PDE4D8 bind to the β_2-adrenergic receptor under resting conditions; however, agonist stimulation induces dissociation of PDE4D9 from the receptor but recruitment of PDE4D8 to the receptor. Agonist stimulation also induces recruitment of PDE4D5 to β_2-adrenergic receptor.[50] Moreover, the receptor-associated PDE4D isoforms play distinct roles in controlling cAMP activities, regulating the protein kinase A (PKA) phosphorylation of the receptor, and modulating myocyte contraction rate responses.[50] Knockdown of PDE4D9 enhances β_2-adrenergic receptor-induced cAMP signaling, whereas knockdown of PDE4D8 only slightly prolongs the receptor-induced cAMP signaling in myocytes. Inhibition of PDE4D9 and PDE4D5 enhances the baseline levels of contraction rates, whereas inhibition of PDE4D9 and PDE4D8 enhances the maximal contraction rate increases on activation of β_2-adrenergic receptor.[50] The decrease in PDE4D8 and PDE4D9 seen in the pediatric DCM myocardium could be associated with the unique down-regulation of β_2-adrenergic receptor in this population. Additionally, PDE4D3 localizes within several multimolecular signaling complexes implicated in cardiac contractility, arrhythmias, and hypertrophy,[51,52] yet no change in PDE4D3 expression was detected in any of the groups examined.

Study Limitations

Because this was a tissue bank–based study, we were not able to determine whether these changes in AC and PDE expression were pathologic or compensatory. Furthermore, expression at the mRNA level may not always reflect a physiologically significant change in either AC or PDE enzyme activity. However, previous studies showed similar increases in PDE RNA and protein levels[53] and have correlated expression of PDE protein isoforms with PDE activity.[54] AC isoforms are regularly investigated at the RNA level owing to the lack of

specific antibodies and inhibitors.[55] Conversely, RNA levels do not always correlate with enzymatic activity. For example, despite up-regulation of PDE3A1-1 and PDE3B in SRV disease, PDE3 activity is not significantly changed in either the microsomal or the cytosolic compartments in SRV myocardium.[8] Additionally, the trend toward decreased PDE3A1-1 in SRV myocardium with PDE3i treatment is not reflected in decreased PDE3 activity in this group.[8] In this case, there is some disparity between mRNA expression and enzyme activity, with enzyme activity being the more physiologically relevant measurement. However, assessment of PDE activity is based on specificity of inhibitors that cannot distinguish between different isoforms of the same family. Furthermore, although enzyme activity can be assessed in large subcellular compartments (ie, microsomal, cytoplasmic, and nuclear), this method may not be sensitive enough to detect changes in enzyme activity in a particular microdomain; therefore, expression changes found with the use of qRT-PCR may still be physiologically important. In addition, because our results were obtained from explanted human tissue, we acknowledge that the expression data are not solely representative of cardiac myocytes; included in our assessment are other cell types, including fibroblasts, vascular smooth muscle cells, endothelial cells, and immune cells which have differing AC and PDE isoform profiles. Finally, owing to the relative rarity of pediatric disease and the current widespread use of PDE3i, some sample sizes were not robust and we acknowledge the possibility of being underpowered to detect differences. Despite these limitations, this study describes significant differences in AC and PDE isoform profiles based on age and disease etiology, which provide the basis for further investigation.

Conclusion

The pediatric HF population continues to pose a unique therapeutic challenge, which is further complicated by differential molecular adaptation based on both age and disease phenotype, as highlighted in our study. Alterations in AC and PDE isoforms that are specific to a disease process and/or age group may assist in devising future studies to elucidate pathogenic mechanisms as well as predict response to PDE3i and possibly other medical therapies, which are particularly needed in the pediatric HF population.

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Table 1

Subject Characteristics

Group	No. of Subjects	% Male	Age at Tissue Collection, years Median [IQR]	Mean EF, %	Proportion on Medication						
					PDE3i	Non-PDEi Inotrope*	Digoxin	ACEi	Beta-blocker	Diuretic	Antiarrhythmic
Adult											
Nonfailing (LV)	20	55	52 [15.5]	59	0.00	0.65	0.00	0.00	0.20	0.10	0.00
Dilated cardiomyopathy	38	74	47 [22.5]	13	0.39	0.59	0.83	0.56	0.36	0.97	0.32
Pediatric											
Nonfailing (LV)	12	50	7.5 [7.9]	54	0.00	0.64	0.00	0.00	0.00	0.00	0.00
Dilated cardiomyopathy	33	36	3.1 [8.4]	23	0.73	0.27	0.42	0.85	0.13	0.94	0.21
Nonfailing (RV)	10	60	7.5 [9.7]	NA	0.00	0.56	0.00	0.00	0.22	0.00	0.00
Single right ventricle	18	67	5.6 [4.7]	NA	0.61	0.13	0.53	0.71	0.00	1.00	0.07

ACEi, angiotensin-converting enzyme inhibitor; EF, ejection fraction; IQR, interquartile range; LV, left ventricle; NA, not available; PDEi, phosphodiesterase inhibitor; RV, right ventricle.

*Non-PDEi inotropes include dopamine, dobutamine, epinephrine, and norepinephrine.

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Table 2

Adenylyl Cyclase (AC) Expression (Mean (SD)) by Disease (Nonfailing [NF] Versus All Disease)

AC Isoform	Adult NF	Adult DCM	Fold Change	Effect Size	*P* Value	Ped NF LV	Ped DCM	Fold Change	Effect Size	*P* Value	Ped NF RV	Ped SRV	Fold Change	Effect Size	*P* Value
AC2	0.472 (0.449)	0.37 (0.235)	−1.276	−0.003	.996	1.210 (0.724)	2.899 (1.862)	2.396	0.441	.007*	1.974 (1.548)	2.816 (2.915)	1.427	0.155	.432
AC3	2.579 (3.711)	0.618 (0.516)	−4.176	−0.173	.269	1.139 (0.521)	0.783 (0.414)	−1.455	−0.294	.069	1.226 (0.656)	1.061 (0.833)	−1.156	−0.186	.545
AC4	1.450 (1.145)	0.934 (0.54)	−1.552	−0.21	.189	1.076 (0.418)	0.871 (0.408)	−1.236	−0.191	.245	1.371 (0.738)	1.679 (0.967)	1.225	0.134	.504
AC5	1.500 (1.391)	3.038 (2.570)	2.025	0.247	.206	0.957 (0.288)	1.225 (0.468)	1.280	0.273	.098	1.079 (0.621)	2.589 (1.594)	2.399	0.589	.002*
AC6	1.218 (0.931)	3.48 (2.629)	2.857	0.386	.045*	1.083 (0.415)	1.352 (0.810)	1.248	0.116	.483	0.508 (0.217)	0.488 (0.371)	−1.041	−0.163	.439
AC7	0.332 (0.224)	0.147 (0.060)	−2.250	−0.427	.007*	1.173 (0.562)	1.394 (0.634)	1.188	0.089	.538	1.110 (0.49)	0.691 (0.384)	−1.606	−0.445	.020*
AC8	1.553 (1.397)	1.113 (0.689)	−1.395	−0.107	.501	1.308 (0.672)	0.969 (0.499)	−1.350	−0.227	.164	1.600 (1.108)	1.269 (0.922)	−1.261	−0.135	.502
AC9	1.927 (1.824)	0.812 (0.478)	−2.373	−0.262	.096	1.156 (0.575)	0.859 (0.29)	−1.347	−0.253	.125	1.116 (0.505)	1.141 (0.805)	1.022	−0.113	.504

DCM, dilated cardiomyopathy; other abbreviations as in Table 1.

*$P < .05$ is considered significant.

Table 3

AC Expression (Mean (SD)) by Phosphodiesterase (PDE) 3i Treatment (Disease Versus Disease Treated With PDE3i)

AC Isoform	Adult DCM	Adult DCM + PDE3i	Fold Change	Effect Size	*P* Value	Ped DCM	Ped DCM + PDE3i	Fold Change	Effect Size	*P* Value	Ped SRV	Ped SRV + PDE3i	Fold Change	Effect Size	*P* Value
AC2	0.463 (0.215)	0.368 (0.37)	−1.260	−0.291	.137	2.407 (1.878)	3.720 (2.625)	1.545	0.254	.177	2.593 (2.342)	2.136 (2.036)	−1.214	−0.081	.784
AC3	0.774 (0.617)	0.454 (0.372)	−1.705	−0.235	.222	0.627 (0.352)	0.843 (0.428)	1.345	0.227	.229	0.789 (0.812)	1.243 (0.842)	1.576	0.548	.036*
AC4	1.020 (0.471)	0.842 (0.611)	−1.212	−0.196	.321	0.853 (0.406)	0.877 (0.419)	1.028	0.018	.917	1.663 (0.803)	1.688 (1.095)	1.015	−0.027	.946
AC5	2.066 (1.678)	4.989 (3.618)	2.415	0.468	.043*	1.074 (0.367)	1.282 (0.497)	1.194	0.227	.229	2.234 (1.217)	2.803 (1.810)	1.255	0.19	.468
AC6	2.612 (2.045)	5.175 (3.348)	1.981	0.487	.035*	1.059 (0.397)	1.656 (1.229)	1.564	0.190	.312	0.568 (0.534)	0.432 (0.216)	−1.315	−0.024	.947
AC7	0.172 (0.063)	0.117 (0.042)	−1.468	−0.438	.029*	0.965 (0.383)	1.702 (0.839)	1.764	0.413	.013*	0.702 (0.447)	0.769 (0.443)	1.095	0.160	.515
AC8	1.194 (0.646)	1.025 (0.749)	−1.165	−0.103	.607	0.94 (0.471)	0.981 (0.520)	1.044	0.036	.842	1.378 (1.267)	1.193 (0.651)	−1.155	0	1.0
AC9	1.007 (0.629)	0.726 (0.463)	−1.387	−0.177	.37	0.783 (0.264)	0.927 (0.341)	1.184	0.172	.361	0.786 (0.252)	1.319 (0.935)	1.678	0.268	.301

Abbreviations as in Tables 1 and 2.

*$P < .05$ is considered significant.

Table 4

PDE Expression (Mean (SD)) by Disease (NF Versus All Disease)

	Mean (SD)					Mean (SD)					Mean (SD)				
PDE Isoform	**Adult NF**	**Adult DCM**	**Fold Change**	**Effect Size**	***P* Value**	**Ped NF LV**	**Ped DCM**	**Fold Change**	**Effect Size**	***P* Value**	**Ped NF RV**	**Ped SRV**	**Fold Change**	**Effect Size**	***P* Value**
PDE1A1											1.156 (0.562)	1.173 (0.714)	1.015	−0.102	.829
PDE1C1	1.185 (0.830)	0.828 (0.634)	−1.432	−0.215	.191	1.195 (0.635)	0.969 (0.408)	−1.233	−0.177	.286	0.713 (0.26)	1.308 (0.93)	1.836	0.475	.032*
PDE3A1-1	1.08 (0.519)	1.116 (0.645)	1.033	−0.015	.928	1.149 (0.594)	1.223 (0.534)	1.064	0.064	.697	0.988 (0.224)	2.267 (1.304)	2.295	0.544	.004*
PDE3A1-2	1.21 (0.552)	1.139 (0.629)	−1.062	−0.018	.911	1.580 (1.521)	1.464 (1.045)	−1.079	0.030	.85	0.947 (0.45)	1.573 (0.971)	1.661	0.31	.068
PDE3B	1.452 (1.105)	2.279 (1.671)	1.570	0.225	.142	1.165 (0.657)	1.657 (0.754)	1.422	0.281	.083	0.618 (0.191)	1.522 (0.949)	2.464	0.671	.001*
PDE4A3	1.305 (0.606)	0.582 (0.507)	−2.240	−0.332	.038*	0.992 (0.355)	0.976 (0.5243)	−1.017	−0.075	.656					
PDE4D3	1.202 (0.884)	1.471 (0.873)	1.224	0.1849	.247	1.076 (0.368)	1.513 (0.975)	1.406	0.12	.464	1.141 (0.568)	1.404 (0.798)	1.230	0.031	.436
PDE4D5	1.161 (0.645)	1.286 (0.575)	1.108	0.08	.487	0.965 (0.304)	0.957 (0.47)	−1.009	−0.064	.714	0.682 (0.343)	1.566 (1.009)	2.296	0.618	.003*
PDE4D8	1.318 (0.804)	0.636 (0.327)	−2.064	−0.47	.002*	1.380 (1.091)	0.479 (0.303)	−2.880	−0.422	.007*	0.790 (0.354)	1.661 (1.373)	2.103	0.285	.062
PDE4D9	1.149 (0.723)	0.884 (0.366)	−1.299	−0.133	.431	0.911 (0.388)	0.322 (0.235)	−2.828	−0.673	<.0001*	1.132 (0.724)	1.405 (0.797)	1.241	0.079	.436
PDE5A	1.205 (0.582)	4.978 (2.669)	4.131	0.63	.001*	1.128 (0.515)	2.902 (1.442)	2.573	0.56	.0002*	1.001 (0.59)	2.8 (1.168)	2.797	0.752	<.0001*

Abbreviations as in Tables 1 and 2.

*$P < .05$ is considered significant.

Table 5

PDE Expression (Mean (SD)) by PDE3i Treatment (Disease Untreated Versus Disease Treated with PDE3i)

PDE Isoform	Adult DCM	Adult DCM + PDE3i	Fold Change	Effect Size	*P* Value	Ped DCM	Ped DCM +PDE3i	Fold Change	Effect Size	*P* Value	Ped SRV	Ped SRV +PDE3i	Fold Change	Effect Size	*P* Value
PDE1A1											1.811 (1.537)	1.073 (0.543)	−1.688	−0.166	.531
PDE1C1	0.695 (0.503)	1.205 (0.998)	1.734	0.232	.25	0.919 (0.45)	1.062 (0.515)	1.155	0.091	.628	0.945 (0.385)	1.271 (0.804)	1.344	0.183	.504
PDE3A1-1	0.965 (0.485)	1.322 (0.831)	1.369	0.197	.291	1.034 (0.538)	1.368 (0.605)	1.323	0.217	.248	3.727 (2.432)	1.660 (0.935)	−2.245	−0.45	.07
PDE3A1-2	1.122 (0.617)	1.160 (0.665)	1.076	0.057	.762	1.092 (1.032)	1.782 (1.464)	1.632	0.276	.157	1.182 (0.936)	1.808 (0.96)	1.530	0.407	.115
PDE3B	2.018 (1.374)	3.230 (2.617)	1.532	0.2	.277	1.299 (0.549)	1.698 (0.724)	1.307	0.236	.218	2.145 (1.985)	1.582 (1.021)	−1.356	−0.108	.706
PDE4A3	0.606 (0.522)	0.556 (0.509)	−1.090	−0.121	.551	0.878 (0.469)	1.065 (0.589)	1.214	0.141	.453					
PDE4D3	1.751 (1.49)	1.699 (1.049)	−1.031	0.057	.771	1.324 (0.488)	1.586 (1.107)	1.198	−0.009	.978	1.173 (0.532)	1.860 (1.334)	1.586	0.219	.385
PDE4D5	1.150 (0.535)	1.475 (0.603)	1.283	0.277	.258	0.639 (0.22)	1.019 (0.455)	1.594	0.383	.046 *	1.859 (1.325)	1.362 (0.726)	−1.365	−0.142	.594
PDE4D8	0.655 (0.354)	0.616 (0.301)	−1.063	−0.071	.731	0.289 (0.105)	0.513 (0.303)	1.772	0.336	.078	2.408 (2.406)	1.336 (0.672)	−1.802	−0.071	.801
PDE4D9	0.868 (0.442)	0.908 (0.227)	1.046	0.033	.884	0.179 (0.07)	0.317 (0.154)	1.773	0.467	.042 *	1.373 (0.869)	1.427 (0.791)	1.039	0.118	.643
PDE5A	4.025 (2.379)	6.202 (2.674)	1.541	0.437	.091	2.229 (0.936)	2.990 (1.448)	1.341	0.218	.247	2.29 (0.44)	2.896 (1.286)	1.265	0.219	.385

Abbreviations as in Tables 1 and 2.

* $P < .05$ is considered significant.