Whole-genome analysis of gene expression associates the ubiquitin-proteasome system with the cardiomyopathy phenotype in disease-sensitized congenic mouse strains

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Aims
Penetrance and phenotypic expressivity of cardiomyopathies are modulated by modifier genes both in model systems and patients. We aimed to dissect the disease-modifying mechanisms by examining genome-wide gene expression in a new set of mouse (Mus musculus) congenic strains.

Methods and results
Mutant alleles of the genes caldescin-1 (Myoz2), sarcoglycan-delta (Sgcd), and muscle LIM protein (Csrp2) were each transferred onto inbred strain backgrounds C57BL/6, C3H/He, 129S1/Sv, and FVB/N, respectively. At 9–10 weeks of age, left ventricular pump function (fractional shortening, FS) was determined by echocardiography in non-sedated congenic animals. Gene expression was then analysed in myocardial tissue using the Affymetrix Mouse 430.2 microarray platform. Variance stabilization, linear mixed-effects modelling, correlations, gene functional classification, and pathway analysis were conducted using the standard software. Strain background FVB/N appeared to protect against the consequences of gene inactivation. Sgcd-deficient congenics showed normal FS, which was consistent with their hypertrophic cardiomyopathy phenotype. Animals with other allele/background combinations developed an impaired ventricular pump function (FS < 65%). Gender did not influence FS significantly, yet it determined the sets of genes that were differentially expressed in mice with low FS. In particular, genes encoding the elements of the ubiquitin-proteasome system (UPS) were strongly correlated with the cardiac impairment (absolute Spearman $r \geq 0.7$) in both males and females.

Conclusion
Gene expression profiling in a novel set of congenic strains revealed an association between the UPS and myocardial contractile function, indicating that the UPS may be an important modifier of phenotypic variability in cardiomyopathies.

Keywords
Cardiomyopathy • Congenic strain • Mouse • Gene expression • Proteasome

1. Introduction
Human cardiomyopathy is considered to be a genetic disorder, and over 40 disease-causing mutations have been identified in patients and model systems. In clinical practice, cardiomyopathies are classified according to their characteristic phenotypes such as hypertrophic cardiomyopathy (HCM), dilative cardiomyopathy (DCM), and restrictive cardiomyopathy, although identical disease genes may cause such diverse phenotypes. Furthermore, in families with inherited cardiomyopathy, the disease phenotype and its penetrance are highly...
variable even in family members carrying the same disease-causing mutation. These observations suggest a critical role of disease modifiers for phenotypic expressivity and the clinical progression of the disorder.

Genetically engineered mice are valuable model systems that are useful in dissecting the molecular pathogenesis of cardiomyopathies. However, similar to clinical observations, the penetrance and phenotypic expressivity of cardiomyopathies may vary considerably depending on the genetic background of the genetically engineered mouse model.3,4 While patients may display a broad range of cardiomyopathy phenotypes as a consequence of genetic heterogeneity, inbred laboratory mouse strains with a given cardiomyopathy gene mutation exhibit a narrow range of phenotypes, like a subset of patients or a family with inherited cardiomyopathy. It is possible to study and compare their phenotypic characteristics in an unlimited number of their progeny. Rockman and co-workers have done pioneering work to identify genetic modifiers of DCM in transgenic mouse models.5

We employed a similar approach and created a set of 10 disease-sensitized congenic inbred strains, including four closely related Mus musculus domesticus backgrounds. The aim was to use this novel resource to elucidate the molecular mechanisms of cardiomyopathy variability, not only for understanding the disease mechanisms, but also for improved risk prediction and therapeutic strategies.

Here, we report the identification of major genetic modifiers in cardiomyopathy mouse models obtained by analysing global gene expression patterns in the heart tissue. Starting with three established knock-out models on a mixed genetic background, we transferred sensitized congenic mouse models exhibited considerable variation in ventricular pump function, which was the central target for the identification of modifier genes by correlation analysis of cardiac gene expression patterns.

2. Methods

2.1 Animals

The inbred strains C57BL/6Ncr (C57BL/6), C3H/HeNcr (C3H/He), FVB/Ncr (FVB/N), and 129S1/Svvlmj@CRL (129S1/Sv) were purchased from Charles River Laboratories (Sulzfeld, Germany). Strain B6.129–Sgcdtm1Mcn/J (B6.Sgcd) was obtained from The Jackson Laboratory (Bar Harbor, MA, U.S.A.).6 Strains B6.129–Myoz2tm1 (B6.Myoz2) and B6.129–Csrp2tm1 (B6.Csrp2) were received on mixed genetic backgrounds (C57BL/6, 129S1/Sv, FVB/N, and C3H/He). These new disease-sensitized congenic mouse models exhibited considerable variation in ventricular pump function, which was the central target for the identification of modifier genes by correlation analysis of cardiac gene expression patterns.

Table I Composition and size of the experimental groups

<table>
<thead>
<tr>
<th>Strain</th>
<th>Allele</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>6M/6F</td>
<td>18M/18F</td>
</tr>
<tr>
<td>C3H/He</td>
<td>6M/6F</td>
<td>5M/4F</td>
</tr>
<tr>
<td>FVB/N</td>
<td>5M/5F</td>
<td>6M/6F</td>
</tr>
</tbody>
</table>

*The number denotes sample size, and the letter stands for sex (M, male; F, female). Not available.

2.2 Echocardiography

A Sonos 5500 (Philips, Eindhoven, Netherlands) with a 12 MHz transducer (12 MHz) was used to determine left ventricular function by transthoracic echocardiography. The echocardiographer was blinded for the mice genotype. All examinations were performed on conscious animals to prevent anaesthesia-related impairment of cardiac function.10 Left ventricular parasternal short-axis M-mode views were obtained at the papillary muscle level. M-mode images were digitally stored for subsequent analysis. The left ventricular end-diastolic (LVEDD) and end-systolic internal diameters (LVESD) were determined and averaged over three consecutive beats using the American Society of Echocardiography leading edge method.11 Fractional shortening (FS) was calculated as FS (%) = [(LVEDD − LVESD)/LVEDD] × 100. In these non-sedated mice an FS <65% was considered indicative of the impaired left ventricular pump function.12 FS measurements in the animals of wild-type strains are presented in Supplementary material online, figure S1.

2.3 Gene expression profiling using microarrays

At the age of 9–10 weeks, sedated mice were sacrificed by cervical dislocation. Hearts were rapidly removed, rinsed with isotonic phosphate-buffered saline (pH 7.4), blotted dry using filter paper, and dissected. Ascending aorta, left and right atrial tissues were removed. The remaining ventricles were shock frozen using liquid nitrogen and stored at −80°C pending isolation of total RNA. Hearts were thawed in Trizol (Invitrogen, Karlsruhe, Germany) and homogenized using a MM301 mixer mill (Retsch, Haan, Germany). After electrophoretic quality control and quantitation, total RNA was sent to a commercial service company (MFT Services,Tuebingen, Germany) that performed genome-wide gene expression profiling using the Affymetrix Mouse 430.2 microarray platform according to their standard protocols.

Preprocessing of the microarray data was conducted in the R using the R packages, which are part of the Bioconductor project.13 Variance stabilizing normalization14 and robust multi-array average-based summarization15 were applied to the Affymetrix Mouse 430.2 microarray platform according to their standard protocols.
of the expression measurements were carried out without adjustment to the perfect match values, i.e. ignoring the values from mismatch probes.\textsuperscript{17} The resulting expression values are presented on the log 2 scale. To reduce multiple testing issues in the subsequent statistical analysis, the data were non-specifically filtered to discard the 10\% lowest variability probe sets (based on the gene-wise standard deviation).

2.4 Statistical analysis
Statistical analysis was conducted in the R v2.11 computing environment\textsuperscript{12} and included three steps.

2.4.1 Step 1
A linear mixed-effects modelling approach was applied to test the statistical significance of the association between FS and factors such as ‘Strain’, ‘Allele’ (i.e. the mutant cardiomyopathy-causing genes), ‘Sex’, ‘Age’, and the interaction between ‘Strain’ and ‘Sex’. As indicated in Table 1, two of the strain/allele combinations were not available for the study, making its design unbalanced by allele. To address this issue, the effect of ‘Allele’ was modelled as a random intercept in the following model:

\[
FS = b_0 + b_1 \text{Strain} + b_2 \text{Sex} + b_3 \text{Age} + b_4 \text{Strain} \times \text{Sex} + \alpha + \epsilon, \tag{1}
\]

where \(b_0\) is the average FS over all observations; \(b_1\), \(b_2\), and \(b_3\) are the coefficients reflecting the effects of ‘Strain’, ‘Sex’, and ‘Age’, respectively; \(b_4\) is the effect of the interaction between ‘Strain’ and ‘Sex’; \(\alpha\) is the random intercept allowed to vary with the levels of ‘Allele’ (it is assumed to be normally distributed with mean 0 and variance \(\sigma_\alpha^2\)); \(\epsilon\) are the model residuals, assumed to be normally distributed with mean 0. An exploratory data analysis revealed high heterogeneity of FS measurements in strain/allele combinations (Figure 1). The residual variance was, therefore, allowed to vary with each combination of strain and allele (see the work by Pinheiro and Bates\textsuperscript{18} for detail on this type of parameterization).

Estimation of the coefficients of Model (1) was conducted with the nlme v3.1–97 package for R using the restricted maximum likelihood method.\textsuperscript{18} Insignificant terms were stepwise backward eliminated from this saturated model. Selection of the optimal model was supported by the log-likelihood ratio test (L) and examination of the Akaike’s Information Criterion AIC (models with smaller AIC values are considered optimal).\textsuperscript{18} Pairwise comparisons of the mean FS levels in the animal groups of interest were performed with the Tukey test.\textsuperscript{19}

2.4.2 Step 2
Spearman correlation coefficients (\(r_s\)) were calculated between the FS measurements and the normalized expression values of each transcript represented on the microarrays. Because we expected gender-specific gene expression patterns,\textsuperscript{20} correlation analysis was carried out separately for male and female animals. \(P\)-values of the resulting coefficients were adjusted to account for the false discovery rate (FDR) following the Benjamini–Hochberg procedure.\textsuperscript{21} Only the genes with \(r_s \geq |0.70|\) and the FDR-adjusted \(P\), \(0.01\) were considered to be strongly associated with FS, and were then passed on to subsequent gene functional classification and pathway analyses.

2.4.3 Step 3
Two types of functional classification were available in the DAVID knowledgebase (Database for Annotation, Visualization and Integrative Discovery).\textsuperscript{22} They were applied to the lists of FS-correlated genes in order to identify biological mechanisms modulating cardiomyopathy. DAVID allows one to organize a list of genes into functionally related clusters. Using default settings, this analysis was run for gene lists obtained from separate analyses of male and female animals. However, we set the classification stringency parameter to ‘lowest’ when examining the relatively short list of FS-correlated genes shared by both males and females. The biological significance of the discovered functional groups was ranked based on the EASE score, an enrichment score that is the log-transformed geometric mean of the cluster members’ \(P\)-values obtained from a modified Fisher’s exact test. High EASE scores suggested a close association between the clustered genes in terms of their biological functions\textsuperscript{22}. The main biological functions of a cluster were derived by visual examination of a 2D gene annotation heatmap offered by the tool.
Pathway analysis was conducted by mapping the lists of FS-correlated genes to the annotation terms from the KEGG database (Kyoto Encyclopedia of Genes and Genomes). This was performed using default settings in DAVID (in particular, the cut-off P-value 0.1 was used in the modified Fisher’s exact test for enrichment). Following the recommendations of the developers of DAVID, we treated the resulting P-values of enrichment tests as scores to rank the importance of a pathway, rather than using them as a statistical decision-making tool. For a more comprehensive ranking, we additionally report fold enrichment for each of the revealed pathways relative to the population background. Fold enrichment values of 1.5 and above are considered biologically important and worth the researcher’s attention.22

3. Results

3.1 Fractional shortening depends on strain background and mutant allele

A mixed-effects statistical modelling approach was applied to determine the factors associated with FS in the examined cardiomyopathy models. FS substantially varied among the experimental groups (Figure 1), ranging from an average of 29.0 ± 6.5% in B6.Myoz2 to 75.0 ± 5.5% in FVB.Sgcd (mean ± SD here and elsewhere). In half of the groups, FS was >65%, indicating that these animals maintained a good ventricular function despite their carrier status.

Sex, age, and their combined effect appeared to be statistically insignificant (P < 0.05, F-tests). They were stepwise-backward eliminated from the initial Model (1), resulting in its reduction to

\[
FS = \beta_0 + \beta_1 \text{Strain} + \sigma + \epsilon. \tag{2}
\]

All parametric terms of Model (2), i.e. the intercept \(\beta_0\) and the strain effect \(\beta_1\), were highly significant (P < 0.001 in both cases, F-tests). In addition, the contribution of mutant alleles to the variance of FS in Model (2) was statistically different from 0 at P < 0.05 (\(\sigma^2 = 54.0\), approximate lower 95% confidence limit = 4.5, upper 95% confidence limit = 653.8). The random effect of alleles was kept in the Model (2) because its removal would have caused a considerable drop in the explanatory power of this model (L = 11.7, P < 0.001; AIC increase from 883.5 to 893.2). Visual examination of the distribution of residuals produced by Model (2) showed no considerable deviation from normality and violation of the homogeneity of variance, suggesting that the model was valid (see Supplementary material online, Figure S2 and S3).

Mean FS in congenic background FVB/N (73.0 ± 4.8%) differed significantly from FS of any other background (49.8 ± 19.6% in C57BL/6, 57.0 ± 20.3% in 129S1/Sv, and 57.9 ± 15.9% in C3H/He). No differences were found in any of the pairwise comparisons between the 129S1/Sv, C57BL/6 and C3H/He strains (Figure 2A). By allele, mean FS in Sgcd-deficient animals (71.1 ± 9.4%) significantly differed from that in Myoz2– (52.0 ± 17.6%) and in Csrp2-deficient mice (44.9 ± 19.3%). No difference was observed between Myoz2 and Csrp2 mutants (Figure 2B).

In summary, the observed FS values were strongly associated with strain background and mutant allele.

3.2 Correlation analysis between gene expression and ventricular function

To test the association between gene expression and ventricular (dys)function, we calculated Spearman correlation coefficients between the FS measurements and the log-expression values of each microarray gene transcript. The correlation analysis was carried out separately for male and female mice because sex is known to be an important confounder at the level of gene expression.20

In male mice, we identified 181 transcripts (corresponding to 159 genes) that were highly significantly correlated with FS (\(r_s \geq 0.70\), FDR-adjusted P < 0.001; see Supplementary material online, Table S1). Most of these transcripts (74%) were negatively correlated with FS, i.e. their expression increased with ventricular dysfunction (Figure 3). Based on the expression levels of these transcripts, males could be divided into two distinctive equally sized groups according to their degree of ventricular impairment (see Supplementary material online, Figure S4). One of the groups had a mean FS of 72.4 ± 9.2%, and was composed of all the Sgcd mutants and the FVB.Myoz2 mice. The other group had a mean FS of 42.6 ± 17.8% and included all other animals.

In female mice, we found 184 transcripts (168 genes) highly correlated with the cardiac impairment (\(r_s \geq 0.70\), FDR-adjusted P < 0.001). As in male mice, most of the transcripts (66%) were negatively correlated with FS (see Supplementary material online, Table S1).
Based on the expression values of these transcripts, all female mice could similarly be divided into two groups according to their degree of ventricular dysfunction (see Supplementary material online, Figure S5). One of these groups had a mean FS of 71.9 ± 5.5% and was composed of FVB.Myoz2 animals and all Sgcd mutants, except for one B6.Sgcd animal that belonged to the second group showing an unusually low FS (43.3%). The second group had a mean FS of 49.2 ± 16.2% and included all other animals.

In total, 280 genes were highly correlated with FS in the examined cardiomyopathy models (see Supplementary material online, Table S1). Of these, only 48 genes (17%) were shared by males and females (see Supplementary material online, Table S1). All shared transcripts had the same sign of correlation in males and females, with the majority (90%) being negatively correlated with FS. Thus, most of these genes were overexpressed in animals with severe heart dysfunction.

### 3.3 Functional classification of genes correlated with FS

We used the gene classification tool in DAVID to organize the lists of genes correlated with FS into functionally related groups and to identify their major biological functions.

Two functionally related clusters were discovered among the 159 genes that were highly correlated with FS in male mice (see Supplementary material online, Table S2). The first cluster was composed of five members involved in metal ion binding. The second cluster listed 16 genes encoding transmembrane proteins and glycoproteins.
In female mice, 26 of the 168 FS-correlated genes were allocated to 4 functional clusters (see Supplementary material online, Table S2) involved in metal ion binding (in particular, potassium and calcium channels), transmembrane transport, protease activity, and ribonucleotide binding. These functional clusters were composed of 4–14 members. In both male and female mice, the highest enrichment scores were observed in the gene groups encoding for metal ion-binding proteins (e.g. zinc finger proteins) and transmembrane transporters related to electrolyte homeostasis and signal transduction (e.g. solute carriers) (see Supplementary material online, Table S2).

Among the genes that were correlated with FS and were shared by both genders, three functionally related clusters were identified, with three to five members each. These genes encoded for leucin-rich repeats, hormone-like proteins, and transmembrane proteins (see Supplementary material online, Table S2).

### 3.4 Pathway analysis of genes correlated with FS

To determine whether FS-correlated genes were enriched with representatives of specific metabolic pathways, we attributed KEGG annotation terms to these genes using the functional annotation tool of DAVID.

In male mice, members of the following five pathways were found to be enriched: proteasome, HCM, DCM, ABC transporters, and focal adhesion proteins. Expression levels of all genes mapped on these pathways were negatively correlated with FS (Table 2, male mice). The proteasome pathway exhibited the most significant enrichment P-value (0.001) and the highest fold enrichment (16.4), suggesting a strong association between this pathway and cardiomyopathy-related impairment of ventricular function. The detection of the HCM and DCM pathways, which were represented...
by the same four genes (Table 2, male mice), was not surprising and reflected the fact that the experimental animals suffered from genetic cardiomyopathy. ABC transporters and focal adhesion pathways showed less significant enrichment P-values but relatively high (>1.5) fold enrichment values, suggesting that their activation does play a role in cardiomyopathy.

In female mice, FS-correlated genes were enriched with representatives of three pathways, namely tyrosine metabolism, proteasome, and amyotrophic lateral sclerosis (Table 2, female mice). All genes of the proteasome and the ALS pathways were negatively correlated with FS. At the same time, the tyrosine metabolism genes were positively related to FS, suggesting a deactivation of this pathway in animals with contractile dysfunction. The tyrosine metabolism had the most significant enrichment P-value (0.047) and the highest fold enrichment (8.5), suggesting a specific, yet unknown role for female cardiomyopathy phenotypes.

Only the proteasome pathway was enriched in the list of the FS-correlated genes shared by males and females (Table 2, both genders). As the proteasome metabolism was also found to be overrepresented in separate analyses of the male and female mice, the activation of this pathway seems to play a particularly important role in the response to genetic cardiomyopathy, irrespective of gender.

4. Discussion

Many authors study gene expression patterns in a murine knock-out model in comparison with appropriate wild-type litter mates controls with the aim of understanding the mechanisms of genetic cardiomyopathies. However, not uncommonly, these mouse models may exhibit an attenuated or no disease phenotype at all. This may be explained by a very weak, pathogenic effect of the gene under study and/or attenuation of penetrance caused by modifier genes in the genetic background of the strain in which the gene under study was inactivated. Although this critical issue can only be resolved by studying the effects of the defective alleles in several strain backgrounds, this is rarely done because it is very cumbersome to produce such disease-sensitized congenic strains. Our study aimed at identifying general genetic modifiers of cardiomyopathy rather than individual cardiomyopathy gene effects. Therefore, we transferred well-known cardiomyopathy genes into four common strain backgrounds to produce the largest group of disease-sensitized congenic cardiomyopathy models published thus far.

At the level of gene expression, one may expect a very complex picture of specific, disease-causing gene fingerprints and compensatory gene expression in response to genetic inactivation of a cardio-myopathy gene. Attempting to identify major genetic modifiers without prior assumptions on candidate genes, our approach was rather ‘statistical’; by comparing many cardiomyopathy models, each on several common inbred backgrounds, we aimed to identify common gene expression patterns shared by the majority of the diverse models. We hypothesized that these common patterns would point to non-specific genetic modifiers of the cardiomyopathy phenotype, whereas gene expression patterns unique to one model would more likely translate the inactivation of a specific cardiomyopathy gene.

Not unexpectedly, mean levels of FS significantly varied among the examined cardiomyopathy models. Use of the linear mixed-effects modelling allowed us to identify strain background as the major factor that determined these differences. For example, mutants on an FVB/N background exhibited normal ventricular pump function with little variance, suggesting that strong compensatory mechanisms existed in this strain background. Notably, FVB/N is a frequently used strain for transgenic gene addition experiments. Also, FS was significantly associated with the type of mutant alleles. Mean FS in Sgcd-deficient mice was consistent with a normal ventricular pump function and exhibited only little variance. This may be related to the finding that the cardiomyopathy showed rather a hypertrophic than a dilative phenotype in these mice.

We identified a total of 280 genes whose expression was highly significantly associated with the level of ventricular dysfunction in the animal models examined. In line with previous findings, we also observed gender-specific gene activation patterns. Most of the identified significant genes were overexpressed in animals with low FS, suggesting that compensatory pathways were activated. In male mice, these genes were enriched with members of five different pathways, with the proteasome pathway showing the highest enrichment. In female mice, three pathways were found, with the tyrosine pathway being most enriched. The administration of anti-oestrogens to the mice may help us to confirm that the observed gender-specific gene enrichments were indeed part of a sexual dimorphism. In contrast, only the proteasome pathway was activated in both males and females.

The ubiquitin-proteasome system (UPS) has already received attention as a potentially important determinant of cardiac dysfunction. An increased expression of the proteasome subunits was previously reported in animal models of hypertrophic and dilated cardiomyopathies and even in cardiomyopathy patients. To explain these findings, the authors hypothesized that impaired pump function produced overload-related cardiac wall stress that may have stimulated the synthesis of excessive amounts of damaged myocardial proteins. These proteins had to be rapidly degraded with the help of the UPS to avoid the activation of pro-apoptotic signals. Preferential degradation of damaged proteins may have, in turn, competitively inhibited UPS proteolytic capacity for other substrates, with the consequence being hypertrophy. Indeed, impaired proteolytic capacity of the UPS, resulting in accumulation of large amounts of ubiquitinated but not degraded proteins, was repeatedly observed in patients and animals with end-stage heart failure. In addition to the hypothesis of competitive inhibition of UPS activity, Predmore et al. provided evidence that the UPS may become dysfunctional due to oxidative post-translational modifications of the proteasome itself. Interestingly, another line of evidence showed that pharmacological inhibition of the proteasome prevented and even reversed (mal)adaptive myocardial hypertrophy and remodelling. In summary, accumulating evidence suggests a certain role of the UPS in cardiomyopathy. However, it remains controversial whether activation or inhibition is favourable for myocardial function.

Additional functionally related genes were correlated with FS. Their biological functions were associated mainly with metal ion binding, transmembrane proteins, proteinase activity, ribonucleotide binding, and signal transduction. These findings emphasize the complexity of the development and progression of cardiomyopathy and are in agreement with a number of studies. For example, a group of four protein kinases (see Supplementary material online, Table S2) correlated with FS in female mice. This group included the mitogen-activated protein kinases Map3k7 and Mapk12, known transducers of extracellular stress signals. Map3k7 mediates signal transduction induced by the transforming growth factor-beta, which has been...
implicated in cardiac fibrosis in mice with mutant sarcosome proteins and HCM.\textsuperscript{33} As a result, we observed an increased expression of both Map3k7 and the transforming growth factor beta-regulated gene 1 (Tbrg1) in females with severe ventricular dysfunction (see Supplementary material online, Table S1). Another example is the atrial natriuretic peptide, a circulating hormone of cardiac origin that is known to compensate for heart failure by its diuretic, natriuretic, and vasodilatory actions and inhibitory effects on renin and aldosterone secretion.\textsuperscript{34} Schoenfels et al.\textsuperscript{20} have previously reported increased concentrations of the atrial natriuretic peptide (Nt-proANP) in a subset of congenic strains with heart failure. Our study confirmed the high expression levels of the natriuretic peptide precursor type A gene (Nppa) in both males and females with ventricular dysfunction (see Supplementary material online, Table S1).

In conclusion, we examined the largest existing collection of disease-sensitized congenic mouse models for cardiomyopathy, allowing the comparison of mutant alleles on several strain backgrounds. We contributed additional evidence implicating a potential role for UPS as a major modifier of the cardiomyopathy phenotype. Elements of the UPS may be pharmacologically targeted in the future to clarify whether their activation or inhibition can improve cardiac function in genetic cardiomyopathy. In contrast to previous reports, our findings resulted from an unbiased, genome-wide analysis of gene expression.

The aim of our work was to generate hypotheses rather than studying candidate gene pathways or confirming previous reports of other groups. Therefore, our study had the limitations of any report on whole-genome analysis of gene expression: a focus on bio-informatics rather than wet lab evidence of molecular mechanisms. Our conclusion is based on a statistical association analysis that cannot differentiate whether myocardial overexpression of the UPS genes is the cause or effect of the impaired pump function in genetic cardiomyopathy. Investigations at repeated time points during the development of cardiomyopathy may help answer this question. Future independent studies should address the UPS activity and protein levels as well as the structure of the 26S proteasome. The determination of oxidized and/or ubiquitinated myocardial proteins should be included in such studies as well as a sophisticated characterization of the new congenic models, e.g. in vivo pressure/volume loops and perfusion of isolated hearts.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

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