**TRPM7 is down-regulated in both left atria and left ventricle of ischaemic cardiomyopathy patients and highly related to changes in ventricular function**

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**Abstract**

**Aims** The kinase ion channel transient receptor potential melastatin 7 (TRPM7) is considered a modulator of cardiac fibrosis progression; nevertheless, we lack of studies analysing its role in human ischaemic cardiomyopathy (ICM). Our objective was to analyse the expression of genes encoding cardiac ion channels in human ICM, focusing on the alterations in mRNA levels of TRPM7 and its relationship with changes in the ventricular function.

**Methods and results** RNA-sequencing was carried out in 13 left ventricular (LV) samples of patients with ICM compared with a control group (n = 10). The analysis revealed a total of 25 ion channel genes differentially expressed. We performed an RTqPCR analysis of the TRPM7 mRNA in LV and left atrial samples and found that it was down-regulated in both cavities (−1.43-fold and −1.52-fold, respectively). Atrial TRPM7 mRNA levels showed an excellent and inverse relationships with the depressed ejection fraction (r = −0.724, P = 0.042) and with the mitral A wave (r = −0.938, P = 0.006).

**Conclusions** We report the down-regulation of TRPM7 in tissue samples from both left atria and left ventricle in patients with ICM. We found an inverse relationship between both cardiac chambers mRNA levels with LV dysfunction, suggesting an important role of TRPM7 in the left atrial and LV functional depression found in this cardiomyopathy.

**Keywords** TRPM7; ischaemic cardiomyopathy; left ventricular dysfunction

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**Introduction**

Transient receptor potential melastatin 7 (TRPM7) is a unique ion channel which has a protein kinase function. It is a divalent cation channel constitutively opened, permeable to Ca\(^{2+}\) and Mg\(^{2+}\). This dual function makes this channel an important regulator of many processes such as cell viability, cytoskeleton organization, magnesium homeostasis and cardiac fibrosis.

Cardiac fibrosis induces an adverse structural remodelling of the myocardium, being a detrimental factor that results in abnormalities in cardiac conduction, loss of contractility, and hardening of ventricular walls, thus contributing to cardiovascular diseases including heart failure (HF).

The role of TRPM7 in the fibrotic process has been suggested to occur via the Ca\(^{2+}\) mediated signals that contribute to TGF-β1-induced fibrogenesis, through ERK1/2 activation due to phosphorylation and Ca\(^{2+}\) influx and by regulation of intracellular Ca\(^{2+}\) and Mg\(^{2+}\) transport in Angiotensin II stimulation.

A deregulation of TRPM7 channel or current has been previously reported in animal models of HF and in atrial fibrillation patients, but there are no studies analysing its expression in human ischaemic cardiomyopathy (ICM). Because of evidences regarding its important role in cardiac fibrosis, we hypothesise that patients with HF of ischaemic origin may display...
changes in TRPM7 gene expression that could be contributing to this deleterious process. Therefore, our aim was to evaluate the tissue mRNA levels of TRPM7 in both the left atria and left ventricle of patients with ICM compared with non-diseased controls (CNTs). We also determined the relationship between the mRNA levels of TRPM7 in the auricular and ventricular myocardium and the left ventricular (LV) dysfunction.

**Methods**

Methods are shown in the Supporting Information (Appendix S1).

**Results**

**Clinical characteristics of patients**

We analysed 13 LV tissue samples from patients with ICM undergoing heart transplantation and 10 LV CNT samples. These ICM patients were all men, with a mean age of 54 ± 7 years. We increased the sample size up to 14 LV tissue and we included 14 left atrial (LA) tissue samples from ICM patients to study the differential expression between cardiac cavities through RT-qPCR. We also increased the pathological sample size up to 17 for Western blot analyses. The sample’s handling was carried out equally in both groups. Table 1 shows the clinical characteristics of the patients included in the study. The CNT group was mainly men (80%), with a mean age of 47 ± 16 years.

**RNA-sequencing analysis**

We carried out a large-scale RNA-sequencing analysis to elucidate the differential expression levels between groups, so as to identify novel genes affecting the development and progression of ICM. This analysis identified 1712 differentially expressed genes between the ICM and CNT groups (≥1.3-fold, \( P < 0.05 \)), of which 815 were up-regulated and 897 were down-regulated. Among these deregulated genes, we found that some belonged to the cardiac ion channel category.

Twenty-five deregulated genes altered in the ICM group were involved in ion fluxes, of which 13 were up-regulated and 12 were down-regulated (Figure 1A). We created a Heat map with hierarchical clustering to visualize the altered expression of genes belonging to the cardiac ion channel category; the plot clearly identified the two groups of study and different expression patterns (Figure 1B).

**RT-qPCR validation**

RT-qPCR was performed to validate the differential gene expression of TRPM7 observed in the RNA-sequencing profiling. For the reaction, the same samples used for the RNA-sequencing technique and an additional ICM sample for a total of 14 ICM and 10 CNT subjects were used. We also measured the mRNA levels of this ion channel gene in LA samples (\( n = 14 \)) from patients with ICM. It was shown that TRPM7 was down-regulated in both LV (−1.43-fold, \( P < 0.05 \)) and LA (−1.52-fold, \( P < 0.05 \)) tissue samples compared with samples from the CNT group (Figure 2).

**Relationship between mRNA levels and cardiac dysfunction**

We analysed the relationships between the differentially expressed genes and the echocardiographic parameters of patients (Table 2). We found that the ventricular levels of TRPM7 were inversely related with EF (\( r = −0.640, P = 0.046 \)). In LA samples, we found that the mRNA levels of TRPM7 were highly and inversely related to EF (\( r = −0.724, P = 0.042 \)). Furthermore, a wave peak velocity of the mitral Doppler spectrum had an outstanding correlation (\( r = −0.938, P = 0.006 \)) when compared with TRPM7 mRNA.

**Western blot analysis**

Western blot experiments were performed to analyse the protein expression of TRPM7 in LV samples of ICM patients. We found that the TRPM7 protein is not differentially expressed between the ICM and the CNT group (\( 123 ± 32 \) vs. \( 100 ± 19 \), arbitrary units, \( P > 0.05 \)).

**Discussion**

Ion channels are important modulators of the cardiac contraction and function, being its alterations implicated in HF.13 In our

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**Table 1 Clinical characteristics of patients with ICM**

| Characteristic                  | ICM (\( n = 13 \)) RNA-sequencing | ICM (\( n = 14 \)) RT-qPCR |
|---------------------------------|-----------------------------------|---------------------------|
| Age (years)                     | \( 54 ± 7 \)                       | \( 55 ± 8 \)               |
| Gender male (%)                 | \( 100 \)                          | \( 93 \)                   |
| BMI (kg/m²)                     | \( 26 ± 4 \)                       | \( 27 ± 4 \)               |
| Haemoglobin (mg/dL)             | \( 14 ± 3 \)                       | \( 13 ± 3 \)               |
| Haematocrit (%)                 | \( 41 ± 6 \)                       | \( 40 ± 8 \)               |
| Total cholesterol (mg/dL)       | \( 162 ± 41 \)                     | \( 160 ± 40 \)             |
| Prior hypertension (%)          | \( 30 \)                           | \( 31 \)                   |
| Prior smoking (%)               | \( 84 \)                           | \( 85 \)                   |
| Diabetes mellitus (%)           | \( 38 \)                           | \( 39 \)                   |
| EF (%)                          | \( 24 ± 4 \)                       | \( 24 ± 6 \)               |
| LVESD (mm)                      | \( 55 ± 7 \)                       | \( 56 ± 8 \)               |
| LVEDD (mm)                      | \( 64 ± 7 \)                       | \( 64 ± 8 \)               |
| Left ventricle mass index (g/m²)| \( 139 ± 36 \)                     | \( 139 ± 36 \)             |
| Duration of disease (months)    | \( 45 ± 40 \)                      | \( 48 ± 40 \)              |

BMI, body mass index; EF, ejection fraction; ICM, ischaemic cardiomyopathy; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter.
patients, RNA-sequencing technique and Heat map analysis revealed a broad set of cardiac ion channel genes deregulated in ICM, indicating a clear separation between the pathological and the CNT group. Of all these altered genes, we found that only the gene encoding for ion channel with protein kinase function, \textit{TRPM7}, has shown an excellent and inverse relationship with the ventricular dysfunction found in patients with ICM.

Cardiac fibrosis is a pathological response in HF, characterized by massive deposition of extracellular matrix proteins, mainly produced by cardiac fibroblasts and myofibroblasts.\textsuperscript{14} Extensive myocardial remodelling disrupts tissue structure and increases its stiffness, leading to ventricular dysfunction. It has been shown that Ca\textsuperscript{2+} ions are associated with this detrimental process, being essential for the proliferation and differentiation of fibroblasts.\textsuperscript{15}

Transient receptor potential melastatin 7 ion channel is responsible for Ca\textsuperscript{2+} and Mg\textsuperscript{2+} trafficking in fibroblasts, as reported in different studies, being an important modulator of cardiac fibrosis.\textsuperscript{10,16} Different pathways activate to promote fibrogenesis, and there is evidence supporting the implication of TRPM7-Ca\textsuperscript{2+} mediated current in the

\textbf{Figure 1} Differential gene expression of cardiac ion channels in patients with ischaemic cardiomyopathy. (A) RNA-sequencing results of mRNA expression levels of cardiac ion channels. (B) Heat map with hierarchical clustering of the transcriptomic analysis. The values of the compared with non-diseased control group were set to 1. The data are expressed as mean ± SEM for the mRNA relative expression levels. *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\) vs. the compared with non-diseased control group. The relative expression level of each gene in the Heat map is indicated by the colour bar.
activation of TFG-β1,7 ERK ½,8 and Ang II10,17 pathways. Previous studies have reported an up-regulation of TRPM7 in HF models12 and the up-regulation of TRPM7-mediated current in atrial fibrillation patients.7 Moreover, it has been shown an up-regulation of this channel in patients with non-ischaemic dilated cardiomyopathy with ventricular tachycardias compared with non-ventricular tachycardia hearts, suggesting an adverse myocardial remodelling in the ventricular tachycardia group.18 Additionally, experiments on silencing TRPM7 gene (shTRPM7) have demonstrated decrease in the progression of cardiac fibrosis.7,8

We report, in contrast to what has been previously published, the down-regulation of TRPM7 in both left atria and left ventricle of ICM patients. Moreover, we found an inverse relationship between both TRPM7 LA and LV mRNA levels with changes in LV function. We also analysed the protein levels of TRPM7 that showed no statistical differences between groups. These results could be explained, as has been previously reported,19–21 due to a mechanism of slowed protein degradation system which may mean that the TRPM7 protein remains invariant despite its gene expression being down-regulated. Although further studies need to be carried out, our results suggest that TRPM7 down-regulation could be an important player in the LA and LV functional depression found in this cardiomyopathy.

A common limitation of studies using human samples is the pharmacological treatment that could influence our results. Moreover, our tissue samples are confined to transmural left ventricle apex, so our findings could not be generalized to all regions of the left ventricle. However, our work was performed using a suitable sample size of both patients and CNTs.

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Conflict of interest
None declared.

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Supporting information
Supporting information may be found in the online version of this article.

Appendix S1 Methods.

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