Common Genomic Response in Different Mouse Models of β-Adrenergic–Induced Cardiomyopathy

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Background—Although β-adrenergic receptor (AR) blockade therapy is beneficial in the treatment of heart failure, little is known regarding the transcriptional mechanisms underlying this salutary action.

Methods and Results—In the present study, we screened mice overexpressing Gsα, βAR, β2AR, or protein kinase A to test if a common genomic pathway exists in different models with enhanced β-adrenergic signaling. In mice overexpressing Gsα, differentially expressed genes were identified by mRNA profiling. In addition to well-known markers of cardiac hypertrophy (atrial natriuretic factor, CARP, and β-myosin heavy chain), uncoupling protein 2 (UCP2), a protein involved in the control of mitochondrial membrane potential, and four-and-a-half LIM domain protein-1 (FHL1), a member of the LIM protein family, were predicted to be upregulated. Upregulation of these genes was confirmed by quantitative reverse transcriptase–polymerase chain reaction at all time points tested during the development of cardiomyopathy in mice overexpressing Gsα. In mice overexpressing βAR, β2AR, or protein kinase A, increased UCP2 and FHL1 expression was also observed at the onset of cardiomyopathy. βAR blockade treatment reversed the cardiomyopathy and suppressed the increased expression of UCP2 and FHL1 in mice overexpressing Gsα.

Conclusions—UCP2 and FHL1 are important candidate genes that correlate with the development of βAR-induced cardiomyopathy in different mouse models with enhanced βAR signaling. In addition to preserving cardiac function, βAR blockade treatment also prevents the genomic regulation that correlates with the onset of heart failure.

(Circulation. 2003;108:2926-2933.)

Key Words: receptors, adrenergic, beta ■ cardiomyopathy ■ genomics

The deleterious effects of chronic β-adrenergic receptor (AR) stimulation in the heart have been documented in several mouse models. Transgenic mice overexpressing Gsα selectively in the myocardium develop cardiomyopathy with age, characterized by left ventricular (LV) dilation and hypertrophy, reduced LV function, fibrosis, apoptosis, and premature mortality.1,2 Mice overexpressing β1AR,3,4 high levels of β2AR,5,6 or protein kinase A (PKA)7 also develop cardiomyopathy with aging. In patients with heart failure, βAR blockade therapy has been proven to be beneficial.8–11 However, little is known regarding the transcriptional mechanisms underlying this salutary action. In the present study, we screened different mouse models with enhanced βAR signaling to identify potential genomic targets of βAR blockade therapy. Using a sensitive, differential gene expression technology12 to study gene expression profiles in Gsα-overexpressing mice, we identified unexpected genes that participate in the development of cardiomyopathy in these mice. Furthermore, we show that these candidate genes are involved in the development of cardiomyopathy in mice overexpressing β1AR, β2AR, or PKA. Most interestingly, the preservation of cardiac function brought by βAR blockade treatment in mice overexpressing Gsα normalizes the expression of uncoupling protein 2 (UCP2) and four-and-a-half LIM domain protein-1 (FHL1), showing that β-blockers alleviate the genomic dysregulation that accompanies heart failure.

Methods

Animals

Mice overexpressing Gsα,1 β1AR,3 β2AR,4 or PKA7 have been previously described. Mice overexpressing β2AR were originally described by Milano et al3 and purchased from Jackson Laboratories (Bar Harbor, Maine). Genotyping5,3,8,7 and βAR blockade treatment13 were carried out as described. Three to 9 animals have been studied in each group.
Differential Gene Expression Analysis
by GeneCalling

Total RNA was prepared using Tri reagent (Sigma) from the hearts of 3-, 6-, 9-, 12-, and 15-month-old Gsα-overexpressing mice or control littersmates. Three animals per age group and genotype were analyzed individually. GeneCalling reactions were performed as described in Figure 1. Briefly, double-stranded DNA was generated from the pools of mRNA and digested with 96 pairs of restriction enzymes. The fragments were amplified by polymerase chain reaction (PCR) in the presence of fluorescent primers and separated according to their size by capillary electrophoresis. The different fragmentation profiles were compared to identify fragments with differential fluorescence, i.e., expression level, between 2 samples. The identification of the gene corresponding to the fragment of interest relied on the size of the fragment and the 6-nucleotide sequence in 5′ and 3′ that corresponded to the restriction sites used to generate the fragment. Confirmation of a gene is done by competitive PCR. If correctly identified, the peak corresponding to the confirmed fragment is blunted (dotted line).

Quantitative Reverse Transcriptase-PCR

Total RNA from transgenic mice and control littersmates was prepared from frozen heart tissues using Tri reagent (Sigma). The mRNA of interest was reverse transcribed according to standard protocol. Real-time quantitative PCR (qPCR) (7700 Prizm, PerkinElmer/Applied Biosystems) was performed with specific primers and fluorogenic probes derived with FAM and TAMRA (Table 1). Internal standards were prepared for each transcript from its PCR-amplified cDNA after ligation of the T7 promoter. Results were normalized to 36B4 or cyclophilin.

Statistics

Results obtained for transgenic mice were compared with those obtained for their age-matched control littersmates using the unpaired Student t test.

Results

Genomic Profiling

We compared the gene expression profiles in mice overexpressing Gsα and control littersmates at 3, 6, 9, 12, and 15 months of age. Candidate genes with at least 2.5-fold predicted differential expression were identified (Table 2). As expected, the transgene, Gsα, was predicted to be high in Gsα-overexpressing mice at all ages analyzed. In addition, genes of the following categories were predicted to have a modified expression pattern: extracellular matrix deposition (collagen III), metabolism (UCP2), transcription factors (FHL1 and cardiac ankyrin repeat protein [CARP]), stress (atrial natriuretic factor [ANF]), and myofilament (myosin heavy chain [MHC] and M-protein). Of particular interest, UCP2, a protein involved in the control of mitochondrial membrane potential, and FHL1, a new member of the LIM protein family, are known genes with unknown function in the heart. Interestingly, UCP2 and FHL1 expression were also found to increase during heart failure in different mouse models and in patients. These genes might therefore bring new insights into the understanding of the development of cardiomyopathy induced by chronic stimulation of βAR signaling.

Uncoupling Protein 2

After capillary electrophoresis, the peak corresponding to UCP2 was found to increase in 3-month-old Gsα-overexpressing mice (Figure 2A) and in 6-, 9-, 12-, and 15-month-old transgenic mice (not shown) compared with
age-matched control mice. Competitive PCR was performed to confirm that the peak indeed corresponds to UCP2 (Figure 2A). Quantification of the changes in UCP2 expression during aging of Gsα-overexpressing mice was done by qPCR. From 3 months of age onward, UCP2 expression was doubled in Gsα-overexpressing mice compared with control littermates (Figure 2B).

**Four-and-a-Half LIM Domain Protein-1**

After capillary electrophoresis, the peak corresponding to FHL1 was increased in 3-month-old Gsα-overexpressing mice (Figure 3A). This was also observed in 6, 9, 12, and 15-month-old transgenic mice (not shown). After competitive PCR, the corresponding peak was blunted, confirming the identity of the gene (Figure 3A). Quantification of the changes in FHL1 expression during aging in Gsα-overexpressing mice was done by qPCR. From 3 months of age onward, a 2- to 3-fold increase in FHL1 expression was seen in Gsα-overexpressing mice compared with control littermates (Figure 3B). In adult striated muscle, 3 isoforms of FHL have been described, FHL-1, -2, and -3.24 We found increased expression of FHL1 in Gsα-overexpressing mice, but no change in expression of FHL-2 or FHL-3 could be detected (Figures 3B through 3D).

**Other Genes**

Additional candidate genes were validated by qPCR during aging in Gsα-overexpressing mice. As expected, expression of the transgene was high in Gsα-overexpressing mice at all ages analyzed compared with control littermates (Figure 4A).

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**Table 1. Characteristics of the qPCR Assays Used in This Study**

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(+) indicates forward primers; (−), reverse primers; FAM-TAMRA, probe. The GenBank accession number was used to number the nucleotides.
The expression of markers of cardiac hypertrophy, ie, ANF, β-MHC, and CARP, were increased in 15-month-old Gsα-overexpressing mice (Figures 4D through 4F). Because changes in the expression of these genes occurred in old transgenic mice, they are likely to be a consequence and not the cause of the cardiomyopathy developed in these mice.

The prediction of an upregulation of β-MHC (Table 2) did not reach a statistical significance after validation by qPCR (not shown). An upregulation of collagen type III was seen in Gsα-overexpressing mice throughout aging and is related to the fibrosis seen in these mice2 (Figure 4B). M-protein, which is part of the M-band, is the only gene found so far with decreased expression in mice overexpressing Gsα (Figure 4C).

**βAR Blockade**

To test whether the increased expression of UCP2 and FHL1 seen in Gsα-overexpressing mice is attributable directly to the chronic stimulation of βAR signaling in these mice, a
AR-blockade experiment was designed. We previously showed that AR blockade was sufficient to arrest myocyte damage and preserve cardiac function in mice overexpressing Gs13. The increased expression of UCP2 and FHL1 seen in Gs1-overexpressing mice in the absence of treatment was decreased significantly by the AR-blockade treatment, as measured by qPCR (Figures 5A and 5B). Because AR blockade can decrease -MHC expression,25 we verified that the treatment did not affect the expression of the Gs transgene, which is under the control of the -MHC promoter1 (Figure 5C).

Common Genomic Pathway
Increased expression of UCP2 and FHL1 in Gsα-overexpressing mice suggests that these genes might play a role in the development of the cardiomyopathy seen in these mice. To test if a common transcriptional pathway leads to cardiomyopathy in other models of enhanced AR signaling, we studied the expression of UCP2 and FHL1 in mice overexpressing AR,2,3,4 AR,5,6 or PKA.7

In AR transgenic mice, overexpression at 15 times endogenous AR levels is sufficient to lead to increased contractility at a young age (7 weeks) and to result in cardiomyopathy with aging (30 weeks).3 Expression of UPC2 (Figure 6A) and FHL1 (Figure 6B) was significantly increased at both time points in AR-overexpressing mice compared with control littermates.

In a thorough study analyzing several lines of AR-overexpressing mice, Liggett et al6 reported that cardiomyopathy is seen only with high (>100-fold) levels of AR overexpression and develops more slowly than in mice overexpressing AR. The severity of the cardiomyopathy and mortality correlates with the expression level of AR. Milano et al5 reported another line of AR-overexpressing mice that we obtained through Jackson Laboratories for this study. These mice have a 200-fold overexpression of AR in the heart.5 At 3 months of age, they displayed enhanced contractility (ejection fraction: wild-type, 69±2%; transgenic, 75±1%; P<0.05; fraction shortening: wild-type, 33±1%; transgenic, 37±1%; P<0.05) and no evidence of cell death.5 At 12 months of age, however, these AR-overexpressing mice showed a significant decrease in left ventricular function (ejection fraction: young transgenic, 75±1%; old transgenic, 59±2%; P<0.05; fraction shortening...
ing: young transgenic, 37±1%; old transgenic, 26±2%; P<0.05), whereas no significant change was seen with aging in control littermates. UCP2 expression was unchanged in 3-month-old β2 AR-overexpressing mice compared with control littermates but dramatically increased with aging in the transgenic mice (Figure 6C). FHL1 was significantly increased at both ages (Figure 6D).

Mice overexpressing PKA show dilated cardiomyopathy and sudden death independent of more proximal events in βAR signaling.7 Expression of UCP2 and FHL1 were significantly increased in 3-month-old PKA-overexpressing mice compared with control littermates (Figures 6E and 6F).

Discussion

In the present study, we used different mouse models with enhanced βAR signaling to identify potential genomic targets of βAR blockade therapy and found that UCP2 and FHL1, two genes with unknown function in the heart, might play an important role in the development of βAR-induced cardiomyopathy.

Uncoupling proteins received their name from their role in uncoupling respiration from ATP production, generating heat in brown adipose tissue (UCP1) and skeletal muscle (UCP3).18 A potential role for UCP2, supported by the corresponding knockout mice,26 is the regulation of reactive oxygen species (ROS) production. Reduction of ROS would be protective but would come at the cost of increased uncoupling activity that might compromise the mitochondrial membrane potential, eventually leading to cell death.16,27

Enhanced UCP2 expression was found in the heart of rats after prolonged thyroid hormone treatment and is thought to have a detrimental effect on cardiac function in that model.21 In mice overexpressing Gsα (Figure 2B) or β1AR (Figure 6A), UCP2 is upregulated before the development of cardiomyopathy and throughout aging, suggesting that it can play a role in the transition of phenotype between young and old transgenic mice. In young transgenic mice, increased expression of UCP2 might decrease the amount of ROS and therefore play a protective role. As mice age, sustained uncoupling might damage mitochondrial membrane, leading to apoptosis. No change in UCP2 expression was detected in young β2AR-overexpressing mice, suggesting that in this model, UCP2 might not be a cause of the cardiomyopathy developed with aging (Figure 6C).

FHL proteins are new members of the LIM-only protein family. The pattern of expression of FHL1 suggests an important role for FHL1 in the heart during development20 and cardiomyopathy.20,23 In this study, we show that FHL1, but not FHL2 or FHL3, is upregulated in mice overexpressing Gsα (Figure 3). FHL1 expression, but not that of FHL2 or FHL3, also was found to increase in 2 mouse models with cardiac hypertrophy and dilated cardiomyopathy20 and in

Figure 4. Confirmation of candidate gene expression changes by qPCR. Expression of the Gsα transgene (A), collagen III (B), M-protein (C), β-MHC (D), ANF (E), and CARP (F) was measured by qPCR in the heart of Gsα-overexpressing mice (solid bars) and control littermates (open bars) harvested at the indicated ages. *P<0.05 between transgenic mice and respective control littermates.

Figure 5. Increased expression of UCP2 and FHL1 seen in Gsα-overexpressing mice is reversed by βAR-blockade treatment. Expression of UCP2 (A), FHL1 (B), and the transgene Gsα (C) was measured by qPCR in the heart of 9-month-old Gsα-overexpressing mice (closed bars) and control littermates (open bars) that were treated (+ propranolol) or not (− propranolol) for 6 weeks with βAR blockade. *P<0.05 between transgenic mice and respective control littermates. ‡P<0.05 between treated mice and respective untreated mice. ND indicates not detectable.
In conclusion, we used genomic profiling to identify unexpected genes that might play a role in the development of cardiomyopathy induced by chronic βAR signaling. We identified UCP2 and FHL1 as important candidate genes that correlate with the development of βAR-induced cardiomyopathy in different animal models. Increased UCP2 expression results specifically from a βAR-Gsa-PKA pathway. The upregulation of UCP2 and FHL1 in mice overexpressing Gsa was reversed by βAR-blockade treatment, suggesting that these 2 genes could be direct targets for the treatment of heart failure.

Acknowledgments

This work was supported in part by National Institutes of Health grants (grants AG14121, HL59139, HL33107, and HL69020 to Drs Stephen and Dorothy Vatner) and the American Heart Association (grant 0130100N to Dr Gaussin). We acknowledge Jill Thaisz-Warden for breeding and genotyping the mice, Jing Liu for echocardiography, and Qian Wang for technical help.

References


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Circulation. 2003;108:2926-2933; originally published online November 17, 2003; doi: 10.1161/01.CIR.0000101922.18151.7B

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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