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RESEARCH ARTICLE | *Systems Biology and Polygenic Traits*

Involvement of human monogenic cardiomyopathy genes in experimental polygenic cardiac hypertrophy

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Prestes PR, Marques FZ, Lopez-Campos G, Lewandowski P, Delbridge LM, Charchar FJ, Harrap SB. Involvement of human monogenic cardiomyopathy genes in experimental polygenic cardiac hypertrophy. *Physiol Genomics* 50: 000 – 000, 2018. First published
AQ: 3 May 18, 2018; doi:10.1152/physiolgenomics.00143.2017.—Hypertrophic cardiomyopathy thickens heart muscles, reducing functionality and increasing risk of cardiac disease and morbidity. Genetic factors are involved, but their contribution is poorly understood. We used the hypertrophic heart rat (HHR), a unique normotensive polygenic model of cardiac hypertrophy and heart failure, to investigate the role of genes associated with monogenic human cardiomyopathy. We selected 42 genes involved in monogenic human cardiomyopathies to study: *1*) DNA variants, by sequencing the whole genome of 13-wk-old HHR and age-matched normal heart rat (NHR), its genetic control strain; *2*) mRNA expression, by targeted RNA-sequencing in left ventricles of HHR and NHR at 5 ages (2 days old and 4, 13, 33, and 50 wk old) compared with human idiopathic dilated data; and *3*) microRNA expression, with rat microRNA microarrays in left ventricles of 2-day-old HHR and age-matched NHR. We also investigated experimentally validated microRNA-mRNA interactions. Whole-genome sequencing revealed unique variants mostly located in noncoding regions of HHR and NHR. We found 29 genes differentially expressed in at least ~~1~~ age. Genes encoding desmoglein 2 (*Dsg2*) and transthyretin (*Ttr*) were significantly differentially expressed at all ages in the HHR, but only *Ttr* was also differentially expressed in human idiopathic cardiomyopathy. Lastly, only two microRNAs differentially expressed in the HHR were present in our comparison of validated microRNA-mRNA interactions. These two microRNAs interact with five of the genes studied. Our study shows that genes involved in monogenic forms of human cardiomyopathies may also
AQ: 4 influence polygenic forms of the disease.

cardiac hypertrophy; cardiomyopathy; DNA sequencing; gene expression; microRNA

INTRODUCTION

Cardiovascular disease (CVD) is the main cause of death and morbidity worldwide, having killed ~17.5 million people in 2012 (23, 37). Both genetic and environmental factors contribute to CVD. The most common genetic contributions are considered to be polygenic, although the exact number and nature of genes involved has been difficult to determine as new genes are yet to be identified with the advancement of technology (16). However, less common monogenic causes of cardiac hypertrophy (CH) have been well characterized in terms of the causative DNA variants and pathophysiology (10, 26). Variation of the expression of genes involved in monogenic CH might provide clues to the causes of polygenic etiology of CH. AQ: 5

Hypertrophic cardiomyopathy (HCM) is the most common inherited form of CVD, affecting 1 in 500 adults, and is the major cause of heart failure and sudden death in young people (21, 25, 26). The condition is characterized by the asymmetric thickening of the cardiac wall, heart failure, and risk of sudden death. A variety of mutations in genes coding sarcomere and cardiac filament proteins account for over 88% of familial HCM (5, 26, 31). Mutations in two genes that encode myosin heavy chain 7 (*MYH7*) and cardiac myosin binding protein C (*MYBPC3*) are the most common causes of monogenic HCM (31, 39). Familial dilated cardiomyopathies form another important group of monogenic CVD characterized by CH and heart failure for which mutations in genes encoding sarcomeric proteins account for almost half of the known forms (15).

The study of genes involved in monogenic hypertrophy and failure have led to an understanding of disease mechanisms and might also provide explanations for more common polygenic forms of heart failure.

We have developed and characterized the hypertrophic heart rat (HHR), a unique polygenic normotensive model of spontaneous ventricular hypertrophy, cardiac failure, and premature death (13). Compared ~~with~~ their genetic control strain, the normal heart rat (NHR), HHR begin life with fewer cardiomyocytes that develop cellular hypertrophy leading to cardiac enlargement and heart failure (29). AQ: 6

Our aim was to study genes previously associated with human monogenic forms of dilated and hypertrophic cardiomyopathies in the polygenic etiology of CH in the HHR. We combined analyses of RNA expression and DNA sequence AQ: 7

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variation to identify those genes that might be of importance in the polygenic setting.

MATERIALS AND METHODS

Sample collection. The HHR and NHR strains have been described in detail elsewhere (13). Two-day-old HHR and NHR (n = 8 HHR, n = 9 NHR) were euthanized by decapitation. At 4 (n = 10 HHR, n = 10 NHR), 13 (n = 10 HHR, n = 11 NHR), 33 (n = 7 HHR, n = 9 NHR), and 50 (n = 12 HHR, n = 10 NHR) wk of age, rats were euthanized using a lethal dose of pentobarbitone (Lethobarb). Hearts were removed and left ventricles (LVs) were immediately dissected from atria. Cardiac weight indexes (mg/g) were calculated as the total heart weight (mg) relative to total body weight (g) of each animal (Ref. 28 and Fig. 1). F1

The 5 age groups investigated represent different developmental stages in their life: prior to hypertrophy (2-day-old), during the development of hypertrophy (4 wk), early hypertrophy (13 wk), established hypertrophy (33 wk), and hypertrophy complicated by heart failure (50 wk-old). This study was approved by the Animal Ethics Committees of the University of Melbourne and Deakin University and ratified at Federation University Australia.

DNA and RNA extraction. DNA from LV was extracted using PureLink Genomic Extraction kit (Thermo Fisher Scientific). RNA from LV was extracted using miRNeasy kit (Qiagen). DNA was quantified by spectrophotometry using NanoDrop 2000, and RNA was quantified by fluorescence using Qubit 3.0 Fluorometer and the RNA high sensitivity assay kit (Thermo Fisher Scientific).

Genes investigated. For these focused studies, we selected 42 genes (Table 1) involved in monogenic forms of familial cardiomyopathies (5, 15, 31, 39). Physiologically, most of these genes are involved in growth and contractility, regulation of mechanical stress, calcium channels, and a variety of muscle development pathways, mainly for cardiac filaments and sarcomere assembly (5, 31). T1

DNA sequence variants in HHR were identified according to methods detailed previously (30). Briefly, we sequenced the whole genome of ~~1~~ male 13-wk-old NHR and ~~1~~ age-matched HHR. Variants were analyzed according to Genome Analysis Toolkit best practices (35), and functional annotation was performed using SnpEff software (4). Results were stored in a database developed in house, and then we identified unique single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels) in the NHR and HHR (30). AQ: 8

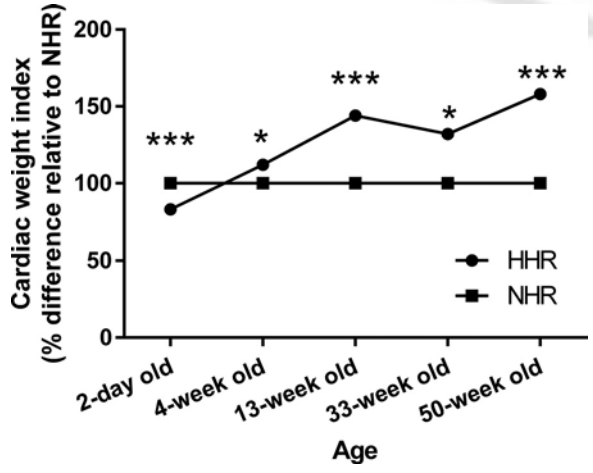

Fig. 1. The hypertrophic heart rat (HHR) has an enlarged heart when compared ~~with~~ its genetic control, the normal heart rat (NHR). Cardiac weight index (mg/g) of HHR is represented as a percentage difference to NHR (normalized to 100%) at 2 days old and 4, 13, 33, and 50 wk old (n = 7–12 per group per age). *P < 0.05; ~~**P < 0.01;~~ ***P < 0.001. AQ:15, AQ:16

mRNA expression of the genes listed in Table 1 was measured using Targeted RNA Expression custom panel in the MiSeq Desktop sequencer (Illumina) and analyzed using MSR: Targeted RNA v2.4.60.8 on Illumina BaseSpace. False discovery rate was set as <0.1.

microRNA (miRNA) arrays were conducted using the Agilent rat microRNA microarray kit 16.0 in LVs of 2-day-old male HHR and age-matched NHR (n = 4/group). The data obtained have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) database with series accession number GSE38710. Differentially expressed miRNAs were identified using Partek Genomics Suite v6.6 with false discovery rate set as <0.05.

In silico investigations. We combined in silico approaches to explore a link between gene expression and DNA sequencing data from the HHR and NHR and human studies available online.

We investigated the possibility that miRNAs might be involved in regulating mRNA expression pre- and posttranscriptionally using three algorithms individually [miRWalk 2.0 (7, 8), miRanda (3), and TargetScan (11)] as a comparative platform to predict possible miRNA binding sites within the sequence in and around each gene of interest. We also used miRWalk 2.0 to investigate experimentally validated miRNA-mRNA interactions and evaluate which miRNAs targeted the genes under investigation.

As DNA methylation can modulate gene expression by compacting DNA sequences, we investigated if any of the DNA sites were differentially methylated in CVD using the Disease Meth database 2.0 (38).

Gene expression in human hearts. We also investigated the mRNA expression of the genes listed identified in HHR in human cardiac samples from the data set "heart failure arising from different etiologies" in the repository GEO reference series GSE1145 (2, 9) (n = 11 control hearts and n = 15 idiopathic dilated hearts). We then determined the expression of the genes investigated using the GEO tool GEO2R.

RESULTS

DNA sequence analyses. DNA sequencing in and around the 42 genes of interest revealed greater number of unique DNA variants discovered in the HHR (compared with the rat reference genome), with 851 SNPs and 491 InDels as opposed to 383 SNPs and 316 InDels in the NHR (Table 1). We found no evidence of DNA sequence variation in six genes in the NHR (namely *Csrp3*, *Emd*, *Gla*, *Mylk2*, *Pln*, and *Taz*) and three genes in the HHR (*Emd*, *Myoz2*, and *Tpm1*, Table 1).

In both the HHR and NHR, most unique variants were located in intergenic or intronic regions. However, we found 11 unique synonymous SNPs in exonic regions in the HHR and ~~2~~ in the NHR. These SNPs were located in genes encoding actinin alpha 2 (*Actn2*), desmin (*Des*), ryanodine receptor 2 (*Ryr2*), troponin T type 2 (*Tnnt2*), and vinculin (*Vcl*) in the HHR and *Ryr2* and *Vcl* in the NHR. Interestingly, only one unique nonsynonymous missense variant (gGt>gTt) in an exonic region was found in the *Vcl* gene in the NHR, changing the amino acid from glycine to valine.

Cardiac RNA expression analyses. The relative fold changes in mRNA expression in the HHR relative to NHR were never greater than fourfold for any of the 42 genes under investigation. We found that 29 of the 42 genes showed significant differential expression for at least ~~1~~ age (Figs. 2, 3, and 4). F2-F4

Genes differentially expressed were not consistent throughout the age groups, possibly reflecting developmental stage-specific regulation. At 2 days old, only four genes were differentially expressed (Fig. 2*A*). In contrast, at 4 wk of age,

Table 1. *Variants found in each gene investigated unique to each strain classified according to type*

		Frequency in Patients, %		Rat Genome			HHR		NHR	
Gene ID	Gene Name	Hypertrophic	Dilated	Chr	Location	Strand	SNP	InDel	SNP	InDel
Abcc9	ATP-binding cassette, subfamily C member 9	n/d	0.6	4	241,019,980–241,139,051		4	10	2	9
Actc1	Cardiac actin alpha 1	<1	Rare	3	112,080,853–112,086,389		3	2	1	1
Actn2	Actinin alpha 2	Rare	0.9	17	68,050,946–68,143,522		61	42	58	39
Ankrd1	Ankyrin repeat domain 1	Rare	1.9	1	262,038,137–262,046,691	+	None	5	2	2
Casq2	Calsequestrin 2	Rare	n/d	2	223,945,611–224,001,893	+	11	8	5	12
Cav3	Caveolin 3	0.6	n/d	4	207,683,202–207,699,176	+	None	2	None	1
Cryab	Crystallin, alpha B	n/d	0.7	8	53,776,100–53,779,780	+	3	6	2	8
Csrp3	Cysteine and glycine-rich protein 3	Rare	0.3	1	105,206,459–105,225,635		1	1	None	None
Ctf1	Cardiotrophin 1	n/d	n/d	1	206,186,511–206,191,930	+	2	3	None	3
Des	Desmin	n/d	0.3–2.1	9	82,325,835–82,333,549	+	34	7	None	1
Dsc2	Desmocollin 2	n/d	n/d	18	11,625,847–11,658,036		9	4	2	3
Dsg2	Desmoglein 2	n/d	2.3	18	15,353,348–15,411,825		5	3	24	4
Emd	Emerin	n/d	n/d	1	152,192,993–152,196,004		None	None	None	None
Fhl2	Four and a half LIM domains 2	n/d	2.1	9	49,591,185–49,664,022		1	5	None	3
Gla	Galactosidase, alpha	1–2 in men	n/d	X	105,295,029–105,306,686		None	1	None	None
Jup	Junction plakoglobin	n/d	n/d	10	88,073,764–88,100,700		1	10	1	6
Lama4	Laminin, alpha 4	n/d	1.1	20	45,786,892–45,926,468	+	37	24	101	17
Lamp2	Lysosomal-associated membrane protein 2	Rare	n/d	X	124,809,053–124,852,509		2	4	1	3
Lmna	Lamin A/C	n/d	6–7.5	2	207,245,237–207,265,928		24	11	3	11
Mybpc3	Myosin binding protein C	20–42	0.2–4	3	86,649,264–86,667,484	+	None	3	None	3
Myh6	Myosin heavy chain 6	Rare	4.3	15	37,492,599–37,516,786		1	4	None	3
Myh7	Myosin heavy chain 7	20–40	4.2–6.3	15	37,512,803–37,544,317		2	None	None	1
Myl2	Myosin light chain 2	<5	n/d	12	41,831,137–41,835,806		6	13	5	7
Myl3	Myosin light chain 3	1–2	n/d	8	118,370,030–118,376,218	+	None	4	3	4
Mylk2	Myosin light chain kinase 2	Rare	n/d	3	154,789,177–154,800,843	+	None	1	None	None
Myoz2	Myozenin 2	Rare	n/d	2	246,542,267–246,569,008		None	None	2	None
Nexn	Nexilin	n/d	1	2	276,129,684–276,161,434		2	5	2	2
Pkp2	Plakophilin 2	n/d	n/d	11	91,966,047–92,031,277		2	4	2	4
Pln	Phospholamban	Rare	Rare	20	36,390,879–36,400,626	+	1	None	None	None
Prkag2	Protein kinase, AMP-activated, non-catalytic subunit gamma 2	<1	n/d	4	6,577,007–6,816,813	+	2	4	None	5
Psen2	Presenilin 2	n/d	1	13	103,521,460–103,547,174		1	0	2	2
Rbm20	RNA binding motif protein 20	n/d	1.9	1	281,783,646–282,003,053	+	12	21	3	12
Ryr2	Ryanodine receptor 2	Rare	n/d	17	67,285,205–67,704,766		518	218	122	106
Sgcd	Sarcoglycan delta	n/d	Rare	10	31,878,904–32,285,036		26	34	15	24
Taz	Tafazzin	n/d	n/d	1	152,161,153–152,169,569		1	None	None	None
Tmem43	Transmembrane protein 43	n/d	n/d	4	187,412,090–187,427,232		3	2	2	1
Tnnc1	Troponin C type 1	Rare	0.4	16	7,220,777–7,223,730	+	0	0	0	0
Tnnt2	Troponin T type 2	3–10	2.9	13	57,711,369–57,729,182	+	48	13	3	4
Tpm1	Tropomyosin 1	<5	0.6–1.9	8	77,147,580–77,174,392		None	None	None	2
Ttn	Titin	Rare	14.1–20	3	70,138,896–70,408,647		24	12	6	10
Ttr	Transthyretin	1–10	n/d	18	15,307,563–15,316,780		None	1	8	1
Vcl	Vinculin	Rare	Rare	15	3,433,521–3,522,441		4	4	6	2
Total							851	491	383	316

Chr, chromosome; HHR, hypertrophic heart rat; ID, identification; InDel, insertion/deletion; n/d, not described; NHR, normal heart rat; SNP, single nucleotide polymorphism.

we observed 50% of genes differentially expressed, the highest prevalence of all the age groups (Fig. 2*B*). Most of the differences at this age presented increased expression. For some genes (such as *Gla*, *Jup*, *Lamp2*, and *Pln*) differential expression was evident only at 4 wk of age. The differential expression of other genes (such as *Actc1*, *Cav3*, and *Fhl2*) became first evident at 4 wk of age and then persisted throughout adulthood. Differences in the expression of other genes (such as *Tnnt2*, *Myoz2*, and *Myl3*) appeared at 4 wk of age but disappeared in later adulthood. Still other genes (such as *Ankrd1*) did not show differential expression until later adulthood (Figs. 2, 3, and 4).

Two genes, those encoding desmoglein 2 (*Dsg2*) and transthyretin (*Ttr*) were significantly differentially expressed at all ages in the HHR compared with NHR. *Dsg2* was underexpressed in the hearts of HHR at all ages sampled, whereas *Ttr* was significantly downregulated at *day 2* in the neonatal period but significantly upregulated at all subsequent ages corresponding to the development of cardiomyocyte hypertrophy (Fig. 2). *Ttr* also showed greater expression in adult human idiopathic cardiomyopathy (Fig. 4).

The analysis of human idiopathic cardiomyopathy revealed that 16 orthologous genes differentially expressed of the 42 genes investigated. We found only 9 of those 16 genes were also differentially expressed in at least 1 rat age.

Predicted miRNA binding sites. We also predicted possible miRNA binding sites within and around each gene region. Potentially there are over 220,000 miRNA binding sites in the gene coding regions alone and almost 54,000 in the promoter regions (Table 2). However, our comparison of validated T2 miRNA-mRNA interactions to miRNAs differentially expressed in HHR compared with NHR in our microarray data found two miRNAs (miR-34a-5p and miR-17–5p) upregulated in the HHR (Table 3). Interestingly, those two miRNAs interact with 5 of the genes investigated, plakophilin 2 (*Pkp2*), RNA binding motif protein 20 (*Rbm20*), *Ryr2*, tropomyosin 1 (*Tpm1*), and *Vcl* (14, 19, 24). Although not statistically significant, the gene expression of *Pkp2*, *Ryr2*, *Tpm1*, and *Vcl* is

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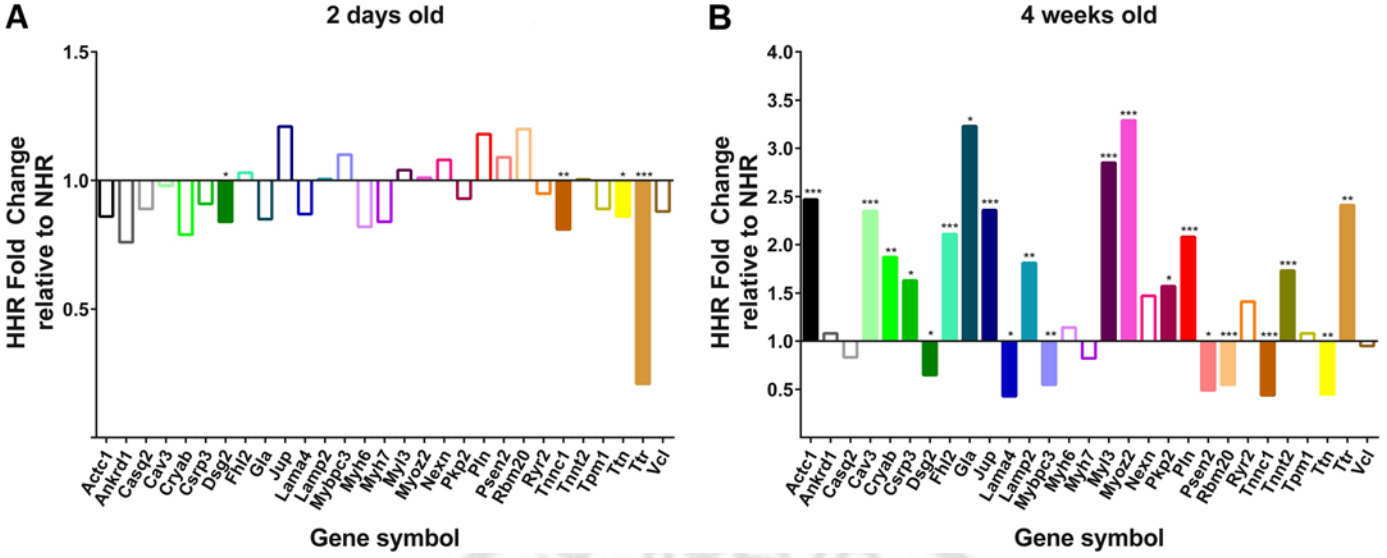

Fig. 2. Heart mRNA expression of genes differentially expressed in at least one age group. There are 4 and 21 genes differentially expressed in neonatal (*A*) and 4-wk-old (*B*) hypertrophic heart rats (HHR), respectively. HHR fold change relative to normal heart rat (NHR) is shown. Genes not differentially expressed are shown in open boxes. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

downregulated in 2-day-old HHR (fold change = 0.93, 0.95, 0.89, and 0.88, respectively).

Other analyses. Scans of published genome-wide association studies [GWAS; using GWASdb v2 (22)] data also indicated the presence of SNPs found in the 42 genes investigated that are associated with CVD traits in humans (Table 4).

T4, AQ:9

Surprisingly no methylation profiles have been reported in human heart-related diseases for any of the genes in our data set.

DISCUSSION

The HHR and their NHR genetic control are derived from the spontaneously hypertensive rat and Fisher 344 rat and provide a unique model of left ventricular hypertrophy independent of high blood pressure and heart failure. Although HHR and NHR have a polygenic background, it is not unreasonable to presume that genes best known for their major mutations causing human ~~cardiac~~ and failure might also encompass DNA variants with a more subtle quantitative impact on cardiac structure and function. Here we sought to determine whether any of the 42 genes implicated in Mendelian human hypertrophic and dilated cardiomyopathies might also be relevant to the polygenic hypertrophy of HHR, either as DNA sequence variants or abnormal gene expression patterns. We had the advantage with our life course approach to examine also the ontogeny of the expressions of these genes and their relationships with the developmental stages of hypertrophy in the HHR.

AQ: 10

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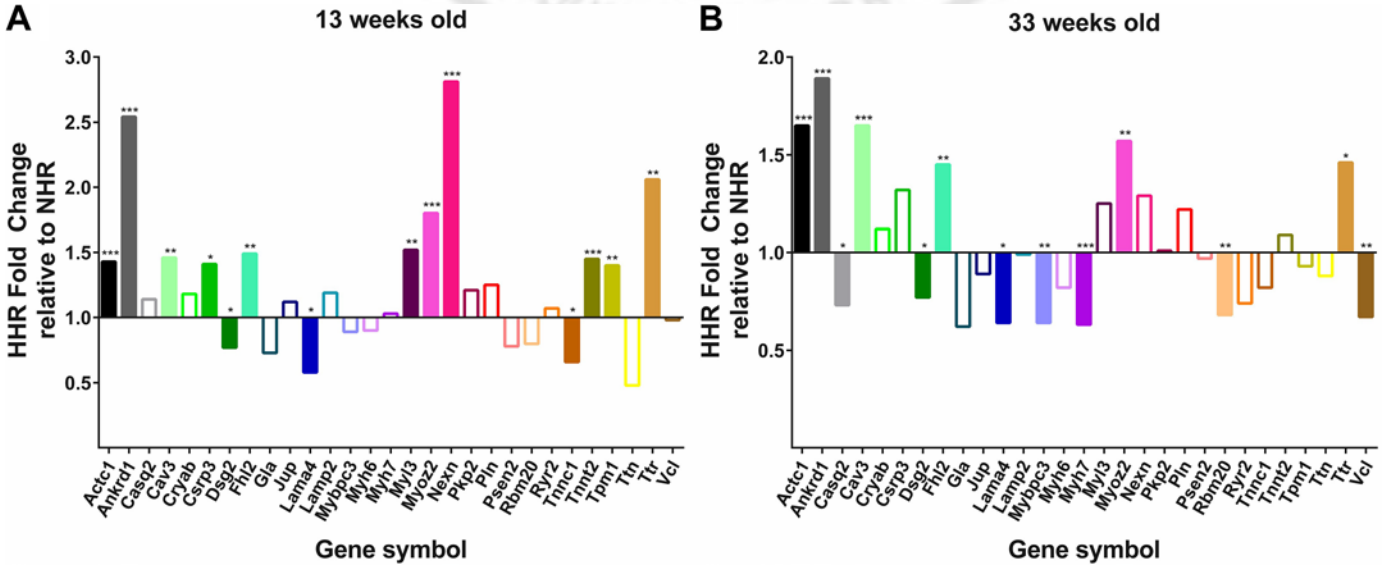

Fig. 3. Heart mRNA expression of genes differentially expressed in at least one age group. There are 14 and 13 genes differentially expressed in 13-wk-old (*A*) and 33-wk-old (*B*) hypertrophic heart rats (HHR), respectively. HHR fold change relative to normal heart rat (NHR) is shown. Genes not differentially expressed are shown in open boxes. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

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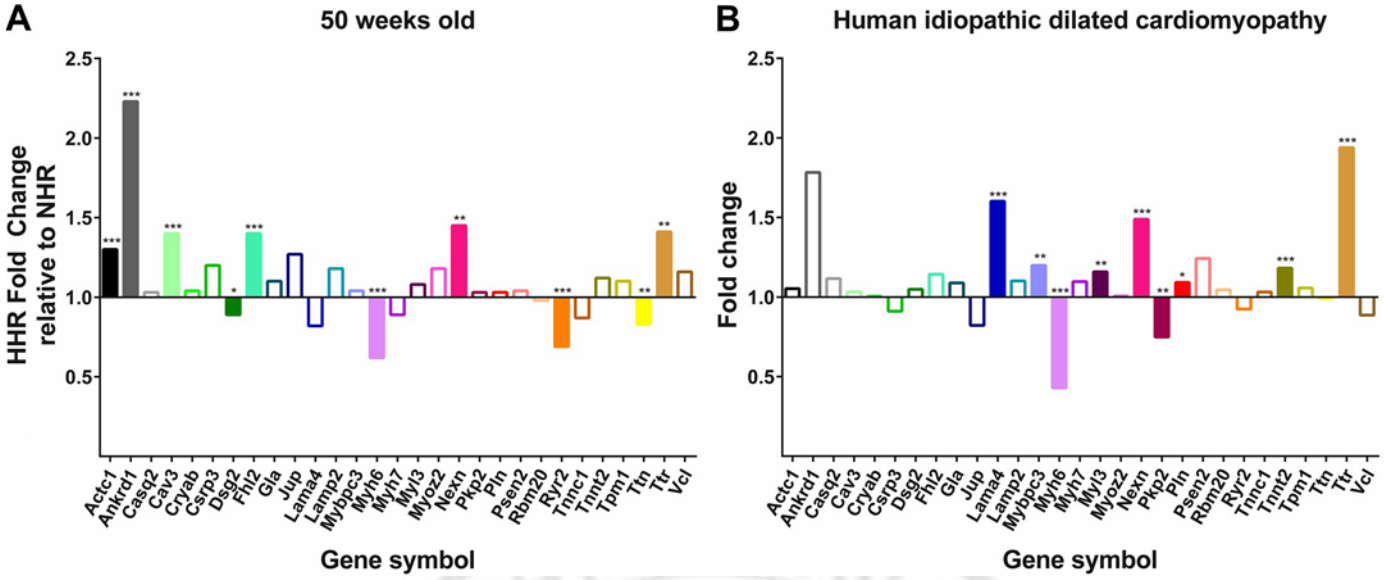

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Fig. 4. Heart mRNA expression of genes differentially expressed in at least one age group. There are ~~10~~ 11 and ~~9~~ 1 genes differentially expressed in 50-wk-old hypertrophic heart rats (HHR; *A*) and human idiopathic dilated cardiomyopathy (DCM; *B*), respectively. HHR fold change relative to normal heart rat (NHR) is shown. DCM patient fold change relative to healthy is shown in humans. Genes not differentially expressed are shown in open boxes. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Molecular genetic studies have identified over 1,400 mutations responsible for inherited cardiomyopathies, and genetic testing is offered to identify genetic causes in families or assess family members at risk (26, 34). However, emerging data suggest that compound mutations have an additive role causing a gene dosage effect influencing the severity and progression of HCM (17, 20, 27). Such interactions at the level of monogenic cardiomyopathic disease provide general support for the hypothesis that interaction between other DNA variants in the same genes might influence polygenic CH.

AQ: 11

Among the sequence variants identified in our sequencing analyses, we found 11 synonymous SNPs in exonic regions in the HHR and ~~1~~ 2 nonsynonymous SNP in the NHR. It is well established that nonsynonymous mutations may impact amino acid sequences and therefore human health (18). However, synonymous variants that had previously been regarded as "silent" mutations and thought to have no effect on disease phenotype have also been reported to cause changes in protein-protein interactions. Importantly, studies suggest that these synonymous mutations contribute to human disease risk and other complex traits (32). The synonymous variants we discovered (Table 1) might result in changes in protein-protein interactions and explain changes in gene expression (Fig. ~~1~~ 2) because of imbalanced availability of tRNA caused by codon bias (12).

Interestingly, HHR are born with smaller and fewer cardiomyocytes than NHR. We believe that this reduced endowment is important for the subsequent development of cardiomyocyte hypertrophy, which becomes evident in early adolescence (4 wk, Fig. 1), when increased pressure and volume loads in the growing animals place proportionately greater stress on the fewer individual myocytes (29). By 13 wk of age, both cardiac and cardiomyocyte hypertrophy are established (Ref. 13 and Fig. 1), but in the end this is counterproductive, as the enlarged cells do not function efficiently and gradually deteriorate, leading to heart failure (evident as early as 30 wk of age) and premature death (toward 50 wk of age) (13, 29). These pathophysiological phenotypic changes can provide context for the developmental stage-specific changes in gene expression we observed.

Our analysis of gene expression showed that at 2 days old, when HHR hearts are smaller than NHR (29), the genes encoding cardiac filaments and sarcomeric proteins are generally underexpressed (Fig. 2). Whether this is a cause or effect of the fewer, smaller cells is not possible to say from these data. As the cells begin to hypertrophy and throughout the rest of their lives, most of these genes are overexpressed. These findings demonstrate that the LV undergoes changes in gene expression that could be related to the pathophysiology of the hypertrophy (36). Interestingly, the switch of the *Ttr* gene from underexpressed in neonates to overexpressed in adults suggest stage-specific regulation. *Ttr* encodes a protein for exosome production, and high protein levels in serum have been previously associated with lung cancer (6) and heart failure caused by accumulation of transthyretin amyloid fibrils in the heart (33). Additionally, the genes for cardiac alpha actin 1 (*Actc1*), caveolin-3 (*Cav3*), and four and a half LIM domains 2 (*Fhl2*) are upregulated in adult HHR and have been associated with cardiomyopathies in mice and humans (1, 28). Conversely, the persistent underexpression of *Dsg2* suggests a constitutive difference between HHR and NHR that appears independent of the pathophysiological changes with age.

Interestingly, the majority of genes identified in HHR were not differentially expressed in the human samples (Fig. 2). This might reflect the nature of the diseases in the human repository that is composed primarily of samples from human idiopathic dilated cardiomyopathy rather than Mendelian human HCM. Furthermore, as we were unable to establish the age of the human samples, we cannot make direct comparisons to our rat data to further our understanding of gene expression patterns with the progression of HCM.

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EQ:1 Table 2. *Predicted number of microRNA binding sites in or around the genes of interest*

Gene	Region			
	Promoter	5=UTR	CDS	3=UTR
AQ: 18 *Abcc9*	1,255	3,618	19,131	8,848
Actc1	1,394	290	1,126	1,721
Actn2	1,195	1,296	6,071	3,848
Ankrd1	1,265	407	1,016	625
Casq2	1,332	835	2,678	2,594
Cav3	1,443	240	1,103	1,411
Cryab	1,356	487	871	116
Csrp3	1,260	332	772	620
Ctf1	1,371	106	1,231	1,607
Des	1,379	137	1,194	907
Dsc2	1,161	1,345	4,605	2,698
Dsg2	1,289	222	1,982	1,224
Emd	1,249	209	946	458
Fhl2	1,319	2,240	7,695	3,197
Gla	1,271	89	1,354	6
Jup	1,280	2,251	25,961	14,746
Lama4	1,233	2,439	16,253	3,119
Lamp2	1,202	732	3,485	4,589
Lmna	1,279	976	5,076	1,977
Mybpc3	1,362	137	2,025	592
Myh6	1,314	118	5,753	234
Myh7	1,341	461	5,832	427
Myl2	1,330	205	646	472
Myl3	1,197	293	819	482
Mylk2	1,403	256	1,552	858
Myoz2	1,235	344	844	933
Nexn	1,242	1,939	7,475	7,584
Pkp2	1,345	234	3,398	2,367
Pln	1,024	254	215	1,062
Prkag2	1,293	3,224	12,154	12,185
Psen2	1,212	1,818	4,518	2,407
Rbm20	1,262	41	2,014	1,835
Ryr2	1,270	565	5,045	1,941
Sgcd	1,216	1,623	3,963	9,768
Taz	1,191	1,139	3,286	3,161
Tmem43	1,362	286	1,218	1,263
Tnnc1	1,370	41	567	347
Tnnt2	1,299	2,222	5,604	2,586
Tpm1	1,302	4,249	15,719	14,114
Ttn	1,117	648	28,263	4,702
Ttr	1,287	224	750	435
Vcl	1,295	396	7,084	6,209
Total	53,802	38,968	221,294	130,275

CDS, coding DNA sequence; UTR, untranslated region.

To provide a comprehensive perspective about the processes involved in this pathology, we investigated DNA methylation profiles and SNPs published in GWAS data of the genes investigated. We were unable to find any relevant methylation data supporting further analyses, as the majority of the studies available were performed in a variety of cancers and only few and individual studies are available in CVD. However, two SNPs found in the GWAS data located in the cardiac troponin T2 (*TNNT2*) gene are associated with cardiac troponin-T levels and directly correlated ~~to~~ heart failure (40). These challenges in merging DNA, RNA, and epigenome data highlight the importance of comprehensive studies publicly available.

Our findings provide evidence of the involvement of monogenic genes in polygenic ~~hypertrophic~~ but might only account for part of the genes responsible for this disease. The prevalence of rare variants and unidentified genes responsible for HCM are yet to be elucidated. Furthermore, the interaction mechanisms used by these genes deserve more attention and might aid professionals in determining diagnostics and prognosis of the disease. AQ: 12

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors. AQ: 13

AUTHOR CONTRIBUTIONS

P.R.P., L.M.D., F.J.C., and S.B.H. conceived and designed research; P.R.P., F.Z.M., G.L.-C., and P.L. performed experiments; P.R.P. analyzed data; P.R.P. interpreted results of experiments; P.R.P. prepared figures; P.R.P. drafted manuscript; P.R.P., F.Z.M., G.L.-C., P.L., L.M.D., F.J.C., and S.B.H. edited and revised manuscript; P.R.P., F.Z.M., G.L.-C., P.L., L.M.D., F.J.C., and S.B.H. approved final version of manuscript. AQ: 14

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Table 3. *Differentially expressed microRNAs experimentally validated to interact with genes under investigation*

miRNA	MIMAT ID	Count	Genes	*P* Value	FDR	Mean Ratio (HHR/NHR)
hsa-miR-34a-5p	MIMAT0000255	3	*PKP2, TPM1, VCL*	0.000117868	0.00977691	1.83
hsa-miR-17–5p	MIMAT0000070	2	*RBM20, RYR2*	0.0000029	0.00076469	1.10

FDR, false discovery rate; HHR, hypertrophic heart rat; miRNA, microRNA; NHR, normal heart rat.

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Table 4. *Single nucleotide polymorphisms found in genes investigated associated with traits related to cardiovascular disease in human genome-wide association studies*

Trait	SNP Count	Genes
Aortic root size	1	*VCL*
Atrial fibrillation	2	*RYR2, TTN*
Blood pressure	5	*RBM20, SGCD, RYR2, PRKAG2*
Blood pressure (response to angiotensin II receptor blocker)	3	*ABCC9, RYR2*
Blood pressure, CVD RF, and other traits (body mass index, waist-to-hip ratio, renin activity and aldosterone concentration in plasma, BNP levels in plasma, alcohol consumption)	9	*TTN, PRKAG2, MYH6, LAMA4*
Body mass index	4	*RYR2, PKP2, ACTN2*
Cardiac troponin-T levels	2	*TNNT2*
Cardiovascular disease	1	*RYR2*
Chronic kidney disease	1	*PRKAG2*
Coronary artery calcification	8	*PRKAG, SGCD, RYR2, MYBPC3, MYH6, RYR2*
Coronary artery disease	1	*VCL*
Coronary heart disease	2	*VCL, TNNT2*
ECG dimensions, brachial artery endothelial function, treadmill exercise responses	3	*PRKAG2, RYR2*
Electrocardiographic conduction measures	1	*RYR2*
Electrocardiographic traits	1	*MYH6*
Glomerular filtration rate	1	*PRKAG2*
Health and aging, CVD, and cancer age of onset	1	*TTN*
Heart failure	1	*SGCD*
Heart rate	2	*CRYAB, MYH6*
Height	1	*DSC2*
Hypertension	2	*SGCD*
Hypertension (early onset hypertension)	2	*ACTN2*
Multiple complex diseases	26	*FHL2, RYR2, LMNA, CSRP3, PRKAG2, SGCD, RBM20, DSC2, ACTN2, LAMA4, MYLK2, MYOZ2*
Myocardial infarction	2	*CASQ2*
Obesity-related traits	7	*ABCC9, RYR2, PKP2*
Red blood cell traits	13	*PRKAG2, CTF1*
Resting heart rate	1	*MYH6*
Sudden cardiac arrest	1	*RBM20*
Triglycerides	5	*MYBPC3, VCL, RYR2, TNNT2, CSRP3*
Ventricular conduction	1	*CASQ2*
Waist circumference	1	*RYR2*

AQ: 19 BNP, brain natriuretic peptide; CVD, cardiovascular disease; ECG, electrocardiogram; RF, risk factor; SNP, single nucleotide polymorphism.

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