Renal Failure is Associated with Driving of Gene Expression towards Cardiac Hypertrophy and Reduced Mitochondrial Activity

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Abstract

Background: The clinical concomitant presentation of cardiac and renal failure (RF) has been referred to as the “cardiorenal syndrome” (CRS), whereby acute or chronic dysfunction of one organ induces acute or chronic dysfunction of the other. To improve our understanding of the cellular and molecular mechanisms underlying the development and diversification of CRS we established a model for CRS and studied the unique gene expression patterns associated with this syndrome.

Methods and Results: We have utilized a rat model in which an acute myocardial infarction (AMI) was induced on the background of subtotal nephrectomy. Changes in cardiac functions and gene expression were analyzed as detailed below. The data demonstrate that chronic RF (CRF) enhances fibrosis of the cardiac tissue. The gene chip array data detect overexpression of genes associated with hypertrophy as well as to reduced mitochondrial activity in the heart. The gene chip data also indicate that chymase inhibitors and coenzyme Q10 may be beneficial in the management of RF- induced cardiac hypertrophy. We further show that in line with the upregulation of the Ngal gene, LCN2, upon acute MI and CRS, Ngal protein levels significantly increase five days post infarction and remain stably elevated when the cardiac disease progresses to CRS.

Conclusions: Acute cardiac injury in the setting of CRF is associated with marked histological and gene expression changes in the heart. Based on our data, predictive biomarkers for the development of RF- induced cardiac hypertrophy as well as novel therapeutic directions may emerge.

Keywords: Cardiorenal syndrome (CRS); Chronic renal failure (CRF); Cardiac hypertrophy

Introduction

Concomitant cardiac and renal dysfunction, termed cardiorenal syndrome (CRS) where acute or chronic impairment of one organ aggravates dysfunction of the other has been thoroughly described [1]. Five types of clinically relevant CRS were described according to the chronicity or abruptness of the development of the syndrome [2] and are all associated with serious outcomes. Impaired renal function, even when mild, represents a major risk factor for cardiovascular disease and significantly affects the long term outcome of the patients [3]. In acute myocardial infarction (AMI) leading to heart failure (HF), a preexisting impaired renal function represents an adverse prognostic indicator for deterioration of the cardiac function. Multiple pathophysiologic mechanisms have been implicated in this crosstalk between organs and the associated progression of the disease both in the kidney and in the heart [1,2]. ACE inhibitors and angiotensin receptor blockers have protective effects on the heart and the kidney when function is impaired. Other approved treatment modalities include beta blockers and the implantation of CRT to improve cardiac synchronization and function.

Van Dokkum et al. [4] and his colleagues established a model in rats where renal dysfunction is induced by unilateral nephrectomy and cardiac dysfunction by coronary artery ligation. In another study, in which MI was induced one week post subtotal nephrectomy (STN), the authors have demonstrated protective role for ACE inhibitors or ACE/NEP (neutral endopeptidase) inhibitors in reducing renal damage [5]. In the same model the group has recently shown that renal failure causes excessive shortening of cardiac telomeres after MI suggesting that genetic modification may underlie the mutual interaction of kidney and heart dysfunction [6].

Numerous groups as well as our laboratory have explored new approaches including enhancement of cardioprotective and regenerative properties of the heart. These two modalities were successfully integrated through genetic modification of stem cells to ameliorate myocyte regeneration as well as angiogenic capacities [7-8]. Additional research is required to elucidate the processes that underlie cardiac and renal dysfunction in order to explore potential treatment modalities.

In the current study we sought to assess alterations in gene expression which may contribute to cardiac dysfunction upon CRF and CRS. Deep understanding of the underlying mechanisms may deliver rational interventions (pharmacological or genetic) which may improve clinical treatment of CRS patients, and improve their prognosis.

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Materials and Methods

Rat model for CRS

Experimental protocol was approved by the IRB committee for animal experimentation at the Tel Aviv Medical Center and conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. The model was based on a previously described model with several changes [5]. Briefly, male Lewis rats underwent subtotal nephrectomy (STN) in two subsequent surgeries: 2/3 of the left kidney was removed initially, followed by removal of the right kidney one week later. Four weeks afterwards the animals were orally intubated and artificially ventilated with a respirator (Harvard apparatus). MI was induced by ligation of the left anterior descending (LAD) coronary artery after chest opening. Prior to the surgical apparatus). MI was induced by ligation of the left anterior descending (LAD) coronary artery after chest opening. Prior to the surgical procedures animals were anesthetized with a mixture of ketamine (50 mg/Kg) and xylazine (10 mg/ml) by an intraperitoneal injection. The animals were terminated on the following day by CO₂ inhalation according to the IRB guidelines. The analyses for the MI and CRS groups were performed on animals in which the echocardiography study confirmed fractional shortening (FS) ranging from 15-28%, which is associated with moderate to severe left ventricular dysfunction (LVD). The experimental design included 4 arms: sham-operated (where the abdomen was cut-open twice in parallel to the time points of the two subsequent STN surgeries and four weeks later the chest was opened and closed in parallel to MI induction in the relevant animals), STN, MI, and STN + MI. For the assessment of gene expression alteration, animals were sacrificed 5 days only after MI induction in order to monitor genes within the acute phase which takes place after AMI (in this experiment the MI was confirmed by visualization only). The time frame for each experimental group is outlined in Figure 1A and B. We started the experiment with n = 15/ arm and after the 3 phase surgeries 8-10 animals survived in each experimental group.

Assessment of cardiac function

Thirty days post LAD ligation, animals were anesthetized with ketamine (40 mg/Kg) and xylazine (8 mg/Kg) and transthoracic echocardiography was performed by use of an Acuson XP-10 cardiac ultrasound machine with a 15-MHz transducer, which has 128 imaging elements configured in a phased-array format in order to assess cardiac function.

Laboratory of RF

Renal failure was confirmed by creatinine (Cr) clearance test (CCT) and sera BUN levels. CCT was calculated using the following equation: [Cr (urine)/Cr (serum)*urine volume]/1440. In addition, urine Ngal levels were determined in all experimental groups using an ELISA kit according to the manufacturer’s instructions (Bioporte Diagnostics, Denmark).

Histological assessment of cardiac and renal damage

The left ventricles (LV) were fixed with 4% paraformaldehyde and sliced into transverse sections and paraffinized. The slices were sectioned (5 μm) and stained with Masson’s trichrome. Photos were taken by Nicon camera using the NIS-elements 3.0 software. All sections were evaluated by a pathologist, blinded to the protocol design.

RNA isolation, amplification, labeling and hybridization to DNA chip arrays

RNA from the cardiac tissue of 4 research arms: sham, STN, MI (day 5 after LAD ligation) and STN+ MI were extracted by phenol/chloroform (Biological industries) and tested for purity by nanodrop and agarose gel electrophoresis. Four samples derived from each one of the 4 arms were divided to two pools of two RNA samples so that the reactions were started with two pools of two RNA samples in each group (8 samples total). RNA samples were reverse transcribed followed by amplification and hybridization steps. For details see Supplementary Methods 1 in “Supplemental file”.

Quantitative Real-Time (RT) PCR

Total RNA was extracted from the harvested hearts by phenol/chloroform/isoamyl alcohol (Biological Industries, Israel). For gene expression studies, 1 μg RNA was transcribed using Verso™ RT-PCR Kits (Abgene, USA). For miRNA RT analysis, 25ng of RNA were transcribed using universal LNA™ cDNA synthesis kit (Exiqon, Denmark). A quantitative PCR was performed with the Syber Green PCR kit (Invitrogen, Israel). Primers used for real-time analysis are listed in Supplemental Methods 2 in “Supplemental file”.

Statistical analysis: Differences in mean values between groups were compared by one way ANOVA followed by Scheffe’s post hoc test, or by two-tailed student’s t-test unless otherwise specified. In all tests, P < 0.05 was considered statistically significant.

Results

Cardiac echocardiography and histology point out to CRF-induced cardiac hypertrophy

Following a 9-week experimental period as outlined in Figure 1A the animals were terminated. Prior to termination we calculated the CCT values which, as expected, were significantly lower following

|          | Sham | STN | LVD | STN+ LVD |
|----------|------|-----|-----|----------|
| Weeks    | 0    | 1   | 5   | 9        |
| STN/sham |      |     |     |          |
| MI/sham  |      |     |     |          |
| Echocardiography&Autopsy | | | | |
| (2 steps) | | | | |

|          | Sham | STN | LVD | STN+ LVD |
|----------|------|-----|-----|----------|
| Weeks    | 0    | 1   | 5   | 5W+5d    |

Figure 1: Experimental protocol-time course.
STN, compared to controls (data not shown). An echocardiography scan confirmed a moderate to severe LVD in animals which underwent LAD ligation (FS ranging from -15-28%). In the CRF group, however, cardiac function was not affected and the fractional shortening values which were similar to those of the sham-operated controls (FS>-40, data not shown), suggesting that cardiac changes occurring upon kidney injury are not detrimental to induce LVD.

Upon termination, the hearts and the remaining kidney stumps were collected. Representative photos show the collapsed and enlarged left ventricle in CRS animals (left, STN+MI) compared to sham (right) (Figure 2A), and a significantly hypertrophic kidney stump, compared to sham-operated control animals (Figure 2B). A representative stain of the LV section performed four weeks following acute MI is given in Figure 2C, demonstrating a massive fibrotic area at the infracted zone as expected. Notably, the histological examination using Masson’s trichrome staining revealed interstitial fibrosis in 4 out of 5 LV sections of STN animals (a representative photo is given Figure 2D) with no similar cardiac fibrosis in the sham-operated animals (not shown). In line with the histological data, the heart weight per body weight ratio was higher upon all experimental groups compared to sham (heart/body of sham =0.37 ± 0.009) (Figure 2E). The weight ratios compared to sham were significantly elevated upon STN+AMI (5 days post MI) (heart/body= 0.57 ± 0.03, p=0.0001) and upon CRS (0.49 ± 0.03, p=0.01. Similarly, the weight ratio of LVD (4 weeks post MI) and the CRF groups were higher compared to sham with a trend towards statistical significance (heart/body=0.47 ± 0.02, p=0.057 and heart/body=0.45 ± 0.03, p=0.13 in the LVD and STN groups, respectively). The data, thus, point out that CRF per se may lead to cardiac hypertrophy.

Alterations in the cardiac gene expression patterns are associated with cardiac hypertrophy

In order to study which genes could account for the pathological cardiac fibrosis and hypertrophy, we applied a gene chip array analysis on RNA samples isolated from the whole cardiac tissue following: acute MI, CRF (STN), CRS (STN+acute MI) and sham operations. The time frame for this experimental design is outlined in Figure 1B. The complete differentially expressed gene lists are given in Supplemental file Table 1. Interestingly, Venn diagram, crossing between the 3 differentially expressed gene-lists compared to sham (MI vs Sham; ii. STN vs Sham; iii. STN+MI vs Sham) according to Panther database (http://www.pantherdb.org/).
33 common genes. No further genes were common to MI and STN (lists compared to sham). On the other hand, 568 genes (approximately 70% of the genes) were common to MI and STN+M1 (Figure 3A). Our analysis points out to different biological cascades initiated in the cardiac tissue upon MI or STN, while the STN+M1 and MI genelists harbor similar biological networks. A list of genes which may be most relevant to cardiac structure and function was made for each treatment (Tables 1, 2 and 3 for MI, STN and STN+M1 versus sham, respectively) based on best differential expressed genes and affiliated KEGG annotations which relate to cardiac structure or function. All further analyses described below were based on these 3 lists of selected genes.

Most of the genes modified upon MI or STN+M1 encode proteins acting at the extracellular environment (Figure 3Bi and 3Bii), while the vast majority of the genes modified upon CRF encode proteins located at the intracellular compartment (Figure 3Biii).

Next, the main molecular pathways modified upon STN, MI or the combined condition STN+M1, were analyzed using five independent gene ontology web-tools. These are shown in Supplemental Table 2. The up-regulated genes modified upon acute MI are related to the following biological pathways: ECM proteolysis, cell adhesion, complement cascades, angiogenesis, inflammation and apoptosis (See differentially expressed gene clustering Figure 4A, Table 1 and Supplementary Table 2 in “Supplemental file”).

Functional annotation of the genes whose expression was significantly changed in the STN gene-list compared to sham revealed two major gene clusters (Figure 4B and 5A). The other major gene cluster, was dramatically downregulated upon STN (blue), and includes genes which are mainly affiliated with proteolysis and proteosome biosynthesis, including the proteosomal genes psma1, psma3, psma4 and psmd6 (Figure 4B and Table 2). Psma3 downregulation was significantly validated by RT analysis (Figure 5B). Three additional genes or gene families belonging to this cluster are (Figure 3B, blue, and Table 1): 1. Rab2a, Rab3ip, Rab10, Rab14 and Rab21. These RAS-related GT-Pases function in apoptotic cell removal by enhancing phagosome maturation [10]. The downregulation of Rab14 was confirmed by RT analysis (Figure 5B). 2. A set of several rat mast cell proteases, which are also known as rat chymases. Out of these, it is worthwhile mentioning MCPT2 which was downregulated by fold-change (FC) of 4.4 compared to sham (p<0.07) and confirmed by real-time analysis (Figure 5B). 3. Complement factor 3 (C3) which was downregulated upon STN (significantly confirmed in RT analysis) (Figure 5B), while upon MI or STN+MI, C3 expression was significantly upregulated (Supplemental Table 1). Taken together, the gene chip analysis suggests an increased synthesis of ribosomal proteins which is accompanied by a marked decrease in intracellular proteolytic activities. These processes may culminate in cardiac hypertrophy which takes place upon CRF.

| Gene ontology                                      | Gene full name/Function | Gene symbol (fold-change and p-value) |
|---------------------------------------------------|-------------------------|----------------------------------------|
| ECM proteolytic activity, Collagen formation & foci adhesion | collagen, procollagen C-endopeptidase enhancer, Lysis oxidase, collagen triple helix repeat containing 1, fibrillin, Riboconectin, versican, hyaluronic acid (HA) receptor, Metallloproteinase, EGF containing fibulin-like extracellular matrix protein 2, elastin, spodin1, extracellular matrix protein, lumican, fibroblast growth factor 5, Tissue growth factor, peristinostoblast specific factor | Col1a1 (5.36, 0.0001), Col5a1 (3.65, 0.0006), Col5a2 (4.84, 0.0004), Col4a1 (3.61, 0.01), Col6a3 (3.64, 0.01), Col1a2 (4.20, 0.0003), Col1a3 (4.08, 0.0006), Polos (4.05, 0.003), COL1A1 (3.27, 0.0037), CHCRC1 (6.17, 0.0005), Fnn1 (4.45, 0.0002), Fn1 (4.83, 0.0005), Vcan (3.63, 0.0001), CD44 (1.83, 0.0031), MMP14 (3.82, 0.0012), MMP19 (2.19, 0.0011), MMP23 (2.38, 0.0022), Timp1 (7.40, 0.0048), Eln (3.36, 0.0031), Eln (3.21, 0.0016), Spon1 (3.37, 0.0007), Lum (2.57, 0.0179), FGF9 (-1.61, 0.0414), CTGF (3.07, 0.0015), TGFb1 (1.50, 0.0315), Postn (19.44, 0.00001) |
| Complement Cascades & Immune response              | Complement factor, Integrin alpha, leukocyte immunoglobulin-like receptor, Fc fragment of IgG, colony stimulating factor 2 receptor, beta | C3ar1 (2.75, 0.0028), C3 (4.13, 0.0016), C6 (2.99, 0.0112), C1r (2.69, 0.0193), C2 (2.72, 0.00410), Igpm (3.80, 0.001), Lirrb4 (3.20, 0.0009), Fcg1 (2.33, 0.0029), Celfrb (3.33, 0.0124) |
| Blood vessel formation & angiogenesis              | tubulin, beta 6, Heme oxidase, Cys-rich angiogenic inducer 61, alanyl aminopeptidase | Tubb6 (3.29, 0.0776), Hmox1 (6.99, 0.0001), Cy6r1 (3.30, 0.0283), Arpp35 (4.05, 0.0003) |
| Muscle contraction & Car-sal signal                | tropomyosin 1, regulates ca-dep interaction of actin- myosin, Myoferlin, Myoerin | Tm2 (3.60, 0.0035), MyoF (2.26, 0.0059), Myo1f (2.80, 0.0707) |
| Lipid                                             | Phospholipase A2         | Plaq2a (2.53, 0.0384), Plaq2a (1.77, 0.0775) |
| Cell cycle arrest & apoptosis                      | FBX osteosarcoma viral oncogene homolog, pleomorphic adenoma gene-like 1, lipocalin 2, lysozyme | fox (1.77, 0.0178), Plag1 (3.49, 0.038), Lcn2 (4.14, 0.029), Lyc2 (2.74, 0.0338) |
| Protein trafficking & phagocytosis                 | RAS oncogene family, lysosomal/endosomal-associated membrane glycoprotein | Rab13 (1.90, 0.011), Rab32 (2.07, 0.0031), CD68 (3.69, 0.0003) |
| Metabolism                                         | glutathione peroxidase 7, pyruvate dehydrogenase kinase, isozyme 2, hemochromatosis type 2, heat shock 70kDa protein 4-like, protein folding | Gpx7 (3.34, 0.0174), Pdk2 (-1.97, 0.0267), Hfe2 (-2.14, 0.026), Hsp44 (-1.67, 0.0365) |
| Mitochondrial activity                             | cytochrome b-245, NADH dehydrogenase (ubiquinone), GipE-like 1, mitochondrial | Cybb (3.12, 0.0023), Ndufs4 (-2.26, 0.0157), Gpelp1 (-1.69, 0.0274) |
| Fatty acid & cholesterol catabolism                | peroxisomal membrane protein 2, apolipoprotein E | Pem2 (-1.77, 0.0347), Apoe1 (1.66, 0.0054) |
| Glucose transport                                  | HtrA serine peptidase may regulate insulin-likeGfG availability, insulin | Htra1 (3.36, 0.0244), Ins1 (-1.67, 0.0441) |

Table 1: A list of genes whose expression is modified compared to sham upon MI only [FC (>1.5) or FC (<1.5), p<0.05] according to the gene chip array. Genes are affiliated with a specific KEGG pathway related to the cardiac structure or function. Only genes demonstrating best differential expression in the MI group relative to sham, are listed.
neutrophil gelatinase-associated lipocalin (Ngal) gene, LCN2 known significantly modified upon MI as well as CRF+MI, was the urine collagen degradation leading to LV dilation and ultimately heart documented in Figure 5C and Table 3. MMP12 elevation may mediate was noted in STN+MI both in the gene array and the RT analysis as In addition, a significant upregulation in the metalloproteinase MMP12 Figure 5C), providing an additional evidence for the potential activation Furthermore, a significant induction in Notch3 was observed (though downregulation compared to sham upon STN (-3.7, p< 0.001), MI RT analysis for a representative gene, Coq7, confirmed a significant the failing heart. The decrease in the ubiquinone expression seems to be additive compared CRF or MI alone (Table 1 'Supplemental'). An RT analysis for Coq7, confirmed a significant downregulation compared to sham upon STN (-3.7, p< 0.001), MI (-2.5, p= 0.001) and to a greater extent upon STN+MI (-10.0, p<0.001). Furthermore, a significant induction in Notch3 was observed (though an RT analysis demonstrated a modest non-significant upregulation, Figure 5C), providing an additional evidence for the potential activation of Notch signaling in cardiomyocytes during post- MI remodeling [11]. In addition, a significant upregulation in the metalloproteinase MMP12 was noted in STN+MI both in the gene array and the RT analysis as documented in Figure 5C and Table 3. MMP12 elevation may mediate collagen degradation leading to LV dilation and ultimately heart failure, as previously demonstrated for other MMPs [12].

On an interesting note, one of the genes whose expression was significantly modified upon MI as well as CRF+MI, was the urine neutrophil gelatinase-associated lipocalin (Ngal) gene, LCN2 known to be associated with an acute idiopathic kidney injury \(|+4.1, p=0.003\) and +3.0, \(p=0.007\) upon MI and STN+MI, respectively). Supplemental Table 1]. Upon the chronic state of STN only, Ngal levels remain unchanged \((-1.4, p=0.17\) in line with previous studies documenting that Ngal levels drop down when the renal injury becomes a chronic one [9]. In view of these data we thought to assess whether urine Ngal levels are indeed elevated upon acute MI and STN+MI. To this end we have utilized an ELISA for Ngal in rats’ urine samples from groups corresponding to the ones used for the gene chip analysis: sham, STN (4 weeks post STN), MI (5 days post LAD ligation) and STN+MI. The data presented in Figure 5D represent mean values of 6 urine samples (4 weeks post STN), MI (5 days post LAD ligation) and STN+MI. The gross observations as well as the histological data and the gene chip array findings presented herein, strongly suggest that a major insult to the kidney enhances pathological changes in the heart, mainly
cardiac hypertrophy accompanied by interstitial fibrosis. Yet, the cardiac global systolic function remains unaffected.

We have also demonstrated that acute MI enhances a rapid moderate kidney injury as determined by Ngal levels five days post MI, both at the protein level (Ngal ELISA) and by the LCN2 gene chip data [13,14]. Using the gene chip array methodology, we have demonstrated that the vast majority of genes modified in the cardiac tissue upon CRF only- encode intracellular proteins, affiliated with cardiac hypertrophy. On the other hand the vast majority of genes modified upon MI or CRF+MI, are mainly translated to proteins affiliated with extracellular activity including matrix degradation, collagen deposition and focal adhesion.

It is already established that highly elevated urine Ngal readouts may indicate an acute kidney injury [15]. Several lines of evidence suggest that the detected Ngal upregulation in the mature kidney represents an intrinsic response of the kidney’s proximal tubule cells to ischemic injury, which may be induced by local release of cytokines from neutrophils trapped in the microcirculation early after ischemic injury [16]. We thus conclude that apart from being a reliable early marker for acute kidney injury, urine Ngal could serve as a laboratory marker for short-term renal injury following acute MI. Urine Ngal could thus serve as a useful marker especially in intensive care units where ST-elevated myocardial infarctions as well as cardiogenic shocks are of everyday practice.

The observed cardiac hypertrophy and interstitial fibrosis occurring upon CRF may probably stem from both elevated preload and afterload in RF due to hypervolemia and increased peripheral vascular resistance respectively. Indeed, preload, induced by hypervolemia, causes serial addition of sarcomers leading to lengthening of myofibers and eccentric hypertrophy, while increased afterload causes parallel addition of sarcomers, thickening of myofibers and concentric hypertrophy [17]. On the other hand, we should take into account when that infarction involves only or mainly the right ventricle, the left ventricular preload is actually reduced while the peripheral vascular resistance is increased in order to counter the systemic hypovolemia [18]. In this case we speculate that cardiac interstitial fibrosis would not be as high as in the LV MI model described here. An additional study has reported a significant increase in cardiomyocyte volume accompanied by a decrease of cardiomyocyte numbers per myocardial volume using a similar model for STN in rats [19]. Nevertheless, cardiac remodeling with interstitial fibrosis in patients with CRF is still poorly understood at the molecular level. Similarly, the uniqueness of genes and molecular pathways modified upon acute MI compared to CRF or combined disease is also poorly established. Yet, it is already recognized that cardiac hypertrophy, especially when diagnosed in the late stages of renal failure is one of the most important risk factors for acute MI, CHF and sudden cardiac death [20] as well as for renal deterioration requiring the use of dialysis [21]. In order to develop novel early biomarkers and potential pharmacologic approaches for left ventricular hypertrophy, it is necessary to recognize the genetic pathways leading to the dramatic changes in the tissue structure.

Amann et al. have previously presented gene chip array data, pointing to cardiac hypertrophy after STN as early as two weeks post surgery [22]. They have shown that genes encoding for collagens and proteoglycans are upregulated two weeks post surgery, and that 10

| Gene Ontology                        | Gene Function                          | Gene Symbols            |
|--------------------------------------|----------------------------------------|-------------------------|
| Differentiation                      | notch 3, may be involved in cardiomyocyte differentiation, presenilin enhancer 2 homolog, required for the Notch signaling pathway | Notch3 (3.11, 0.0345), Psen2 (-1.61, 0.0170) |
| Coenzyme Q biosynthesis              | cytochrome c oxidase, coenzyme, required for Ubiquinone (coenzyme Q) biosynthesis, coenzyme | Cox18 (-2.04, 0.0036), Cox3 (-1.77, 0.0129), Cox7 (-1.74, 0.0063), Cox2* (-1.60, 0.057), Cox6 (-1.67, 0.0226), Cox10a (-1.55, 0.013) |
| ECM proteolysis                      | matrix metalloproteinase 12, may have a role in glomerular injury, Disintegrin and metalloproteinase domain 12, ADAMTS-like 2 | MMP12 (3.95, 0.0443), Adam12 (2.39, 0.0083), Adam9 (1.66, 0.0073) |
| Cell migration                       | hyaluronan synthase                    | Has2 (2.03, 0.0337)    |
| Cell growth and proliferation        | latent transforming growth factor beta binding protein 2, transforming growth factor b, early growth response 1, transcription, cyclin-dependent kinase 1, cyclin L2, EGF-like-domain, NDRG family member 3, negative regulation of cell growth | Ltbp2 (3.61, 0.001), TGFβ1 (1.68, 0.0195), Egfr (2.57, 0.0050), CDC2 (1.65, 0.0207), Ccnl2 (2.03, 0.0085), Egf (1.87, 0.0327), Ndrk3 (-1.60, 0.0091) |
| Ca-binding                           | transient receptor potential cation channel, subfamily V, member 2, two pore segment channel 1, Ca-channel activity | Trpv2 (1.66, 0.0035), Tpcn2 (1.56, 0.0341) |
| Transcription factors                | nuclear transcription factor, nuclear receptor corepressor 2, transcriptional silencing, runt-related transcription factor | Nrk1 (1.69, 0.0159), Ncor2 (1.51, 0.0209), Runx1 (2.13, 0.0314) |
| Proteolysis                          | mast cell protease 1, mast cell protease 10, mast cell protease 8 | Mcpt1 (2.20, 0.0495), Mcpt10 (-1.87, 0.0221), Mcpt8 (-1.65, 0.0284) |
| Phagosome maturation                 | member RAS oncogene family            | Rab20 (2.09, 0.017), Rab27a (1.61, 0.003) |
| Inflammation                         | interleukin 33, interleukin 17, interleukin 10 | Il1b (1.51, 0.0412), Il1r1a (1.84, 0.0125), Il1r1a (1.73, 0.0203) |
| Macromolecule metabolism             | aldehyde dehydrogenase 3 family, alcohol dehydrogenase 1a | Aldh3a1 (-1.61, 0.0135), Aldh3a2 (-1.53, 0.0273), Aldh1 (-1.77, 0.0367) |
| Ribosome                             | ribosomal protein, exosome component 5, ribosome biogenesis | Rpl3 (1.88, 0.0254), Exosc5 (-1.57, 0.0481) |

Table 3: A list of differentially expressed genes (FC> (+1.5) or FC< (-1.5), p<0.05), revealed from STN+MI treatment versus sham according to the gene chip array (Exceptional p-values are marked in asterisk). Genes that are affiliated with KEGG pathways specific to the cardiac structure or function are presented. Only genes demonstrating best differential expression in the STN+MI group relative to sham are listed. The turquoise highlight refers to genes whose relative expression was also tested by RT PCR; the green highlight demonstrates statistical significance in real-time analysis.
weeks later (12 weeks post surgery), a striking upregulation in laminins and integrins is noted. At our time point for animal termination (4 weeks and 5 days post STN surgery) neither one of these genes was modified in the cardiac tissue of the STN group. However, to the best of our knowledge we are the first ones to report an increase in numerous ribosomal genes as well as a decrease in proteolytic pathways, including pathways which are phagosome and complement-dependent. This tissue remodeling may induce endoplasmic reticulum stress as a response to an increased demand for protein synthesis as previously suggested [23]. The modification in the expression of these genes as well as the marked downregulation in several chymase genes point out to enhanced cardiac hypertrophy upon CRF.

Consequently, the gene chip data point out to a new set of biomarkers in the cardiac tissue which could indicate cardiac hypertrophy resulting from the various insults: 1. Upon CKD: downregulation of chymase genes and the complement factor C3 may probably reflect an inhibition of cellular catalytic processes; an inhibition which is reversed upon acute MI, once removal of the necrotic cells becomes a must. 2. On acute MI: induction of several metalloproteinases could indicate early cardiac remodeling. It was previously suggested that MMPs could also serve as a prognostic marker in the circulation for cardiomyocyte injury and subsequent cardiac remodeling [12]. 3. In CRS- a marked downregulation in ubiquinon expression indicates that the cardiac metabolic function is severely damaged.

The ubiquinone which is also known as coenzyme Q is present in most eukaryotic cells, primarily in the mitochondria and serves...
as a critical component of the energy-generating electron transport pathways. Two additional studies have described a reduction in the expression of crucial mitochondrial components during the development of cardiac hypertrophy in rats or in human [24,25]. In line with these reports we demonstrate herein that these ubiquinone-forming genes are downregulated upon MI, upon STN only, and to a larger extent- upon CRS. The data may point to the potential therapeutic potential of Coq10 therapy for patients who are at high risk for developing cardiac hypertrophy including conditions of CKD, CHF and above all, upon CRS.

As for the cardiac chymase expression, a previous biochemical study demonstrated that 24% of Angiotensin II (Ang II)-forming capacity in rat cardiac sections could be attributed to chymase-3 which converts Ang I to Ang II by cleaving the Phe8-His9 bond of Ang I. This activity was found to be chymostatin-inhibitable. On the other hand, β-chymases, i.e. rat chymases 1 and 2 were shown to be angiotensinases because they readily cleave the Tyr4-Ile5 and the Phe8-His9 bonds in angiotensins, thus degrading Ang I to inactive fragments [26]. Our study demonstrated that 24% of Angiotensin II (Ang II)- forming genes are downregulated upon MI, upon STN only, and to a larger extent- upon CRS. The data may point to the potential therapeutic potential of Coq10 therapy for patients who are at increased risk for CRS complications including frequent hospitalizations and death.

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Figure 6: The potential molecular events leading to CRF and eventually to CRS.

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