Genome-wide Approach to Identify Novel Candidate Genes for Beta Blocker Response in Heart Failure Using an Experimental Model

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Abstract

Background—We explored use of a canine model of heart failure (HF) for pharmacogenomic discovery, specifically analyzing response to beta blockers (BB)

Methods—Dogs with HF that received BB (n=39) underwent genome-wide genotyping to test the association with changes in left ventricular (LV) volume and ejection fraction after treatment. Resulting candidate genes underwent RNA quantification in cardiac tissue from normal (n=5), placebo-HF (n=5), and BB-HF (n=7) dogs.

Results—Three markers met whole-genome significance for association with improved LV end-systolic volume after BB therapy (each $p<5\times10^{-7}$). RNA quantification of three candidate genes near these markers -- *GUCA1B, RRAGD*, and *MRPS10* --revealed that gene expression levels in BB-HF dogs were between that of placebo-HF dogs and normal dogs.

Conclusion—Genome-wide pharmacogenomic screening in a canine model of HF suggests 3 novel BB response candidate loci. This approach is adaptable to discovering mechanisms of action for other drug therapies, and may be a useful strategy for identifying candidate genes for drug response in the pre-clinical setting.

Introduction

Heart failure (HF) continues to be an enormous public health problem despite the many advances in its pharmacotherapy over the past two decades (AHA, 2009). The availability of so many therapeutic options for HF makes optimization of therapy for an individual challenging due to the risks of polypharmacy (Francis and Young 2001; Tang and Francis,

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2001) and because diversity of response to therapy between individuals or population subgroups is well recognized (van Campen et al., 1998). The expansion of human genomic information has led to exciting new opportunities for pharmacogenetics to improve the selection of drug therapy. Beta adrenergic antagonists (beta-blockers, BB) are an illustrative example where there have been numerous studies relating genetic sequence variants to a variety of BB effects (Johnson et al., 2003; Lanfear et al., 2005) including reverse-remodeling (Kaye et al., 2003; Terra et al., 2005) and survival in HF patients (Shin et al., 2007; Bristow et al., 2010).

Despite progress in discovering drug-response determinants, the candidate gene approach is limited to genes with *a priori* associations to the phenotype of interest, such as beta receptor variants with BBs. On the other hand, genome-wide association studies (GWAS) may be able to identify important genetic targets without preexisting knowledge or hypotheses. Advances in genotyping technology have made whole genome arrays more accessible, but despite this, pharmacogenomic progress remains costly and challenging due to the extensive genotyping needed for adequate genomic coverage in humans and the large sample size requirements due to the high number of multiple comparisons and the inherent "noise" in human clinical phenotypes. In addition, the number of studies required (to first detect and then validate an association), and the complexity of drug-response phenotypes, add to this difficulty. As a result almost all studies to date, save a few recent examples (Link et al., 2008), have taken a candidate gene approach. Specifically for BB, to our knowledge there are no published studies of genome-wide analysis of BB response in HF. If pharmacogenomics could take advantage of animal models of human disease, it may be able to accelerate the pace of discovery. Animal models have several advantages such as allowing for more uniform experimental conditions, study designs, and phenotype assessments that are not possible in humans (such as placebo-controlled trials of BB or routine myocardial sampling), as well as the potential advantage of increased efficiency of genetic mapping in some organisms. For example, an animal model would allow myocardial genotype:gene expression correlative experiments, but in contrast similar studies in humans would be extremely difficult to carry out due to ethical concerns (e.g., risks of myocardial biopsy in heart failure patients) and the inability to get a sufficient sample size to assess inter-individual variability by treatment assignment. For example, there are published studies examining gene expression pattern changes in humans with heart failure after BB therapy or cardiac resynchronization but the sample sizes in these studies are roughly 10–15 subjects and are all treated (i.e., no comparison between treated and untreated subjects).

Canine models offer many of these advantages; dogs display a number of diseases that are very similar to humans (Tsai et al., 2007), most known canine genes have human homologues (Lindblad-Toh et al., 2005), and the canine genome has long blocks of linkage disequilibrium which have enabled efficient mapping of genetic traits (Karlsson et al., 2007; Karlsson and Lindblad-Toh, 2008). Importantly, an established canine model of HF exists which has been tested extensively and is an excellent reflection of human HF (Kono et al., 1992; Sabbah et al., 1992), particularly in terms of response to drug therapies (Sabbah et al., 1991; Sabbah et al., 1994; Morita et al., 2002). Given this high-fidelity to human HF drug response and that the dog is a favorable model for genetic study, the canine HF model becomes an important setting to explore the possibility for pre-clinical pharmacogenomic study. Another advantage is the fact that there are existing data and samples from completed studies that could be leveraged. Thus, we sought to explore whether this canine model of HF could be used for pharmacogenomic discovery, focusing on response to BB as a test case.

Methods

Overview

This study utilized tissue from previously conducted studies of BB therapy in the canine model. Our group has conducted 4 previous experiments of BB therapy in dogs with heart failure, the preserved samples and data from which were used to complete the present studies. Samples from all BB treated animals (39 total), 5 control group animals (treated with placebo), as well as 5 normal dogs (i.e., no HF) were used in the described experiments.

Canine subjects

The studies were approved by the Institutional Review Board. The canine micro-embolic model of chronic HF has been previously described in detail (Morita et al., 2002). Briefly, HF is produced in dogs by multiple sequential intracoronary embolizations with polystyrene latex microspheres (~90 μm in diameter) that lead to loss of viable myocardium. These embolizations are performed weekly and the LV ejection fraction is repeatedly assessed until the goal level of LV dysfunction is reached, in this case an EF 35%. The model manifests many of the sequelae of HF in humans including profound systolic and diastolic left ventricular (LV) dysfunction, LV dilation and compensatory hypertrophy, increased LV filling pressures, increased systemic vascular resistance, and decreased cardiac output (Gengo et al., 1992; Sabbah et al., 1992; Sabbah et al., 1993). The HF cohort for this study is made up of 44 male dogs which had HF induced as above and then were treated with either BB (39 dogs, included several agents and doses, see below) or placebo (8 dogs). They were subsequently evaluated for response to treatment in terms of LV dimensions and ejection fraction after 3 months of treatment. In addition, samples from 5 dogs without HF were utilized in the gene-expression experiments (detailed below).

Beta blocker studies

There were 4 similar BB experiments from which the included animals were drawn. The time to achieve the goal EF (35%) varies from animal to animal, ranging from 2 to 8 weeks. Once the goal EF is achieved BB therapy was initiated after 2 weeks of recovery. The BB were initiated at half of the target dose for one week and then increased to the target dose which was continued for 3 months. The agent and goal dose in the 4 groups were as follows: 1) Metoprolol succinate 100 mg daily for 3 months (n=17); 2) Carvedilol 0.3 mg/kg Bid for 3 months (n=8); 3) Carvedilol 1 mg/kg Bid for 3 months (n=8); and 4) Atenolol 50 mg daily for 3 months (n=6). Outcomes assessed included the change in LV size and function from baseline (after inducing HF but before BB initiation) to the end of study period (3 months). This was achieved using cardiac catheterization with ventriculogram and the area-length method (Dodge et al., 1966) to assess LV end-systolic volume, end-diastolic volume, and ejection fraction. The dogs weighed between 20 and 25kg at baseline.

Genotyping and gene expression

DNA isolated from frozen tissue which had been stored at −70°C. genome-wide genotyping was accomplished using a commercially available canine genotyping array from Illumina® (CanineSNP20). The array utilizes the Infinium platform (Steemers et al., 2006) and tests ~22,000 validated polymorphisms across the canine genome, roughly equally spaced at an average distance of roughly 8kb. It has been validated in 10 different breeds and containing many breed informative markers. Markers meeting whole genome significance for association with LV reverse remodeling after BB treatment were further investigated, and 5 nearby genes were chosen for gene expression analysis. Canine myocardial samples were lysed and RNA was isolated according to standard techniques. Real time reverse

transcriptase polymerase chain reaction (RT-PCR) was performed to quantify transcript abundance of genes of interest as well as a house keeper gene (*GAPDH*). The target gene RNA abundance was normalized to that of *GAPDH*. These ratio values were then divided by the ratio in the normal dog samples, to find a relative change in expression for heart failure dogs versus normal dogs.

Statistical analysis

The association of change in LV end-systolic volume (ESV), LV end-diastolic volume (EDV), and LV ejection fraction (EF) with genotype was tested using the ANOVA. Bonferonni correction was used to adjust for the 22,000 multiple comparisons, yielding a critical p value of 2.2×10^{-6}

Results

Compared to placebo, BB treatment led to improvement in LV ESV and EF, regardless of BB regimen. Average changes in LV size and function (compared to baseline) during treatment were as follows: ESV −4.33 ml in BB treated dogs vs. +10.6 ml in placebo dogs (p<0.001); for EF it was +4.9 vs. −6.9 (p<0.001); and for EDV it was −1.6 ml vs. +8.5 ml (p<0.001). Among dogs receiving BB there was a substantial amount of variation in terms of improvement in LV size and function (Table 1). For example, the change in ESV over the treatment period ranged from −15 ml to +1 ml.

Association of beta blocker response with genotype

genome-wide markers were tested for association with changes in ESV, EDV, and EF after BB treatment. This revealed 3 markers meeting whole genome significance after Bonferonni correction for association with changes in ESV (Figure 1). There were several more markers in the near-significant range. No markers met genome-wide significance for change in EDV or EF. Upon interrogation of the locations of the statistically significant variants it was discovered that they were not nearby expected BB response elements (i.e., *ADRB1, ADRB2*) but instead were in genes/regions not previously associated with BB response. Further examination of the genes in these regions revealed that several had potential biologic plausibility in terms of effecting myocardial physiology or BB effects (summarized in Table 2).

Gene expression analysis of genes indicated by genome-wide genotyping

Gene expression quantification using real time RT-PCR was attempted for each gene listed in Table 2 due to their proximity to an associated marker. Despite multiple attempts working probe sets were not able to be generated to interrogate Guanylate Cyclase Activator 1A (*GUCA1A*) and Lim and cysteine rich domain protein 1 (*LMCD1*). The remaining three genes of interest [*GUCA1B*, mitochondrial ribosomal 28s protein S10 (*MRPS10*), and ras-related GTP binding D (*RRAGD*)] successfully underwent further testing. We quantified RNA using myocardial tissue samples from 7 BB treated dogs (from high-dose carvedilol group), 5 placebo treated dogs, and 5 normal dogs (i.e., no HF). This was quantified via real-time RT-PCR, normalizing RNA abundance of the gene of interest to that of *GAPDH*. These ratio values were then divided by the ratio in the normal dog samples, to find a relative change in expression for heart failure animals (ΔΔCt method). The results revealed that each of the genes tested showed higher expression in placebo treated HF dogs compared to normal dogs, with roughly 3 to 4 fold greater expression (Table 3). This reached statistical significance for *GUCA1B* (p=0.02) and was borderline significant for *MRPS10* (p=0.09). These expression differences appeared attenuated in HF dogs treated with carvedilol, which showed expression 2 to 3 fold that of normal dogs.

Discussion

In this study we were able to identify genetic markers associated with favorable remodeling in response to BB treatment in a canine model of HF. Several markers met genome-wide significance after Bonferonni correction, which tends to be conservative, and this was achieved with a relatively low number of animals (n=39). Subsequent gene expression analysis showed differences in transcript abundance between BB treated dogs, placebo treated HF dogs, and normal dogs which, while modest in magnitude, were consistent in direction with the hypothesis that these novel loci are relevant to BB response in HF.

To varying degrees, each of these three genes has some biologic plausibility. The function of RRAGD is not completely established, but it is a member of the ras-like GTPase superfamily of proteins which are known to regulate a wide range of intracellular processes. These include gene expression, cytoskeletal processes, translation, and cell division. Its specific function and role in HF are not established. The function of GUCA1B is better known, it being a recognized stimulator of guanylate cyclase in the retina. Whether it could have effects elsewhere is not known, though some guanylate cyclases are known to have important cardiovascular effects (GCA/NPRA) and GUCA1B appears to be expressed in heart, skeletal muscle, and kidney, though not at the level of the retinal expression. On the other hand, *MRPS10* is known to code for the small subunit of mitochondrial ribosomes. Myocardial energetics is being increasingly recognized to play an important role in HF pathophysiology (Huss and Kelly, 2004; Qanud et al., 2008), and BB treatment is known to impact this (Wallhaus et al., 2001; Sabbah 2004). Thus, it would not be difficult to hypothesize that variability in how individuals' mitochondria function may impact the development of HF or reverse-remodeling in response to BB therapy.

Our data are the first we are aware of demonstrating pharmacogenomic discovery in a large mammal disease model. Our model system has the distinct advantage that it is known to be an accurate predictor of drug response in human HF, across multiple drug classes and specifically for BBs. While pharmacogenetic and pharmacogenomic studies have been pursued in mice, the ability to use the canine model would be a significant advance because the findings may be more directly applicable to human disease and treatment, as evidenced by the high fidelity of this canine model to human heart failure. Furthermore, the potentially favorable genomic structure in dogs may allow genome-wide screening for pharmacogenetic interactions using a relatively small number of animals, thus increasing speed while decreasing cost of pharmacogenomic discovery. This may also allow subsequent human studies to utilize an expanded candidate gene approach (rather than whole genome), greatly accelerating the pace of progress while not limiting discovery to genes with *a priori* associations to the drug of interest. Finally, this is of high potential impact because the approach could be applied to any drug of interest or conceivably even medical devices.

Our study has limitations that should be noted. First, we did not study a large enough sample of placebo treated HF dogs to perform drug x genotype interactions. Despite this, predicting favorable response within treated animals (i.e., pre-post change) is an important end-point, and this study design has been often used in comparable human studies. Second, this analysis includes four individual BB studies that were previously carried out. While the drug and dose regimens were different, the heart failure induction, duration of treatment, and outcomes assessed were all identical, making it suitable to merge them in order to increase power. Conversely the individual study sizes were not large enough to allow evaluation of individual agents. Another concern is that the dogs were mongrels, so that population stratification could theoretically lead to false-positive associations. Confirmatory studies are needed to define this further. Finally, two of five candidates could not have RNA quantified

so the relationship of gene expression to HF and BB response for these genes remains unknown.

Conclusion

We described the first use of a canine model of HF in a genome-wide search for genetic predictors of response to drug therapy, identifying novel candidate genes for BB response. Our data suggest that this approach may be useful, having the potential to yield significant results with a modest sample size in a pre-clinical research setting. This type of approach requires further scrutiny, but if proven useful, could greatly accelerate pharmacogenomic discovery.

Acknowledgments

Abbreviations

BB	beta blocker
EDV	end diastolic volume
EF	ejection fraction
ESV	end-systolic volume
GWAS	genome-wide association studies
HF	heart failure
LV	left ventricular

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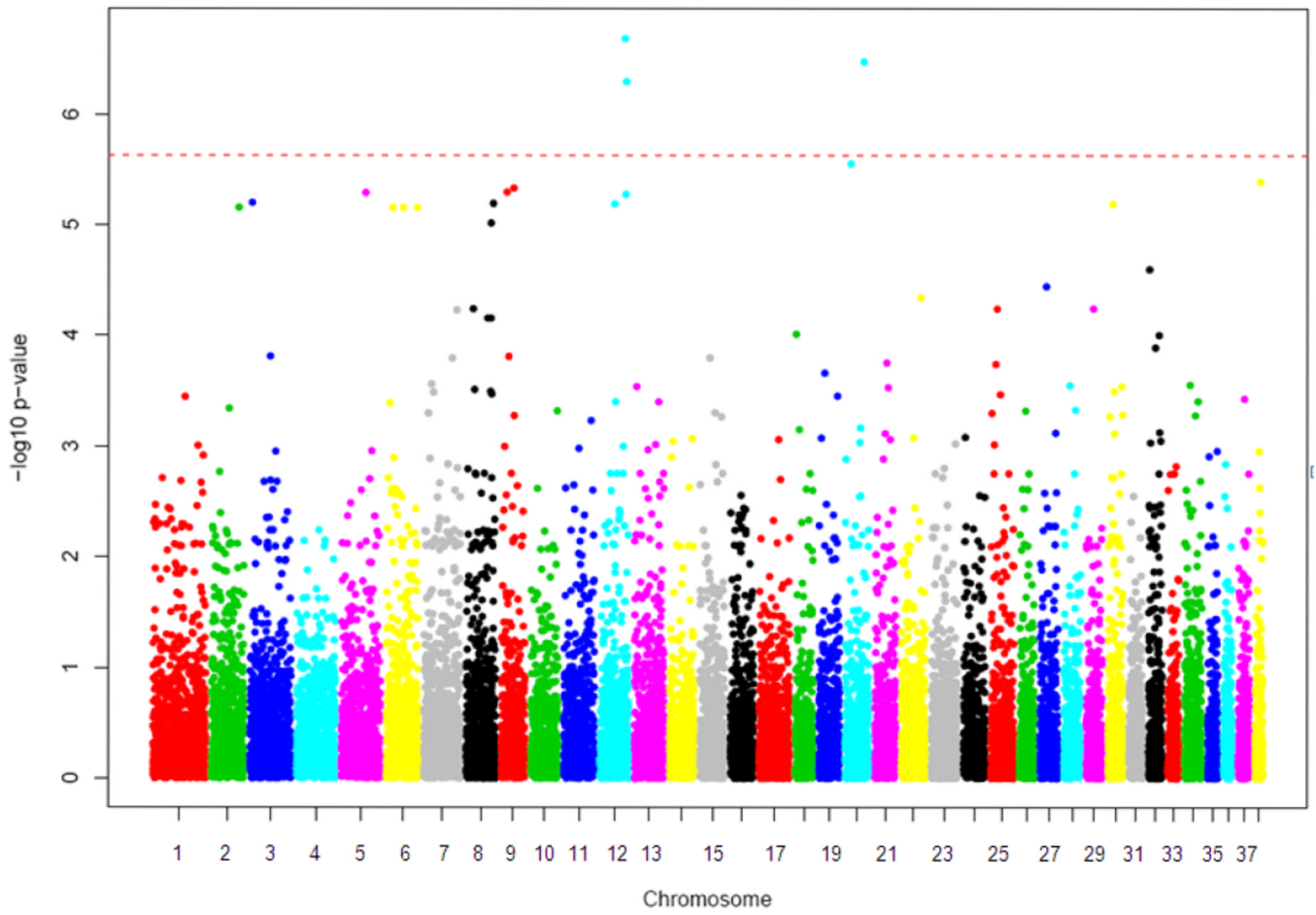
Figure 1.
Manhattan plot of log(p) vs. chromosomal position of genetic markers. (Red dashed line indicates whole-genome significance level by Bonferonni correction.)

Table 1

Changes in Ventricular Size and Function in HF Dogs Treated with BB (n=39)

BB Group	Parameter	Mean Change	Std. Deviation	Range
Combined BB Group (n=39)	Δ Ejection Fraction (%)	**4.92**	2.97	−1, +11
	Δ End-Systolic Volume (ml)	**−4.33**	3.62	−15, +1
	Δ End-Diastolic Volume (ml)	**−1.62**	2.93	−11, +7
Carvedilol 1mg/kg bid (n=8)	Δ Ejection Fraction (%)	**6.38**	2.97	+2, +11
	Δ End-Systolic Volume (ml)	**−7.75**	3.49	−5, −15
	Δ End-Diastolic Volume (ml)	**−5.00**	2.73	−2, −11
Carvedilol 0.3mg/kg bid (n=8)	Δ Ejection Fraction (%)	**2.38**	1.41	0, +5
	Δ End-Systolic Volume (ml)	−1.63	2.07	−4, +1
	Δ End-Diastolic Volume (ml)	**0.13**	3.23	−3, +7
Metoprolol Succinate 100mg daily (n=17)	Δ Ejection Fraction (%)	**6.65**	1.73	+3, +10
	Δ End-Systolic Volume (ml)	**−5.18**	2.98	−15, −1
	Δ End-Diastolic Volume (ml)	**−1.47**	1.91	−5, 0
Atenolol 50mg daily (n=6)	Δ Ejection Fraction (%)	1.50	2.07	−1, 5
	Δ End-Systolic Volume (ml)	−1.00	1.26	−3, 0
	Δ End-Diastolic Volume (ml)	0.17	1.33	−1, +2
Placebo (n=8)	Δ Ejection Fraction (%)	**−6.88**	1.64	−10, −5
	Δ End-Systolic Volume (ml)	**10.63**	6.94	+4, +24
	Δ End-Diastolic Volume (ml)	**8.75**	8.73	1, +25

Note: $p < 0.05$ vs. baseline show in bold.

Table 2

Genes in Region Near Markers That Reached Genome-wide Significance

Variant ID	Chr.	p-value	Gene Symbol	Gene Name	Classification	Function
BICF2P138111	12	2.07E-07	Predicted gene	Unknown	Unknown	Unknown
			GUCA1A	Guanylate cyclase activator 1A	Calmodulin Superfamily	Regulator of guanylate cyclase activity
			GUCA1B	Guanylate cyclase activator 1B	Calmodulin Superfamily	Regulator of guanylate cyclase activity
			MRPS10	Mitochondrial Ribosomal 28s protein S10		Small subunit of mitoribosomes, mitochondrial protein synthesis
BICF2P1313841	12	5.07E-07	RRAGD	Ras-related GTP binding D	Ras Superfamily	Unknown
BICF2P351263	20	3.36E-07	No known genes – lies within conserved intergenic region			
			near by (<1Mb) LMCD1	Lim and cysteine rich domain protein 1	transcriptional cofactor expressed in myocardium	involved in cell differentiation and cytoskeletal remodeling

Table 3

Candidate Gene Expression in BB Treated HF Dogs, Placebo Treated HF Dogs, and Normal Dogs for Novel Candidate Genes

Gene	Normal (N=5)	Placebo (N=5)	Carvedilol (N=7)
	Expression relative to Normal		
GUCA1B (Guanylate cyclase activator 1B)	1	3.7[*]	2.8
RRAGD (Ras related G protein D)	1	2.95	2.75
MRPS10 (Mitochondrial ribosomal 28s protein S10)	1	3.8[†]	2.6

Note: Shown as average relative expression compared to normal dog samples, using $\Delta\Delta Ct$ method to normalize to simultaneous sample GAPDH expression

[*] $p < 0.05$ vs. Normal

[†] $p < 0.01$ vs. Normal