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Heart diseases

ROLE OF THE NOVEL CARDIOMYOKINES FGF21 AND METRNL ON CARDIAC DAMAGE INDUCED BY ALCOHOLISM AND ARTERIAL HYPERTENSION

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1. Summary of the project

Currently, alcohol and arterial hypertension (AHT) are well-identified risk factors for progressive heart damage, leading to a great impact on heart failure and a significant level of morbidity and mortality. Since alcohol misuse is not expected to be controlled and arterial hypertension will be highly prevalent in the general population, common strategies to avoid progressive heart damage should be developed. Heart damage induced by both AHT and alcohol misuse is highly relevant among cardiac diseases and it probably will not be decreased in the next decades because of the difficulty of controlling the primary population risk factors. There are common mechanistic processes leading to cardiac damage and heart failure in alcohol and AHT-induced cardiac damage. Common pathogenic pathways involving local cardiomyokine activation, mitochondrial disruption, oxidative damage, myocyte hypertrophy, fibrosis and apoptosis are implicated and interrelated in alcohol and AHT-induced cardiac damage.

Experimental results and some clinical data point to novel, recently identified, cardiomyokines involved in the regulation of cardiac damage and remodelling. Among them, fibroblast growth factor-21 (FGF21) appears to play an important role whereas preliminary data point to the involvement of meteorin-like (Metrnl) as well. We hypothesize that these emerging cardiomyokines may be relevant in the pathogenesis of AHT and alcohol-induced cardiac damage. Moreover, FGF21 and Metrnl may constitute therapeutic tools by themselves in order to modulate the progression of alcohol and AHT-induced cardiac damage.

OBJECTIVES

1) Define the role of FGF21 and Metrnl myocardial expression in alcohol and in hypertension induced cardiac damage. Characterization in a mouse model of experimentally-induced cardiomyopathy (alcoholism and hypertension), how the FGF21 and Metrnl system (systemic, and specific in heart) is affected by the intervention, and to relate it to the cardiac alterations induced (cardiac hypertrophy, fibrosis, functional alterations, and induction of cardiac inflammatory and metabolic dysregulation).

2) Establish whether the lack of a functional FGF21 and Metrnl systems ("knockout" mice) exacerbates the cardiac deleterious effects induced in the previous model.

3) Analysis and validation of FGF21/Metrnl gene expression and protein in human cardiac samples from hypertensive and alcoholic patients developing cardiomyopathies.
4) Evaluate the influence of mitochondrial apoptosis and function in alcohol and hypertension-induced cardiac damage
5) Evaluate the effects and mechanistic basis of a potential therapeutic intervention to diminish the effects of alcohol misuse and AHT on cardiac damage. Establish whether treatment with FGF21 or Metrnl restores/protects cardiac function in a cellular model (cardiomyocytes) of AHT-induced cardiac hypertrophy and mouse models described above.

METHODOLOGY

1. Animal studies: We will generate alcohol and hypertension-induced cardiac damage experimentally in mouse lacking (generated in collaboration with MMRC) or overexpressing (by injection of adeno-associated virus AAV) Fgf21 or Metrnl gene by the following commonly used methods:
-Adult mice will be subjected to a liquid diet supplied with isocaloric 4% (vol/vol) ethanol for a 12-week period to induce cardiac damage.
-Hypertension will be induced by angiotensin-II infusion (0.7mg/kg/day) by subcutaneously implantation of osmotic minipumps for one week.

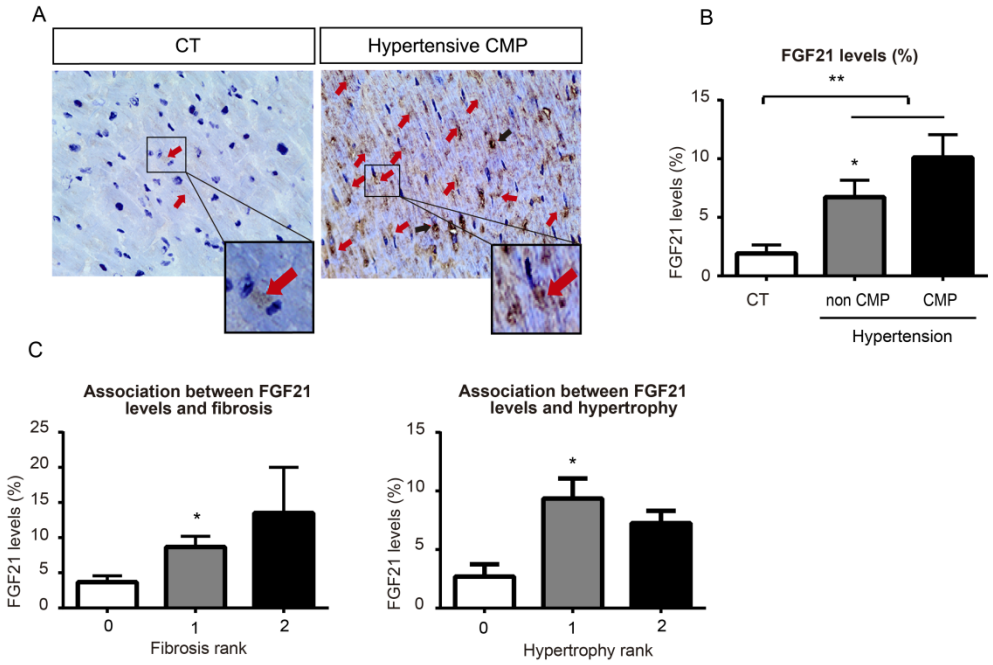
2. Human samples: Over a 5-year period, hearts from subjects who suffered brain death of either traumatic or cerebrovascular origin were consecutively collected in the Hospital Clinic. 52 hearts from donors younger than 70 years were not suitable for transplantation and available for scientific study purposes. From these donors some suffer from alcoholism and others from hypertension. In addition, 7 hearts from previously healthy persons were not eligible for implantation because of lack of a matched recipient or size inadequacy; these donors were treated as control subjects.

2. Results obtained

1) ROLE OF FGF21 DURING HYPERTENSION-INDUCED CARDIOMYOPATHY

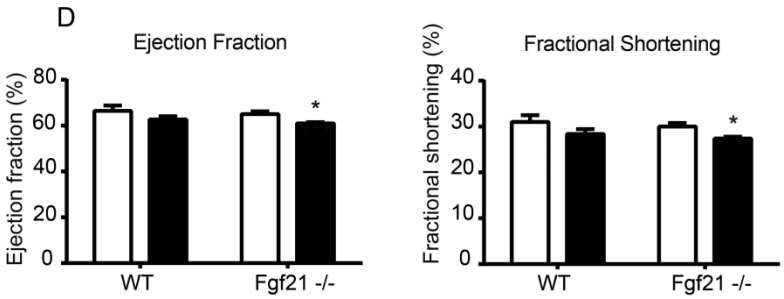
FGF21 expression is up-regulated in the human heart of hypertensive heart donor patients

FGF21 protein levels were significantly higher in heart tissue from hypertensive donors, especially those that developed cardiomyopathy compared with control hearts (A, B). FGF21 protein levels in the myocardium were correlated with the degree of cardiomyocyte hypertrophy and interstitial fibrosis (C).

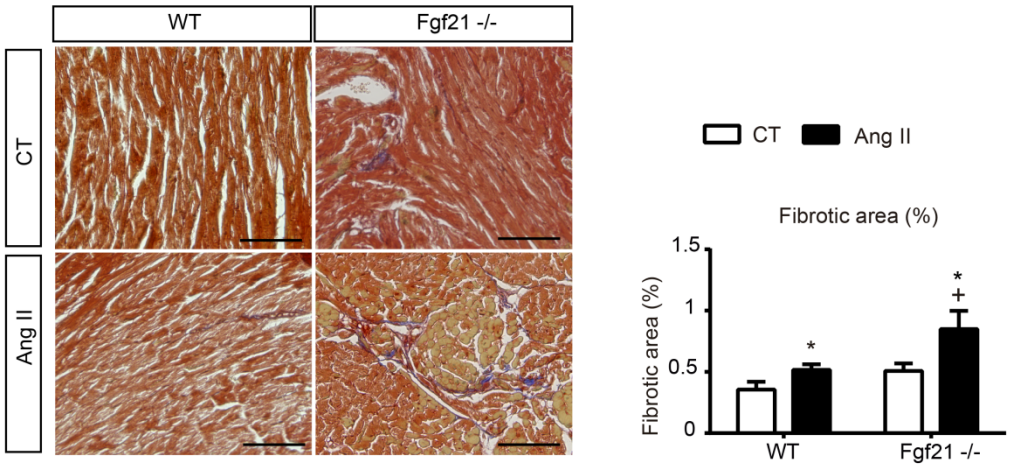


Lack of FGF21 impairs cardiac remodelling and fibrosis in hypertensive heart disease

We next analyzed the impact of FGF21 deficiency on cardiac morphology after 1 week AngII infusion in wt and *Fgf21*$^{-/-}$ mice. Echocardiography measurements revealed that both the ejection fraction (EF) and the fractional shortening (FS) were significantly reduced in hypertensive *Fgf21*$^{-/-}$ mice, compared to their corresponding non-hypertensive controls, indicating a clear cardiac dysfunction in AngII-treated *Fgf21*$^{-/-}$ mice.

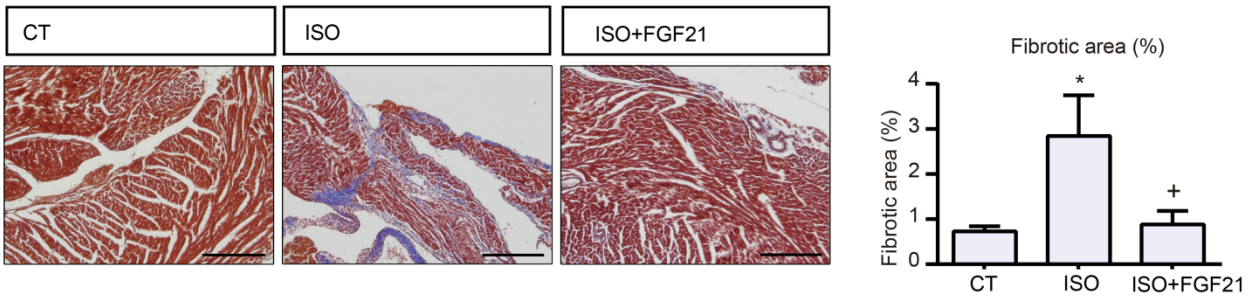


An analysis of hearts by Masson's trichrome staining showed evidence of some fibrosis in hypertensive wt mice, but showed a significant increase in fibrotic areas in the hearts of *Fgf21*$^{-/-}$ mice after AngII-treatment compared with hypertensive wt mice.



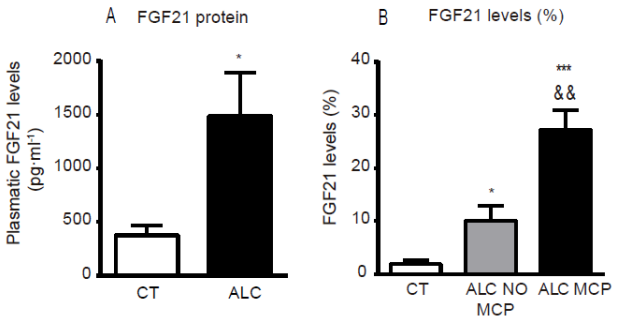
FGF21 protects against cardiac fibrosis

In vivo experiments in a mouse model of isoproterenol (ISO)-induced cardiac fibrosis showed that FGF21 treatment for 7 days protected against fibrosis development assessed by Masson's trichrome staining.

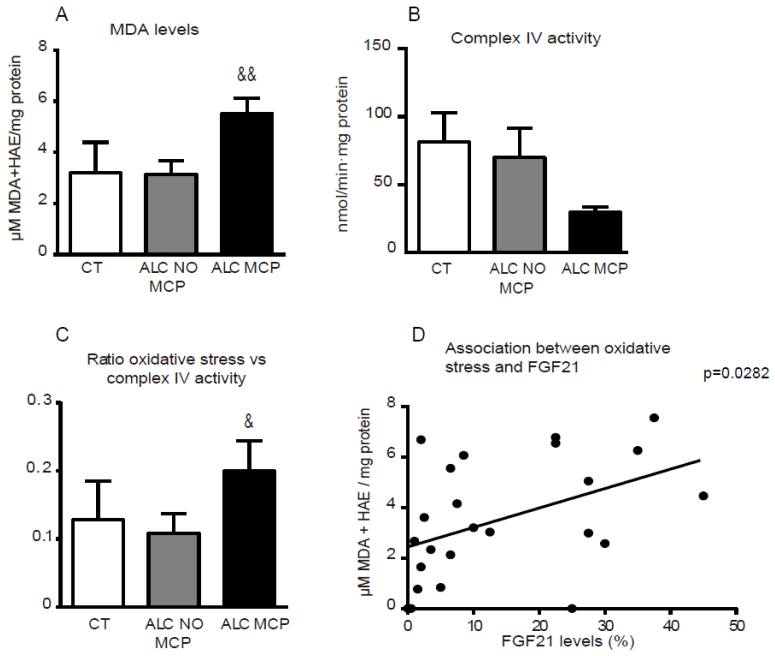


2) ROLE OF FGF21 DURING ALCOHOLISM-INDUCED CARDIOMYOPATHY

FGF21 expression is up-regulated in the human heart of alcoholic patients. We found a significant increase of circulating FGF21 levels in alcoholic versus non-alcoholic patients (A). Alcoholic heart donors showed a significant increase in FGF21 staining in both perinuclear areas and cytoplasmatic areas of cardiomyocytes (B). Finally, FGF21 protein levels in the myocardium were associated with the degree of cardiomyocyte hypertrophy and interstitial fibrosis.



Oxidative stress is induced in alcoholic patients developing cardiomyopathy and is positively associated to FGF21 protein levels in the myocardium Measurement of lipid peroxidation in these hearts by measuring MDA as an indicator of oxidative damage, revealed a significant increase of MDA in alcoholic hearts developing cardiomyopathy compared to healthy hearts (A). Subsequent analysis of mitochondrial complex IV activity indicates a clear trend to be reduced in the myocardium of alcoholic patients with cardiomyopathy (B). The oxidative stress versus complex IV activity was also significantly increased in alcoholic cardiomyopathic hearts (C). We found that oxidative stress assessed by MDA levels was directly, positively and significantly associated with FGF21 expression in the myocardium (D).



3) ROLE OF METEORIN-LIKE (Metrnl) IN CARDIAC DISEASES

Metrnl is a new prognostic biomarker for human heart failure

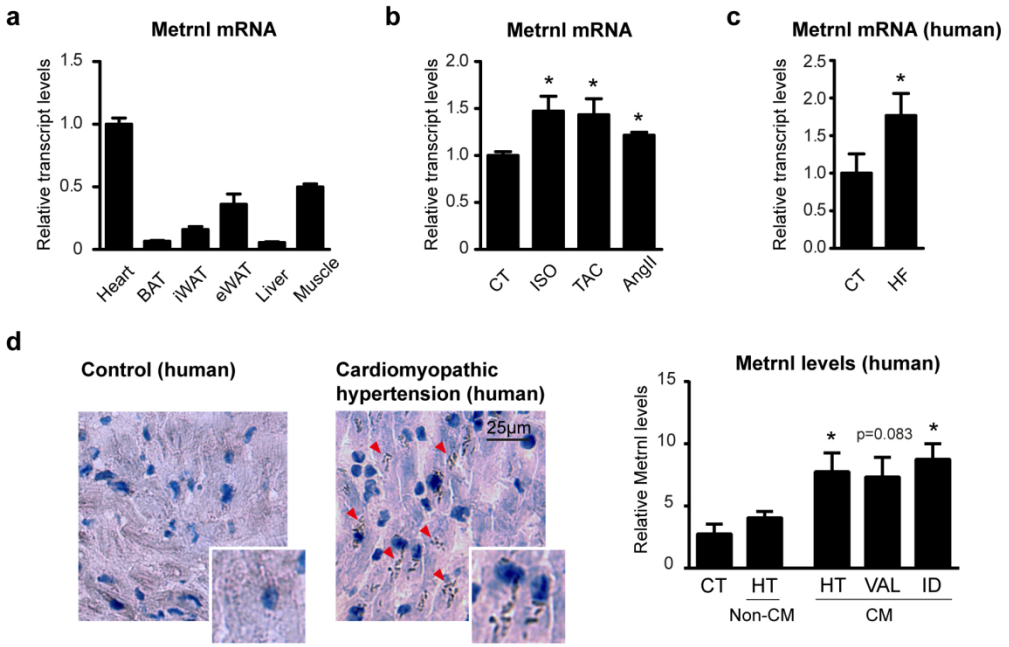
We examined the predictive value of circulating Metrnl in a large cohort of 446 patients with heart failure (mean age 66.7 years (59-76 years), 72.4% male, and median left ventricular ejection fraction (LVEF) of 34.8%) to assess mortality risk. In a comprehensive multivariable analysis that included Metrnl, age, sex, LVEF, ischemic aetiology, and the presence of diabetes and hypertension, only Metrnl (HR 1.175 (95% CI 1.009-1.369); p=0.038), age (HR 1.055 (95% CI 1.039-1.071); p<0.001) and sex (HR 0.690 (95% CI 0.483-0.985); p=0.041) were found to be independent predictors of all-cause death. Interestingly, only age (HR 1.05 (95% CI 1.03-1.07); p<0.001) Metrnl (HR 1.12 (95% CI 1.08-1-71); p=0.008) and ischemic aetiology (HR 1.70 (95% CI 1.10-2.65); p=0.018) were found to be independent predictors of cardiovascular death.

Metrnl is highly expressed in heart and up-regulated upon cardiac stress in mice and humans

We found the highest expression level in the heart, where the Metrnl mRNA level was higher than that found in any other tissues, including those previously considered to be sources of Metrnl, such as adipose tissue and skeletal muscle (A). Induction of cardiac hypertrophy in distinct experimental models in mice based on treatment with

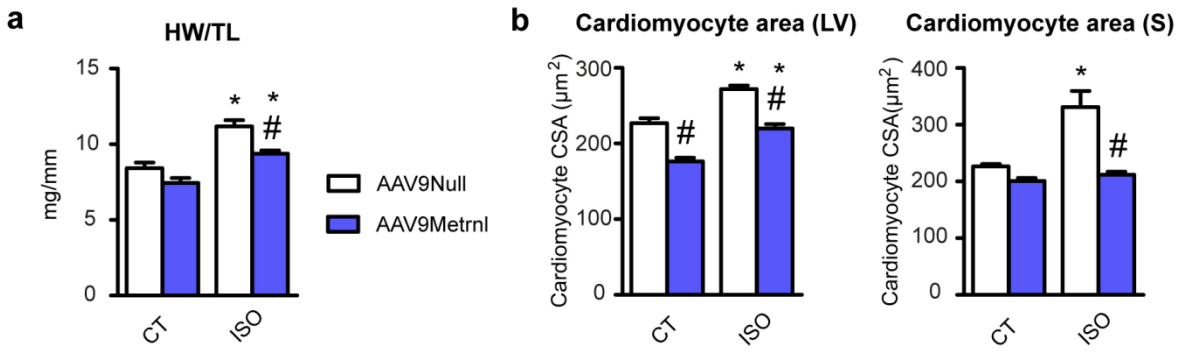
the hypertrophic agent isoproterenol (ISO), transversal aortic constriction (TAC), and angiotensin II (AngII)-induced hypertension, significantly increased the expression levels of Metrnl in mouse heart (B).

Furthermore, Metrnl transcript expression levels were significantly induced in heart from human patients suffering from heart failure (C). Immunohistochemical analysis revealed a significant increase in the Metrnl protein levels in patients affected by distinct types of cardiomyopathy (hypertensive, valvular and idiopathic) (D).



Cardiac-specific overexpression of Metrnl prevents cardiac hypertrophy development

The HW/TL ratio (indicative of the development of cardiac hypertrophy) was significantly reduced in mice in which Metrnl expression in the heart had been induced by AAV9-Metrnl injection (A). Histological examination of haematoxylin and eosin (H&E)-stained LV and septum tissue sections confirmed that the CSA was significantly smaller in ISO-treated mice injected with AAV9-Metrnl than in corresponding ISO-treated mice injected with AAV9-null vector (B). We confirmed the cardioprotection by echocardiography.



3. Global relevance of results

This study has evaluated the role of the cardiomyokines FGF21 and Metrnl in alcoholism and hypertension-induced cardiac damage in a coordinated design with human and experimental samples.

Main findings and relevance:

We discovered that Metrnl is a new cardiokine that shows promise as a new potential therapeutic agent for cardiac diseases and also as a new biomarker for prognosis in heart failure patients.

FGF21 is also increased in response to the cardiac remodelling for alcohol injury and to hypertensive cardiomyopathies. This allows consideration of the use of this cardiokine as a therapeutic agent in clinical trials to avoid progression to alcoholic or hypertensive cardiomyopathies and/or new clinical biomarker to assess the severity of the cardiomyopathy.

FGF21 directly targets the heart and protects it from hypertensive heart disease especially affecting fibrosis development. Preventing, or at least inhibiting, the accumulation of pathologic levels of fibrotic tissue in the myocardium is a major therapeutic goal. Present conceptual approaches for therapeutic interventions are focused on avoiding and inhibiting fibrosis therefore we propose Fgf21 as an anti-fibrotic agent for the treatment of hypertensive heart-disease

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