Transcriptional regulation of cardiac genes balance pro- and anti-hypertrophic mechanisms in hypertrophic cardiomyopathy

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Abstract

Hypertrophic cardiomyopathy (HCM) is characterized by unexplained left ventricular hypertrophy. HCM is often hereditary, but our knowledge of the mechanisms leading from mutation to phenotype is incomplete. The transcriptional expression patterns in the myocardium of HCM patients may contribute to understanding the mechanisms that drive and stabilize the hypertrophy.

Cardiac myectomies/biopsies from 8 patients with hypertrophic obstructive cardiomyopathy (HOCM) and 5 controls were studied with whole genome Illumina microarray gene expression (detecting 18 189 mRNA).

When comparing HOCM myocardium to controls, there was significant transcriptional down-regulation of the *MYH6, EGR1, APOB* and *FOS* genes, and significant transcriptional up-regulation of the *ACE2, JAK2, NPPA* (*ANP*), *APOA1* and *HDAC5* genes.

The transcriptional regulation revealed both pro- and anti-hypertrophic mechanisms. The pro-hypertrophic response was explained by the transcriptional down-regulation of *MYH6*, indicating that the switch to the fetal gene program is maintained, and the transcriptional up-regulation of *JAK2* in the JAK-STAT pathway. The anti-hypertrophic response was seen as a transcriptional down-regulation of the immediate early genes (IEGs), *FOS* and *EGR1*, and a transcriptional up-regulation of *ACE2* and *HDAC5*. This can be interpreted as a transcriptional endogenous protection system in the heart of the HOCM patients, neither growing nor suppressing the already hypertrophic myocardium.

Introduction

Hypertrophic cardiomyopathy (HCM) is characterized by cardiac hypertrophy of the septum and/or left ventricular walls in the absence of other pro-hypertrophic diseases.[1] The disease is often monogenetic, caused by mutations in sarcomeric genes in approximately 56% of cases.[2] At the cellular level, there is an increase in cardiomyocyte size, myocyte disarray and fibrosi.[3-4] HCM is associated with an increased risk of mortality and morbidity and is a common cause of sudden cardiac death in young adults.[5] In a subset of patients with hypertrophic obstructive cardiomyopathy (HOCM), surgical myectomy may be indicated to alleviate symptoms.

To date, numerous signalling pathways and transcription factors have been related to cardiac hypertrophy, including the janus kinase - signal transducer and activator of transcription (JAK-STAT) pathway,[6] the fetal gene program[7] and transcription influenced by the immediate early genes.[8] Analysis of the transcriptome can be made by using microarray gene expression techniques which give a picture of the gene activity at a given time point. In HCM, such studies have been carried out in experimental animal models as well as in human hearts. In 2001, Lim *et al.* performed a DNA subtraction hybridization analysis of tissue from septal myectomies in a single human heart with HCM and from a normal heart, followed by confirmation of the results in 6 more HCM hearts. Up-regulation of markers of cardiac hypertrophy, such as alpha skeletal actin, isoforms of myosin light chains and brain natriururetic peptide was found.[9] In 2002, Hwang *et al.* investigated the gene expression in end-stage heart failure due to HCM (2 patients) or dilated cardiomyopathy (DCM) (3 patients) by using an in-house spotted cDNA microarray with 10,272 unique clones from various cardiovascular cDNA libraries in their laboratory. A total of 192 genes were highly expressed in both HCM and DCM (*e.g. ANP*, decorin, elongation factor 2 and heat shock protein 90) and 51 genes were down-regulated in both conditions (*e.g.* elastin and sarco-/endoplasmic reticulum Ca ATPase, SERCA).[10] Rajan *et al.* studied ventricular tissue from two different transgenic mouse models carrying mutations in the alpha-tropomyosin gene (TPM1). Out of 22,600 genes studied, 754 were differentially regulated in the transgenic mice compared to controls. Genes with the highest expression belonged to secreted/extracellular matrix category and genes with the most significant decrease were associated with metabolic enzymes.[11] Thus, a number of differentially expressed genes have been identified in HCM.

In HCM, cardiac hypertrophy often progresses for a number of years and then subsides, as shown in a study by Maron *et al.* where left ventricular wall thickness increased by 6 to 23 mm during a mean follow up of four years in children with HCM.[12] It is important to identify how different pathways interact to cause, control and maintain the hypertrophy so that the progression of the heterogeneous disease can be better understood and a target for future therapy might be developed depending on the stage of hypertrophy. Today, the whole transcriptome can be assessed at once by the use of whole genome microarray analysis, which has proven to be useful in portraying molecular events in a specific tissue sample. It is not known what starts the hypertrophic process in HCM, nor is it known what stops it in order to prevent growth in *absurdum*. By combining results from animal experiments and human material from patients from different stages of the disease, microarray data might shed light on what starts the process. The aim of this study was to examine the transcriptional expression in the myocardium of patients with symptomatic HOCM in an attempt to explain the mechanisms that drive and maintain the hypertrophy.

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Materials and Methods

Study population

This study includes myectomies from 8 unrelated patients with non-end stage HOCM. The myectomies were collected between November 2005 and April 2007 on obtaining written consent. The HOCM patients were diagnosed using criteria proposed by McKenna *et al.*.[13] Three of the patients were also diagnosed with mild hypertension, although this was not sufficient to cause this severe phenotype. The electrocardiographic left ventricular hypertrophy (LVH) was interpreted using the Romhilt-Estes score.[14] As controls, myectomies/biopsies were collected from 5 respirator patients, without cardiac disease who were deceased following accidents. Consent was given to the hospital administration of Hôpital Lariboisière in Paris according to French legislation. The study was approved by the ethical committees of Umeå, Uppsala and Paris.

Sample preparation

The myectomies/biopsies from the patients were excised and immediately placed in RNAlater (Qiagen, Hilden, Germany). Total RNA was isolated using the RNeasy Fibrous Tissue Kit (Qiagen, Stanford, CA, USA). The RNA concentration was measured with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Inc, Wilmington, USA) and the integrity of the RNA was analyzed with a 2100 Bioanalyzer (Agilent Technologies Inc, Palo Alto, CA, USA).

Microarray gene expression

Using the Illumina Totalprep RNA Amplification Kit (Ambion, Austin, TX, USA), aliquots of total RNA were converted to biotinylated double-stranded cRNA. The biotin labeled cRNA samples were hybridized on a Sentrix HumanRef-8 Expression Beadchip (Illumina, San Diego, CA, USA), including 18,189 gene transcripts, and incubated with streptavidin-Cy3. The microarray analysis was scanned with the Illumina Beadstation GX (Illumina).

Data analysis

To find differentially expressed genes in the microarray data, Illumina GenomeStudio software (version 3.3.8) was used. The data were normalized and significant differential expression was calculated using Beadstudios cubic spline algorithm. The gene expression fold change for the patient group was calculated as the average signal value relative to the average signal value for the control patient group. A significant up-regulation was defined as a fold change of 1.5 or over and a significant down-regulation was defined as fold change of -1.5 or under. P<0.05 was considered statistically significant. To avoid selecting genes with high fold change due to low signal intensity a minimum signal intensity value was used; the signal intensity was set at over 50. The statistical filtering can be found in Table 1.

MetaCore™ (GeneGo Inc., USA) was used to find common transcriptional relations between transcription factors and genes in HCM to generate a direct interaction network.

Quantitative real time polymerase chain reaction

To validate the microarray expression data, we performed relative quantification of mRNA expression of four genes, differentially expressed in the microarray analysis using an Applied Biosystems Prism 7900HT Sequence Detection System (Foster City, CA, USA). Omniscript RT Kit (Qiagen) was used to convert an aliquot of the remaining total RNA to cDNA. Human cDNA-specific TaqMan Gene Expression Assays for the genes: angiotensin converting enzyme 2 (*ACE2*) (Assay ID Hs01085333_m1), apolipoprotein A-I (*APOA1*) (Assay ID Hs00163641_m1), early growth response 1 (*EGR1*) (Assay ID Hs00152928_m1) and myosin heavy chain 6 cardiac muscle alpha (*MYH6*) (Assay ID Hs00411908_m1) from Applied Biosystems were used in the study. The human cyclophilin A (PPIA) gene was chosen as an endogenous control (part n. 432631E; Applied Biosystems Inc, Foster City, CA, USA) after confirmation as the most stable in myectomies when comparing with glyceraldehyde-3-phosphate dehydrogenase (*GADPH*) and large ribosomal protein (*RPLPO*).[15] All samples were run in triplicates and amplification was analyzed using Applied Biosystems Prism Sequence Detection software (version 2.3). Relative quantification was calculated according to the comparative CT method (Applied Biosystems Inc) using a statistical confidence of 95%. The amount of target gene mRNA, normalized to an endogenous control and relative to a calibrator, is given by $2^{-\Delta\Delta C_T}$. The gene expression fold change of the patients is the average $2^{-\Delta\Delta C_T}$ value relative to the average $2^{-\Delta\Delta C_T}$ value for the control patients.

Statistics

All analyses of the real time polymerase chain reaction (PCR) data were performed with Predictive analytics software (PASW) (version 18.0, SPSS Inc., Chicago, Ill, USA). Differences between two groups were compared using Mann-Whitney U test. P<0.05 was considered statistically significant.

Multivariate data analysis

SIMCA-P+ 12.0.1 software (Umetrics, Umeå, Sweden) was used to perform principal component analysis (PCA) to find[16-17] clusters and outliers in the sample population. A PCA model was calculated on the X-matrix (*i.e.* the microarray data with the signal intensity >50) to provide an overview of the data. In the data matrix X [NxK], N defines the number of rows or samples and K the number of variables or gene transcripts. A small number of latent variables (principal components) are calculated; these variables reflect the largest systematic variations by describing the largest variation in the X-matrix. As a result, the influence of noise is reduced and the dimensionality of the data is greatly reduced, which simplifies interpretation. *Scores* are the coordinates of the samples in the reduced space (the principal component space) whereas *loadings* are the relations between the original variables and the principal components. In the score plots, trends and outliers can be found and clusters of samples can be revealed.

Results

Clinical analysis of cohort

The demographics and patient history of the HOCM cohort (n=8; 3 men and 5 females) are summarized in Tables 2 and 3. The mean age at diagnosis was 60.5±17.0 years with a mean maximum wall thickness of 18.6±2.5 mm and a mean left ventricular outflow tract-obstruction gradient of 79±46 mmHg. The mean NT pro BNP was 5253±8906 ng/L. Information on cause of death, age and sex of the control patient group can be found in Table 4. All patients had a preserved systolic left ventricular function with normal fractional shortening.

Table 1. Number of differentially expressed genes after different filtering steps.

Filtrating step	mRNA expression Number of genes	
Differential P-value<0.05	343↑	254↓
↓		
Detection P-value<0.05	330↑	233↓
↓		
Avg. sign. P-value>50	330↑	233↓
↓		
Fold change (mRNA)±1.5	283↑	210↓

Statistical significance was set to P<0.05. A significant up- and down-regulation was defined as a fold change ±1.5. A minimum signal intensity value of 50 was utilized.

Table 2. Patient characteristics.

Sex	ID	Age (yrs)	Blood pressure (mmHg)	ECG[20]	NTpro BNP	LA (mm)	IVSD (mm)	LVPWD (mm)	Max. wall thickness (mm)	LVEDD (mm)	LVOT-obstruction (mmHg)
Woman	H1	75	150/75	LVH	1 181*	47	16	10	16	44	60
Man	H2	51	145/80	LVH	295*	49	18	15	19	51	36
Woman	H3	79	150/75	LVH	1 412*	53	17	17	17	45	159
Man	H4	50	130/80	LVH, QT-prolongation	25 139	95	18	15	20	50	16
Woman	H5	70	100/60	LVH	-	46	16	15	19	39	117
Woman	H6	76	130/70	LBBB	-	39	13	11	15	42	64
Man	H7	31	130/70	LVH	1 633	38	22	12	22	50	91
Woman	H8	52	130/80	LVH, inferior Q-wave	1 862	44	12	10	21	44	89

ECG, Electrocardiography; LA, left atrium; IVSD, interventricular septum dimension; LVPWD, left ventricular posterior wall dimension; LVEDD, left ventricular end diastolic diameter; LVOT, left ventricular outflow tract; LVH, left ventricular hypertrophy; LBBB, left bundle branch block. *post myectomy.

Sample preparation

The integrity analysis of the RNA from both patients and controls showed no breakage or digestion.

Microarray gene expression

The microarray data was filtrated as described in the Methods section (Table 1). There were 493 gene transcripts (283 were up-regulated and 210 were down-regulated) that were found to be significantly differentially regulated in patients compared to controls. The MetaCore™ (GeneGo Inc., USA) bioinformatics software was used to find genes with correlation to heart disease, hypertrophy and transcriptional changes among the 493 gene transcripts. Relevant regulated gene transcripts were the up-regulation of *ACE2*, *JAK2*, and the down-regulation of *EGR1*, *FOS* and *MYH6* (Table 5). MetaCore™ was also used to generate a direct interaction network. The direct interaction network analysis resulted in a network with direct connection between 101 of the 493 (~20.5%) significant transcripts, the network was completed by adding CCAAT/enhancer-binding protein alpha (*C/EBPalpha*), *EGR1* and ATP-binding cassette, sub-family A (ABCA1) (Figure 1). All data are MIAME compliant and available through NCBIs Gene Expression Omnibus (GEO) (GEO Series accession n. GSE32453).

Quantitative real time polymerase chain reaction

The levels of *ACE2*, *APOA1 EGR1* and *MYH6* mRNA were measured using real time PCR (Table 6). Both the fold change and the P values showed higher significance in 3 cases (*MYH6*, *APOA1* and *ACE2*) than with the Illumina microarray beadchip. In one case (*EGR1*), the fold change showed a similar down-regulation while the P value was not significant. Further real time polymerase chain reaction (RT-PCR) analysis was not possible due to the limited size of biopsies/myectomies.

Table 3. Patient medications and other diseases.

ID	Other diseases	Medication
H1	Diabetes mellitus, arterial hypertension	Candesartan/ hydrochlorothiazide, metoprolol, simvastatin, metformin, insulin
H2	Diabetes mellitus, hypercholesterolemia, arterial hypertension	Metoprolol, simvastatin, ramipril, insulin
H3	Atrial fibrillation	Acetylsalicylic acid, metoprolol
H4	Arterial hypertension	Acetylsalicylic acid, metoprolol, losartan
H5	Hypercholesterolemia, angina pectoris	Metoprolol, acetylsalicylic acid, atorvastatin
H6	Diabetes mellitus, previous myocardial infarction	Metformin, quinapril, acetylsalicylic acid, simvastatin
H7	-	Metoprolol
H8	-	Bisoprolol, losartan

Multivariate data analysis

A PCA analysis was performed to clarify whether patient history (*e.g.* medication, other diseases, age or sex) affects the gene expression result in such a way that clusters of individuals can be traced back to factors other than the disease (Figure 2). The PCA analysis revealed a dispersed control group and three clusters in the HOCM patient group (Figure 2).

Discussion

We studied the transcriptional expression in the myocardium of patients with symptomatic HOCM. The results are compared with known gene expression responses in cardiac hypertrophy to generate a hypothesis explaining the mechanisms that drive and maintain the pathological growth, leading to a balanced chronic hypertrophy.

Due to limited access to samples (especially control tissue), myocardial gene expression data in human HCM is scarce, performed on very few patients in different stages of the disease, with somewhat diverging methodology and results. However, there have been some common findings such as increased expression of natriuretic peptide genes. Our whole genome expression study on 8 non-end stage HOCM patients provides additional information on this subject. Although lacking the support of longitudinal data, we present a hypothesis that when expressions of pro- and anti hypertrophic genes are in equilibrium, a steady state is achieved with no further development of hypertrophy.

In the characterization (network and biomarker analysis) of the 493 differentially expressed transcripts, 101 (20.5%) were connected with direct interactions indicating a common transcriptional regulation (Figure 1). The result also revealed significant regulation of two important biomarkers for cardiac hypertrophy: *MYH6* and *ANP*. This characterization validates microarray as an established method and indicates that the findings are associated to the hypertrophic process in the myocardium of the HOCM patients.

To evaluate the influence of patient history (*e.g.* age, sex, medication, other diseases) on the observed gene expression patterns in HOCM, a multivariate data analysis was per-

formed. The analysis showed that patient history can not be associated to the differentially expressed gene transcripts (Figure 2A and B, Tables 2 and 3), indicating that the differences seen are related to the disease. The fact that fractional shortening was normal in all patients suggests that systolic function was preserved and that the differential gene expression seen is due to the hypertrophy itself and not to systolic dysfunction.

The patients in the control group have a somewhat lower age range than the patients in the HOCM group (Tables 2 and 4); nevertheless, the PCA analysis revealed no clustering according to age, indicating that age is not a factor for separating the groups. The same can be concluded about the 3 HOCM patients with hypertension (Table 3), no clustering according to blood pressure could be found.

Data regarding the underlying pathophysiological mechanisms in HCM are diverging, but one of the current hypotheses suggests that the dysfunction of the sarcomere may lead to a rightward shift on the Frank Starling curve and thus increased wall stress. The adaptive response of the cardiomyocyte would be to initiate pro-hypertrophic signals, where two important, initial responses in a sequence may be the secretion of angiotensin II (AngII)[18] and endothelin 1-3 (*EDN1, EDN2* and *EDN3*)[18] After this initial response an increased expression of genes would be a second response. In this study, the *ACE* gene and the endothelin gene transcripts (*EDN1, EDN2* and *EDN3*) were not differentially expressed compared to controls, which indicates that the initial hypertrophic response is no longer present. Another early response at the transcriptional level is the activation of the immediate early genes (IEGs), which are known as early regulators of cell growth and differentiation activated in response to stimuli mediated by AngII and/or other mechanical factors.[19] IEGs are the first response to stimuli, activating the genome, before the onset of protein synthesis. Examples of known IEGs are, *EGR1*, *FOS* and *JUN*. In our study the IEGs were either down-regulated (*FOS* and *EGR1*) or not differentially regulated at all (*JUN* and *JUN*B), indicating a reduced need for IEGs in this stage of the hypertrophic process. An additional transcriptional regulator is histone deacetylase 5 (*HDAC5*), which is known to repress the expression of pro-hypertrophic genes, such as myocyte enhancer factor-2 (MEF2). *MEF2* was

Table 4. Control patient characteristics.

Sex	ID	Age (years)	Cause of death
Man	C1	46	CO intoxication
Man	C2	37	Head trauma
Woman	C3	31	Head trauma
Man	C4	29	Traffic accident
Man	C5	58	Head trauma

Table 5. Gene characteristics and results of gene expression.

Designation		Description	Illumina GEX P-value	Illumina GEX Fold change
ACE	↓	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	0.968	-1.09
ACE2	↑	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	0.0016	6.50
ACTA2	↑	Actin, alpha, cardiac muscle 1	0.739	1.07
ACTC1	↓	Actin, alpha 2, smooth muscle, aorta	0.907	-1.02
EDN1	↓	Endothelin 1	0.703	-1.18
EDN2	↓	Endothelin 2	0.648	-1.30
EDN3	↓	Endothelin 3	0.708	-51.19
EGR1	↓	Early growth response 1	0.057	-2.27
FOS	↓	v-*fos* FBJ murine osteosarcoma viral oncogene homolog	0.0007	-9.57
HDAC5	↑	Histone deacetylase 5	0.008	2.52
JAK2	↑	Janus kinase 2 (a protein tyrosine kinase)	0.00001	2.85
JUN	↓	*Jun* oncogene	0.216	-1.34
JUNB	↓	*Jun* B proto-oncogene	0.892	-1.26
MYH6	↓	Myosin, heavy chain 6, cardiac muscle, alpha	0.000005	-3.51
MYH7	↓	Myosin, heavy chain 7, cardiac muscle, beta	0.86	-1.03
NPPA	↑	Natriuretic peptide precursor A	0.029	6.28
NPPB	↑	Natriuretic peptide precursor B	0.340	2.30

↑ and ↓ indicates change in gene expression in patients compared to controls. GEX, gene expression.

Table 6. Gene characteristics and results of real time polymerase chain reaction analysis in patients *vs.* controls.

Designation	Description	Accession number	Applied biosystems assay number	P-value	Fold change
ACE2	Angiotensin I converting enzyme 2	NM_021804.2	Hs01085333_m1	0.005	5.42
APOA1	Apolipoprotein A-I	NM_000039.1	Hs00163641_m1	0.048	2.84
EGR1	Early growth response 1	NM_001964.2	Hs00152928_m1	0.202	-2.18
MYH6	Myosin, heavy chain 6, cardiac muscle, alpha	NM_002471.2	Hs00411908_m1	0.018	-4.07

not differentially regulated).[20] In the study, *HDAC5* was up-regulated indicating activation of an anti-hypertrophic program. These results show that the early and instant hypertrophic response is not present in the HOCM patients, suggesting that the acute response is attenuated and an anti-hypertrophic pattern of the transcriptional regulation is detected. This is in agreement with the fact that the disease is at a significant stage and required surgical intervention (myectomy).

At birth, there is a switch from the fetal gene program which involves the regulation of a number of different genes,[7,21-23] including MYH6, skeletal -actin (*ACTA1*) and natriuretic peptide precursor A (*NPPA*). Part of the adaptation to increased workload is the development of hypertrophy and reinduction of the fetal gene program, including a relative increase of MYH7 compared to *MYH6*. We found *MYH6* to be significantly down-regulated in the HOCM patients (with preserved systolic function). Similar regulations have also been shown in studies of cardiac failure.[22-23] *MYH6* is regulated, amongst other, by the transcription factor *EGR1*[24] which was transcriptionally down-regulated in the present study, which could be a cause for the low expression of *MYH6*. Another component of the fetal gene program is the increased expression of *NPPA*, commonly regarded as driven by the RAAS activation. In our data, *NPPA* was significantly up-regulated and natriuretic peptide precursor B (*NPPB*) was non-significantly up-regulated to the same level with corresponding increases in serum levels of NT pro BNP (Table 2). This up-regulation of *NPPA* is seen despite a possible counter acting RAAS blockade by *ACE*-inhibitors or angiotensin II type 1 receptor blockade (ARB) in 5 of the HOCM patients. Another regulation of the fetal gene program, that is normally seen is that from cardiac (ACTC1) to skeletal (*ACTA1*) α-actin.[25] In our patients, this switch was not detected. Together, these results suggest that the fetal gene program is still partially activated even in the chronic state HOCM.

Several signalling pathways have been identified to contribute to the increased gene expression and protein synthesis in cardiac hypertrophy. Examples of such pathways are the JAK-STAT pathway, the Ca^{2+}/calmodulin (CaM)-dependent calcineurin pathway and the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway.[21] In this study, the only differentially regulated signalling pathway detected was the JAK-STAT pathway (where *JAK2* was up-regulated). *JAK2*, previously shown to have a parallel activation to the onset of cardiac hypertrophy, has an important role in mediating signals from the sarcolemma cytokine gp130 receptor[26] and angiotensin receptor AT_1[27] to the nucleus for transcriptional regulation. The results suggest that maintenance of hypertrophy seen in our patients might well be preserved mainly via the JAK-STAT pathway.

The RAAS is known as a regulator of cardiac growth. Ang II is a pro-hypertrophic octapeptide formed by conversion of the decapeptide angiotensin I (Ang I) by angiotensin-converting enzyme (*ACE*). Angiotensin 1-7 (Ang 1-7) is a peptide that exerts actions opposite to those of Ang II and is formed by conversion of Ang I by *ACE2*.[28] An increased *ACE*-activity may contribute to the harmful effects of RAAS in cardiac diseases. The purpose of an activated local RAAS might be to assist in the develop-

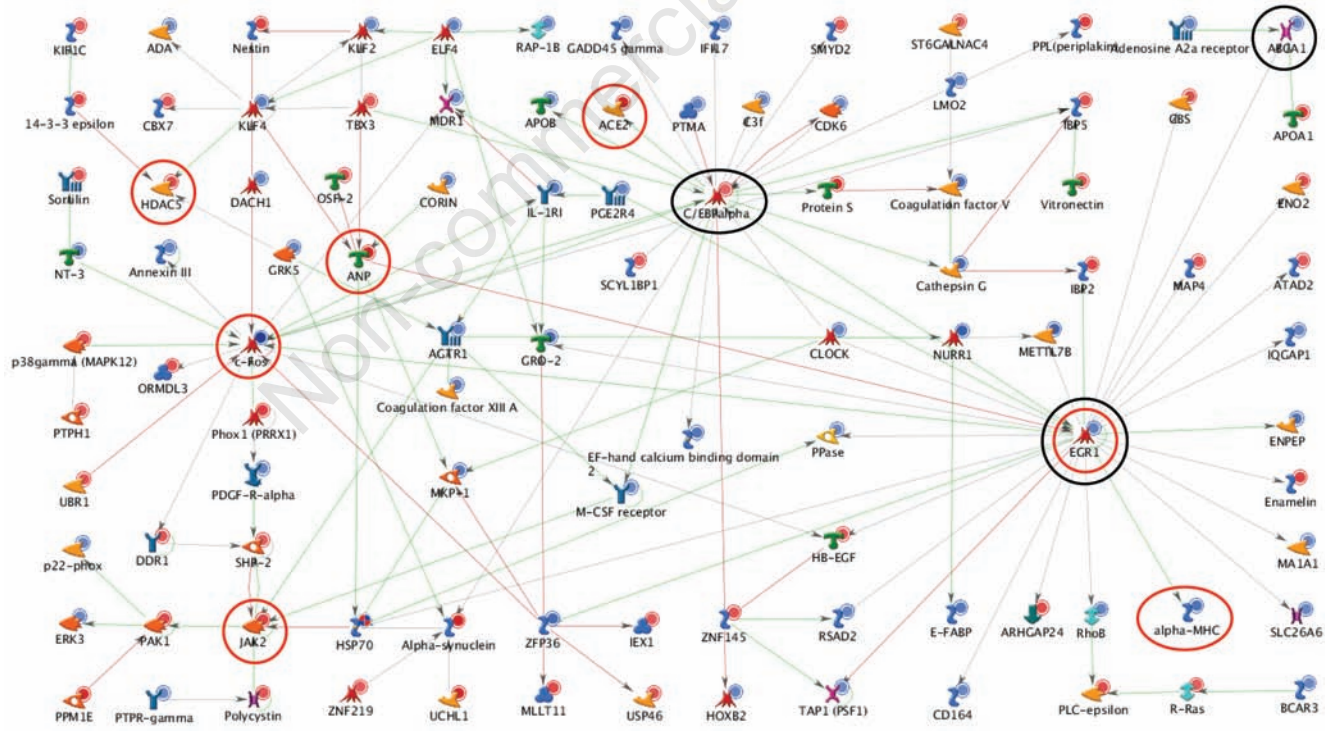

Figure 1. MetaCore™ direct interaction network analysis. 101 of the 493 (~20.5%) differentially expressed transcripts could be connected by direct interaction in the network analysis. Encircled with red are important genes and transcription factors, further discussed in the discussion. Encircled with black is not present in the 493 transcripts. Green arrows indicate activation and red arrows inhibition. The symbols are as follows: transcriptional up-regulation; transcriptional down-regulation; enzyme; kinase; protease; phosphatase; protein; generic binding protein; transcription factor; GTPase; G-protein; G-protein adaptor; receptor; receptor ligand; channel; transporter.

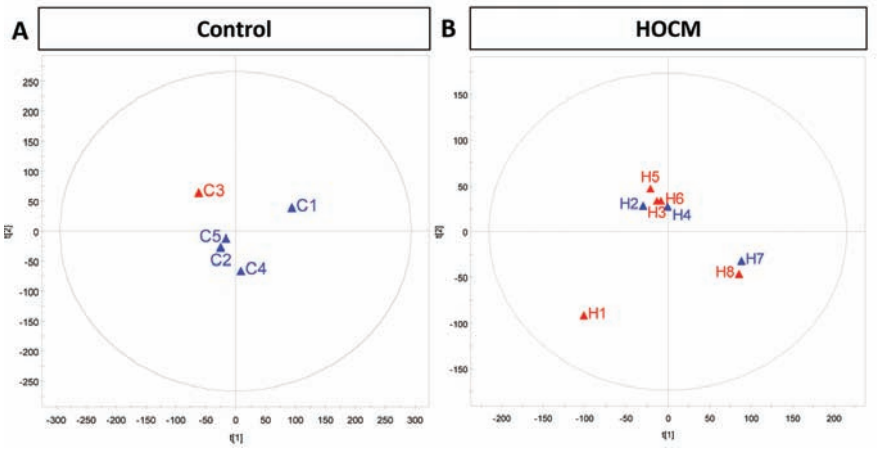

Figure 2. The PCA analysis revealed no groupings in the control group (A, control patient history can be found in Table 4) and three clusters in the hypertrophic obstructive cardiomyopathy (HOCM) patient group (B, HOCM patient history can be found in Table 2 and 3). Females are shown in red and males in blue.

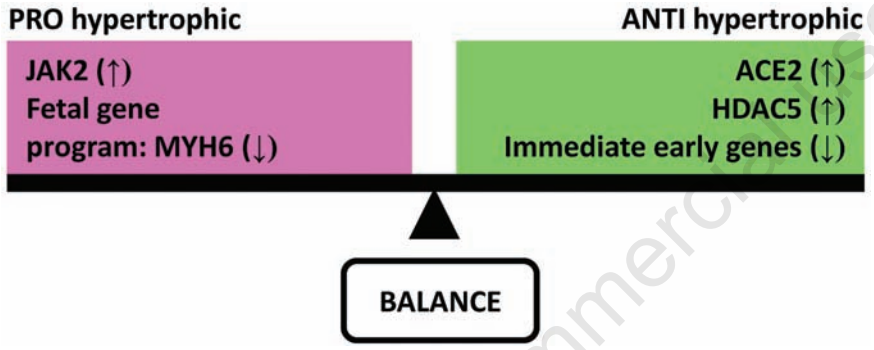

Figure 3. The balance of pro (pink box) and anti (green box) hypertrophic genes, pathways and mechanisms. Pro hypertrophic mechanisms are highlighted by the transcriptional down-regulation of *MYH6*, indicating that the switch to the fetal gene program is maintained, and the transcriptional up-regulation of *JAK2* in the JAK-STAT pathway. The anti hypertrophic mechanisms were seen as a transcriptional down-regulation of the IEGs, *FOS* and *EGR1*, and a transcriptional up-regulation of *ACE2* and *HDAC5*. ↑ and ↓ indicates change in gene expression in patients compared to controls

ment of a compensatory hypertrophy needed to adjust to the acute dysfunction of the heart. It seems that the observed regulation of the corresponding genes, with up-regulation of *ACE2* and no differential regulation of *ACE*, reflects an anti-hypertrophic response in the chronic state hypertrophy that is maintaining a balance between pro- and anti-hypertrophic effects/forces.[29-30] The observed anti-hypertrophic *ACE/ACE2* balance does not support the use of *ACE*-inhibitors as left ventricular (LV) remodelling treatment in HCM. This is in line with current therapy guidelines for HCM, where *ACE*-inhibitors are only recommended in a minority of the patients for treatment of end-stage heart failure[31]

Staging of the hypertrophy as a selection tool for treatment may be of clinical importance, as indicated by a recent experimental study by Seidman *et al.* They reported that losartan seemed to be of value in delaying the hypertrophic process in the early stages, but was without effect in reversing an already manifest cardiac hypertrophy.[32] In children with HCM, progression of LVH can be attenuated by high doses of beta blockers which has so far not been shown in adults. Such studies may result in future combination therapies to induce regression of the hypertrophy. Although myocardial biopsy is not a routine procedure, such gene expression data could be of value to determine the stage of the disease.

In summary, this is a whole genome expression study comparing myectomies from non-end stage HOCM patients and controls. The data show evidence for both pro- and anti-hypertrophic transcriptional regulations, which suggests the possibility of adjusting the pharmacological treatment according to the stage of hypertrophy. An interesting aspect is the simultaneous up-regulation of the *ACE2* gene and the *ANP* and BNP genes (*NPPA* and *NPPB*). *ANP* and BNP have diuretic and anti hypertrophic effects while *ACE2* has a repressive effect on RAAS[33-34] This can be interpreted as an *auto medication* of the hypertrophic heart in the chronic disease state, contributing to an endogenous protection system, neither growing nor suppressing the already hypertrophic myocardium (Figure 3).

Limitations

Differences between the two methods, Illumina microarray and real time PCR, can be explained by the fact that the handling of the RNA before hybridization is more extensive compared to the single RT-PCR step before the real time PCR. This could possibly cause more sample variation in the microarray analysis. Another limiting factor for this study was that the size of the biopsies/myectomies only allowed for the Illumina micro array and four real time PCR runs.

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