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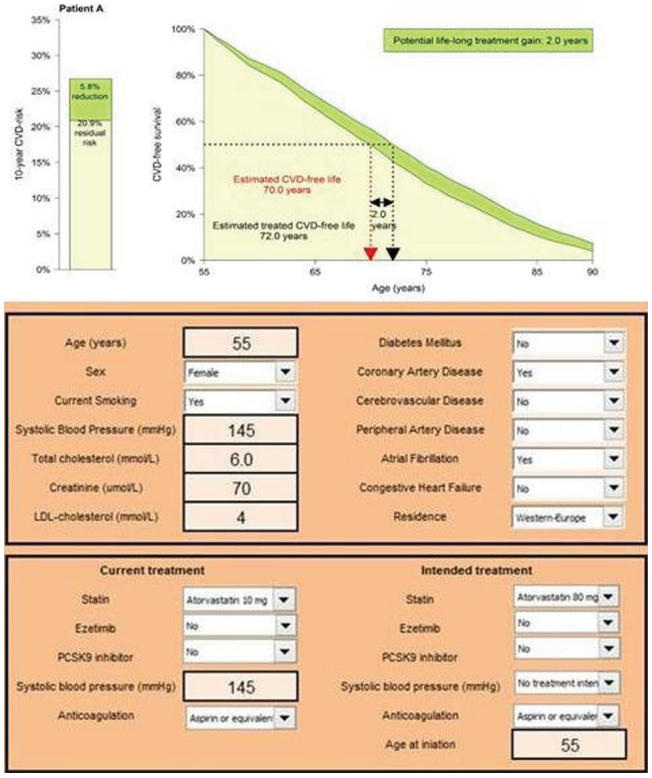
Background: Patients with established cardiovascular disease (CVD) are all at risk of recurrent cardiovascular events. Yet, preventive treatment effects differ between patients depending on their risk factor levels. Individual estimates of 10-year risk and potential number of years gained from preventive therapy could help to identify patients that benefit most from intensive lipid, blood pressure and antithrombotic treatment. Also, such personalized treatment effect estimates may facilitate doctor-patient communication, shared decision-making, and potentially motivate patients to adhere to therapy.
Purpose: To develop and geographically validate a decision-support tool for individualizing lipid, blood pressure and antithrombotic treatment in patients with coronary, cerebrovascular, and/or peripheral artery disease in Western-Europe and North-America based on individual estimates of 10-year CVD-risk reduction and years gained without recurrent cardiovascular events.
Methods: The prediction algorithm was developed in the REACH Western-Europe cohort (n=14,259, enrolment between 2003–2004, median follow-up 1.8 years) using pre-specified risk variables that would be readily available in clinical practice (figure). The algorithm consists of two complementary Fine and Gray competing risk adjusted models with left truncation (i.e. using age as the time-scale) and right censoring. Competing outcomes were: 1) cardiovascular events (myocardial infarction, ischemic stroke, cardiovascular death) and 2) non-cardiovascular death. Model performance was assessed using c-statistics (measure of model discrimination) and calibration (predicted versus observed survival). The international validation was performed using data from the REACH North-America cohort (n=19,170, enrolment between 2003–2004, median follow-up 1.8 years) and Dutch SMART cohort (n=6,959, enrolment between 1996–2014, median follow-up 6.5 years). Individualized preventive treatment effects for lipid, blood pressure and antithrombotic treatment were estimated by combining respective hazard ratios from randomized trials or meta-analyses.
Results: Predicted and observed survival showed good agreement in both external validation sets. The c-statistic was 0.68 in SMART (95% CI 0.67- 0.70), 0.67 (0.66–0.68) in REACH North-America. An example of how the decision-tool can be used to estimate an individual's CVD-free life-expectancy gain is shown in the figure.



Decision-support tool screenshot

Conclusion: The externally validated REACH-SMART model could be used for estimation of anticipated effects of lipid, blood pressure and antithrombotic treatment in individual patients with cardiovascular disease in Western-Europe and North-America. Clinicians should be aware of the discrepancy in anticipated benefit of treatment using 10-year risk versus life-expectancy, as these may result in different clinical decisions about the appropriate preventive strategy for the individual patient with cardiovascular disease.

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HEART FAILURE: NEW TARGETS FOR TREATMENT

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Angiotensin II-dependent activation of NADPH oxidase 4 contributes to muscle wasting in mice via downregulation of NF-E2-related factor 2

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Background: Muscle wasting is promoted by cardiovascular diseases such as chronic heart failure (CHF). Angiotensin II (Ang II), a key mediator of the renin–angiotensin system, plays an important role in the pathogenesis of CHF. We previously reported that Ang II-dependent NADPH oxidase activation is associated with muscle wasting in mice. In this study, we hypothesized that Ang II-dependent Nox4 isoform activation is associated with muscle wasting in mice via downregulation of nuclear factor erythroid 2-related factor 2 (Nrf2).
Methods: Twelve-week-old male Nox4 knockout (KO) mice and age-matched male wild-type (WT) mice were used in this study. Either saline (vehicle) or Ang II (1000 ng/kg/min) was infused into WT and Nox4 KO mice via subcutaneously implanted osmotic minipumps for four weeks. Experiments were performed in the following four groups: WT + vehicle, Nox4 KO + vehicle, WT + Ang II, and Nox4 KO + Ang II. Gastrocnemius muscle was removed from the lower limbs and used to analyze the total Nox activity, quantitative real-time PCR and western blot. To identify the function of Nox4 in skeletal muscles, we used mouse skeletal muscle-derived myocytes (C2C12) transfected by electroporation with Nox4 small interfering RNA (siRNA) or scrambled control siRNA. Once confluent, the cells were treated with or without Ang II ($10^{-}6$ M) and collected 24 h after treatment.
Results: Four weeks after Ang II infusion, the WT + Ang II mice showed increased total Nox activity, which was attenuated in the Nox4 KO + Ang II mice, in the skeletal muscles ($p<0.05$). Body mass, gastrocnemius muscle mass, and myocyte cross-sectional area were all significantly decreased in the WT + Ang II mice compared with the WT + vehicle mice (all, $p<0.05$). These changes were significantly attenuated in the Nox4 KO + Ang II mice (all, $p<0.05$). The levels of phosphorylated protein kinase B (Akt), a key molecule involved in protein synthesis, decreased in the WT + Ang II mice, whereas the protein expression levels of muscle RING finger-1 (MuRF-1) and muscle atrophy F-box (MAFbx)/Atrogin-1, key molecules in protein degradation, significantly increased in the WT + Ang II mice compared with the WT + vehicle mice. Moreover, Nrf2 mRNA and protein expression levels in the nuclear fraction significantly decreased in the WT + Ang II mice compared with the WT + vehicle mice. These effects were all significantly ameliorated in the Nox4 KO + Ang II mice (all, $p<0.05$). Nox4 knockdown by siRNA treatment in C2C12 cells significantly attenuated Ang II-induced downregulation of phosphorylated Akt and upregulation of MuRF-1 and MAFbx/Atrogin-1 ($p<0.05$).
Conclusion: Ang II-dependent Nox4 activation is associated with muscle wasting via downregulation of Nrf2 signaling, suggesting that the Nox4–Nrf2 axis plays an important role in the development of muscle wasting.

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Excessive mitochondrial reactive oxygen species emission from circulating blood cells is associated with severity of heart failure and exercise intolerance

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Background: Mitochondrial dysfunction and oxidative stress play a crucial role in the development of heart failure. However, the role of reactive oxygen species (ROS) originating from peripheral blood mononuclear cell (PBMC) mitochondria in chronic heart failure (HF) is unknown.
Purpose: To determine whether mitochondrial ROS emission from PBMCs is increased in symptomatic HF patients and whether enhanced mitochondrial oxidative stress in these cells is linked to HF severity and exercise intolerance.
Methods: We enrolled 36 patients with chronic HF (62±13 years, New York Heart Association [NYHA] functional class I-III) and the patients were divided into two groups; NYHA I-II group (n=17) and NYHA III group (n=19). To assess exercise capacity, we performed cardiopulmonary exercise testing with bicycle ergometer. We measured mitochondrial respiratory capacity and mitochondrial ROS emission in permeabilized PBMCs with the use of high-resolution respirometry and spectrofluorometry.
Results: Plasma levels of B-type natriuretic peptide (BNP), a biomarker for severity of HF, were significantly higher in NYHA III group than in NYHA I-II group (286.0±251.0 vs. 93.4±101.2 pg/mL, $P<0.01$). HF patients with NYHA III had a lowered peak oxygen uptake (VO2) compared to HF patients with NYHA I-II (14.4±4.1 vs. 19.7±4.5 mL/kg/min, $P<0.01$). Mitochondrial oxidative phosphorylation capacities with complex I, complex II, or complex I+II-linked substrates in PBMCs were comparable between groups, however, maximal capacity of mitochondrial electron transfer system with complex II-linked substrates in PBMCs

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was significantly reduced in NYHA III group in association with the lowered peak VO2. Notably, HF patients with NYHA III had a significantly higher mitochondrial ROS emission from PBMCs, and this was closely correlated with the increased plasma BNP levels and the lowered peak VO2, respectively.
Conclusions: Enhanced mitochondrial oxidative stress characterized by increased emission of mitochondrial ROS in PBMCs is associated with HF severity and exercise intolerance in patients with chronic HF.

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Positive cardiac inotropy by carvedilol via unique beta-arrestin2-dependent SERCA2a stimulation

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Background: Heart failure (HF) is the number one killer in the western world and β-blockers [β-adrenergic receptor (AR) antagonists] are part of the cornerstone pharmacotherapy for post-myocardial infarction (MI) chronic HF. We have recently shown that cardiac β1AR-activated βarrestin2, a G protein-coupled receptor (GPCR) adapter protein, promotes Sarco(endo)plasmic reticulum Ca^2+ATPase (SERCA)2a SUMO (small ubiquitin-like modifier)-ylation and activity, thereby directly increasing cardiac contractility. βarrestin2 also exerts anti-inflammatory and anti-apoptotic effects in the post-MI heart.
Purpose: Given that certain β-blockers, such as carvedilol and metoprolol, have been shown to activate βarrestins and SERCA2a in the heart, we sought to investigate, in the present study, the effects of these drugs (if any) on cardiac βarrestin2-dependent SERCA2a SUMOylation & activity.
Methods: We studied SERCA2a interaction with the βarrestins and its SUMOylation & activity in response to therapeutic doses of carvedilol or metoprolol in the cardiomyocyte cell line H9c2. We also measured contraction amplitude (cell shortening) of neonatal rat ventricular myocytes (NRVMs) transfected with βarrestin2 and treated with either drug.
Results: Carvedilol, but not metoprolol, acutely induces βarrestin2 (but not βarrestin1) interaction with SERCA2a in H9c2 cardiomyocytes, resulting in enhanced SERCA2a SUMOylation. However, this translates into enhanced SERCA2a activity only in the presence of the β2AR-selective inverse agonist ICI 118,551 (ICI), indicating an opposing effect of this βAR subtype on carvedilol-bound β1AR-stimulated, βarrestin2-dependent SERCA2a activation. In addition, the amplitude of spontaneous cell shortening of NRVMs transiently transfected with βarrestin2 is acutely enhanced by carvedilol, again in the presence of ICI only (122+9% of control, ICI only-treated NRVMs; $p<0.05$, n=10). In contrast, metoprolol had again no effect on NRVMs' shortening amplitude with or without ICI (98+6% of control, ICI only-treated NRVMs; $p<0.05$, n=10).
Conclusions: Carvedilol, but not metoprolol, stimulates βarrestin2-mediated SERCA2a SUMOylation and activity through the β1AR in cardiac myocytes. This translates into direct positive inotropic effects of this beta-blocker drug in the heart, which, however, might be masked by the opposing actions of the cardiac β2AR. Thus, contrary to the conventional wisdom that all β-blockers exert negative inotropy in the heart, complicating their use in chronic HF therapy, carvedilol may actually have direct positive inotropic actions in the myocardium. This makes carvedilol particularly useful for HF treatment and unique among the β-blocker class of drugs.
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Empagliflozin potently reduces sarcoplasmic Ca leak and increases Ca transient amplitude of human failing ventricular cardiomyocytes

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Background: The EMPA-REG OUTCOME trial showed a reduction in heart failure (HF) hospitalization and reduced mortality in a high CV risk population upon treatment with empagliflozin (empa). It is unclear if renal and/or cardiac effects are responsible for the clinical benefits. Increased activity of Ca/Calmodulin kinase II (CaMKII) is a hallmark of HF, leading to increased pro-arrhythmogenic sarcoplasmic (SR) Ca leak and depressed Ca transients.
Purpose: We investigated the effects of empa on isolated human failing ventricular cardiomyocytes (CM) and murine ventricular CM, specifically excitation-contraction coupling, SR Ca leak, and CaMKII-activity.
Methods/Results: Ventricular CM were isolated from explanted hearts of heart transplant recipients (end-stage HF) by established protocols and cultured in the presence of empa (1μmol/L) for 30min or 24h. Intracellular [Ca] was measured in Fluo-4 or FURA2-loaded CMs (10μmol/L, 15min) by confocal or epifluorescence microscopy, respectively. Interestingly, compared to vehicle, 24h exposure to empa potently reduced SR Ca leak as assessed by Ca spark frequency (CaSpF 1/100 μm^{-1} s^{-1}: empa 1.0±0.24 vs. vehicle 3.26±0.93, n=16 vs 11 cells, 4 patients, Mann-Whitney-test $p<0.05$). Importantly, this empa-dependent reduction in SR Ca leak was associated with a significantly increased Ca transient amplitude (0.5 Hz, ΔF/F0: empa 0.53±0.06 vs. vehicle 0.36±0.02, n=13 vs 12 cells, 3 patients, Mann-Whitney-test $p<0.05$). In contrast, acute, 30 min, exposure to empa did neither alter CaSpF (empa 1.83±0.3 vs. vehicle 1.55±0.27, n=20 vs 18 cells, 3 patients, p=N.S.) nor Ca transient amplitude (n=16 vs 17, 3 patients, p=N.S.). These results were confirmed in a murine model of HF by transverse aortic constriction (TAC). Compared to vehicle, 24h exposure to empa significantly reduced SR Ca leak and significantly increased Ca transient amplitude in ventricular CMs isolated 6 weeks after TAC (n=4 mice, n=28 cells each for Ca leak, n=17 vs n=13 cells for Ca transients). Consistent with Ca spark data, empa also significantly reduced diastolic [Ca] upon 24 h but not after 30 min exposure. Diastolic [Ca] is a major regulator for CaMKII activity. In accordance, CaMKII-activity (measured by highly specific CaMKII-HDAC4 association) was unaltered upon 30min empa-exposure, but potently reduced after 24h. Mean densitometric values for CaMKII-HDAC normalized to HDAC were 1.56±0.66 (empa) vs. 4.15±0.95 (vehicle, 10 mice, Mann-Whitney-test $p<0.05$).
Conclusions: We show for the first time that 24h (but not 30min) exposure of human or murine failing CM to empa significantly increased the diminished Ca transient amplitude in HF. The underlying mechanism may involve decreased CaMKII-dependent SR Ca leak. Our data may help understanding the clinical benefit of empagliflozin treatment.

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HECTD3 attenuates cardiac hypertrophy acting as an E3 ligase of SUMO2

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Background: Small ubiquitin-like modifiers (SUMO) are essential for numerous cellular processes including cell growth, proliferation, apoptosis, as well as protein trafficking. We recently reported SUMO2 as an inducer of cardiomyocyte hypertrophy via activation of calcineurin-NFAT signaling in vitro and in vivo.
Purpose: Utilizing a Yeast-two hybrid screen, HECTD3, an E3 ubiquitin ligase, was found to interact with SUMO2 via its "APC10" domain, a necessary domain for ubiquitination of HECTD3 substrates. We therefore hypothesized that SUMO2 is one of the bona fide cardiac substrates of HECTD3.
Methods and results: Adenovirus-mediated expression of HECTD3 in neonatal rat ventricular cardiomyocytes (NRVCMs) resulted in degradation of endogenous as well as overexpressed SUMO2. Proteasome inhibitor MG132 treatment inhibited HECTD3-mediated SUMO2 degradation, further substantiating the involvement of ubiquitin-proteasome system. HECTD3 impeded SUMO2-dependent prohypertrophic effects and activation of calcineurin-signaling in NRVCMs. Importantly, these in vitro findings were recapitulated in mice, where, AAV9-mediated overexpression of HECTD3 not only reduced cardiac SUMO2 levels, but significantly attenuated pathological hypertrophy induced by transverse aortic constriction or Angiotensin-II. Moreover, inverse correlation of SUMO2 and HECTD3 expression was observed in human patients suffering from hypertrophic cardiomyopathy (HCM). Mechanistically, we found that HECTD3 disrupts recently proposed SUMO2-calcineurin interaction, inhibiting nuclear localization of Calcineurin and thereby suppressing downstream signaling and consequent hypertrophy.
Conclusions: Taken together, these data show that ubiquitination of SUMO2 via HECTD3 regulates cardiomyocyte hypertrophy. To the best of our knowledge, this is also the first report showing regulation of a SUMO protein by an E3 ligase enzyme involved in ubiquitination. The present findings may therefore not only have an impact on the understanding of cardiac disease pathophysiology, but also on pathophysiology of other tissues as well.

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RNA methylation in cardiac hypertrophy and heart failure

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Introduction: During the last decade, knowledge of gene regulation beyond transcription factors has increased dramatically by studying epigenetic processes and modifications. Their deregulation was found to be critically involved in the pathogenesis of heart failure. Among these, the recently described N6-adenosine methylation (m6A) is the most prevalent modification found in many classes of RNA (mRNA, noncodingRNA (ncRNA), microRNA (miR)). Being both dynamic and reversible, it is a promising new mechanism of post-transcriptional gene regulation. The degree and pattern of mRNA methylation can affect their splicing, transport, storage, and/or decay, whereas methylation of ncRNAs might influence signal transduction directly.
Purpose: As the role of m6A-RNA methylation in the heart is poorly understood, we aimed to elucidate the state and influence of RNA methylation on cardiac hypertrophy and heart failure.

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