

# New Genetic Variants Associated with Acquired Dilated Cardiomyopathy. Towards a New Predisposing Polygenic Panel

*Nuevas variantes genéticas asociadas a miocardiopatía dilatada adquirida. Hacia un nuevo panel poligénico predisponente*

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## ABSTRACT

**Background:** Heart failure with reduced left ventricular ejection fraction (HFrEF) is one of the cardiovascular pathologies with the highest mortality. The existence of a genetic predisposition for the development of this pathology in the presence of a noxa is still uncertain.

**Objectives:** The aim of this study was to identify associations between genetic variants in polymorphic alleles, single nucleotide polymorphisms (SNPs) with the presence of rEF and intraventricular conduction disorders (IVCD) in a model of acquired cardiomyopathy such as chagasic cardiomyopathy.

**Methods:** A study model including patients seropositive for Chagas disease with a history of infection of more than 20 years, one group with rEF ( $\leq 35\%$ ) and another group with preserved LVEF (pEF,  $\geq 50\%$ ). A blood collection was performed and processed to obtain deoxyribonucleic acid (DNA), which was sent to an international genotyping laboratory. A panel of gene SNPs was prepared using the international TheGenomeAggregationDatabase (GnomAD), choosing SNPs with a frequency in the population of 10% to 40%. The SNPs chosen are related to genes responsible for the process of ventricular contraction and relaxation (TTN, BAG3, MTSS1), myocardial metabolism (PPARGC1A, SIRT1, AKT1 mTOR, AMPK), adrenergic receptor beta 1 (ADRB1), cholinergic receptor muscarinic 2 (CHRM2), angiotensin II type 1 receptor (AGTR1B), and atrial natriuretic peptide (NPPA) Using various artificial intelligence models for supervised learning (Logistic Regression, Support Vector Machines, Artificial Neural Networks, NaiveBayes, Classification Trees, and RandomForest) 68 SNPs were evaluated as predictors of the presence of two phenotypes: rEF, and IVCD. The performance of the models for the prediction of rEF was evaluated by cross-validation technique, with the F1 metric as a measure of accuracy to select the best model.

**Results:** One hundred and eighty-two patients were included, with a median age of 62 years, 39.6 % men. Thirty-one percent had rEF and 53% had some IVCD. The model with the best performance was logistic regression ( $F1=0.85$ ), so it was used to express the association by Odds Ratio and its 95% CI. It was observed that the variants rs2076300, rs61772962 and rs7071853 were independent predictors of rEF, whereas the only predictor for IVCD was the SNP rs72840788. The SNP rs61772962 corresponds to the PRKAA2 gene, which encodes the alpha-2 catalytic subunit of 5'-AMP-activated protein kinase. On the other hand, rs7071853 and rs72840788 correspond to the gene encoding the BAG3 protein (chaperone regulator 3). Finally, the SNP rs2076300 corresponds to the DSP gene encoding a desmoplakin, a protein of the desmosome binding plate.

**Conclusions:** In this derivation model, in a set of patients with positive serology for Chagas disease, 3 SNPs predictive of rEF and one SNP predictive of IVCD were identified. The reproducibility of these results should be confirmed in a validation model with a larger sample of individuals. Furthermore, this finding could be useful in dilated cardiomyopathies of other etiologies.

**Key words:** Cardiomyopathy, Dilated - Heart Failure - Genetics - Chagas Disease

## RESUMEN

**Introducción:** La insuficiencia cardíaca con fracción de eyección ventricular izquierda reducida (FEVIr) es una de las patologías cardiovasculares con mayor mortalidad. La existencia de una predisposición genética para el desarrollo de esta patología ante la presencia de una noxa es aún incierta.

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**Objetivos:** El objetivo de este estudio fue identificar asociaciones entre variantes genéticas en alelos polimórficos, polimorfismos de nucleótido único (SNPs) con la presencia de FEVIr y de trastornos de conducción intraventricular (TCIV), en un modelo de miocardiopatía adquirida como es la chagásica.

**Material y métodos:** Se incluyó como modelo de estudio a pacientes seropositivos para enfermedad de Chagas con data de infección de más de 20 años, un grupo con FEVIr (FEVI  $\leq 35\%$ ) y otro con FEVI preservada, FEVIp ( $\geq 50\%$ ). Se realizó una toma de sangre que fue procesada para la obtención del ácido desoxirribonucleico (ADN), que se envió a un laboratorio internacional de genotipado. Se preparó un panel de SNPs de genes utilizando la base internacional TheGenomeAggregationDatabase (GnomAD), eligiendo SNPs con una frecuencia en la población del 10% al 40%. Los SNPs elegidos están relacionados con genes responsables del proceso de contracción y relajación ventricular (TTN, BAG3, MTSS1), el metabolismo miocárdico (PPARGC1A, SIRT1, AKT1 mTOR, AMPK), receptor adrenérgico beta 1 (ADRB1), receptor colinérgico muscarínico 2 (CHRM2), receptor de angiotensina II tipo 1 (AGTR1B), y el péptido natriurético auricular (NPPA) Mediante diversos modelos de inteligencia artificial para aprendizaje supervisado (Regresión Logística, Máquinas de Vectores de Soporte, Redes Neuronales Artificiales, NaiveBayes, Árboles de Clasificación y RandomForest) se evaluaron 68 SNPs como predictores de la presencia de dos fenotipos: FEVIr, y TCIV. El desempeño de los modelos para la predicción de la FEVIr se evaluó mediante la técnica de validación cruzada (*cross-validation*), con la métrica F1 como medida de precisión para seleccionar el mejor modelo.

**Resultados:** Se incluyeron 182 pacientes, con una mediana de edad de 62 años, el 39,6 % hombres. El 31 % presentó FEVIr y el 53 % algún TCIV. El modelo con el mejor desempeño fue la regresión logística (F1 = 0,85), por lo que se la utilizó para expresar la asociación mediante el Odds Ratio y su IC del 95%. Se observó que las variantes rs2076300, rs61772962 y rs7071853 fueron predictores independientes de reducción de la FEVI. Mientras que, para la presencia de TCIV, el único predictor fue el SNP rs72840788. El SNP rs61772962 corresponde al gen PRKAA2, que codifica a la subunidad catalítica alfa-2 de la proteína quinasa activada por 5'-AMP. Por su parte el rs7071853 y el rs72840788 corresponden al gen que codifica a la proteína BAG3 (regulador 3 de chaperona). Finalmente el SNP rs2076300 corresponde al gen DSP que codifica una desmoplaquina, proteína de la placa de unión de los desmosomas.

**Conclusión:** En este modelo de derivación, en un conjunto de pacientes con serología positiva para Chagas se identificaron 3 SNPs predictores de reducción de la FEVIr y un SNP predictor de TCIV. La reproducibilidad de estos resultados debería ser confirmada en un modelo de validación con una mayor muestra de individuos. Además, este hallazgo podría ser de utilidad en miocardiopatías dilatadas de otras etiologías.

**Palabras claves:** Miocardiopatía dilatada - Insuficiencia cardíaca - Genética - Enfermedad de Chagas

## INTRODUCTION

Heart failure with reduced left ventricular ejection fraction (LVEF) represents one of the pathologies with the highest mortality. Among its main etiologies is dilated cardiomyopathy (DCM). The predictors of DCM development and prognosis are subject to ongoing research. Despite advances in understanding the underlying pathophysiological mechanisms, the reasons why certain conditions predispose to the progression of dilated cardiomyopathy are still not fully understood. (1)

Genotypic (familial) alterations that are responsible for a small group of cases with the ventricular dilatation phenotype have been identified.

A notable aspect is that the same noxa can generate variable effects among different patients. This suggests the possible existence of a genetic predisposition that would influence a differentiated clinical evolution. In this context, single nucleotide polymorphisms (SNPs) could play a key role in the variability of patients' response to the same noxa.

To investigate the existence of genetic variants associated with the predisposition to DCM, we used Chagas disease as model. (2) Chagas disease is responsible for DCM with the highest morbidity and mortality. It presents two clinical phases: an acute phase, which is usually asymptomatic in 95% of patients, and a chronic phase, with a long latency period of 10-30 years. In this group, between 25% and 30% of individuals develop signs or symptoms of cardiac involvement (Chagas cardiomyopathy). (3)

Chronic Chagas cardiomyopathy (CCM) has been

considered the most frequent cause of non-ischemic cardiomyopathy in Argentina. However, there is no consensus on the risk indicators for the development of this condition and other cardiovascular events, regardless of the effect of traditional risk factors. (4)

## OBJECTIVE

The aim of our study was to determine the genetic variants related to the presence of DCM by performing an association analysis with SNPs.

## METHODS

Prospective single-center study that included patients from June 2016 to January 2020.

Patients from 21 to 80 years of age, seropositive for Chagas disease with DCM, from the provinces of northwestern Argentina and southern Bolivia, and other serologically positive patients of similar age, from the same geographic areas without DCM, were included as a control group.

All patients should have a known history of infection of more than 20 years certified by the treating physician, serological analysis, and abandonment of the endemic area for more than 20 years as well as a LVEF measured within 12 months prior to inclusion,  $\leq 35\%$  or  $\geq 50\%$ .

Patients who refused to sign the informed consent form, those with known serious pathologies (excluding cardiovascular) that generate a life expectancy of less than one year, and those who were participating in research protocols in the 30 days prior to sample collection were excluded. Those in whom personal or telephone contact could not be assured, with alcohol or drug abuse in the last 6 months, with clinical and laboratory evidence of liver failure (transaminase value  $\times 3$  and total bilirubin  $> 2$  mg/dL), with LVEF between 36% and 49% (the objective was to separate the groups avoiding patient crossover), and those receiving drugs with a known

action on cardiovascular parameters (immunosuppressants, nitrates, estrogens) were also rejected. Patients with acute or chronic coronary artery disease, with an indication for revascularization or with coronary angioplasty or myocardial revascularization surgery in the previous 6 months, patients with renal failure (serum creatinine  $>2.5$  g/dL), with severe obstructive pulmonary disease, with DCM of other etiologies (e.g. rheumatoid arthritis, diabetes, hypertension), with significant valvular diseases (except those secondary to mitral and/or tricuspid valve annulus dilatation), with pacemakers with stimulation greater than 50%, or with autoimmune pathologies (lupus, scleroderma, hepatitis C, etc.) were also excluded.

Patients who met the inclusion criteria and did not present exclusion criteria were divided into 2 groups:

- Group 1: patients with LVEF $\leq$  35%.
- Group 2: individuals with LVEF $\geq$ 50%.

After signing the informed consent, a complete clinical history and physical examination were performed, according to their underlying pathology and the corresponding diagnostic and prognostic procedures.

An echocardiogram to confirm the LVEF value and a 12-lead ECG were performed. According to the ECG results, patients were classified into two groups: with intraventricular conduction disorders (IVCD), when they had one or more of the following criteria: right bundle branch block, left bundle branch block, left anterior hemiblock and left posterior hemiblock; or without IVCD.

Patients were coded with an alphanumeric system to ensure the identity of the groups.

A 10 mL blood sample was taken by trained personnel for genomic determination. The sample was placed in tubes with the presence of ethylenediaminetetraacetic acid (EDTA) according to ISO standards to prevent coagulation and stored at  $-20^{\circ}\text{C}$  until processing. This sample was processed to obtain the corresponding deoxyribonucleic acid (DNA) in a specialized center in our country, leaving safety samples.

The DNA was sent via a courier authorized by international legislation for this purpose to the international laboratory Xenética Cardiovascular, Instituto de Investigación Sanitaria de Santiago, laboratory n°1, Complejo Hospitalario Universitario de Santiago de Compostela, where genotyping and analysis were performed (Table 1). The laboratory remained blind to which group the patients belonged. This was only known by the statistical team at the time of the analysis.

An SNPs panel of genes was prepared related to the following:

- Ventricular contraction and relaxation process (TTN, BAG3, MTSS1). (5-7)
- Myocardial metabolism (PPARGC1A, SIRT1, AKT1 mTOR, AMPK, PRKAA2).
- Beta 1 adrenergic receptor (ADRB1). (8-11)
- Cholinergic receptor muscarinic 2 (CHRM2).
- Angiotensin II type 1 receptor (AGTR1B).
- Atrial natriuretic peptide (NPPA).
- Cell cycle regulators (CDKN1A, RYR2)
- Sarcomeric structure (ATP2A2, DSP, JUP)
- Cellular energy (PRKAB2, PRKAB1)
- Mitochondrial activity (SOD 2)
- Apoptosis (AKT1)
- Cellular hypoxia regulator (HIF1A)
- Respiratory chain (LDHA, lactate dehydrogenase (LDH))
- Nitric oxide (NOS1, NOS2P3)
- Growth and inflammation factors (SH2B3)

The international database TheGenomeAggregation-Database (GnomAD) was used, choosing SNPs with a frequency of 10% to 40% in the population incorporated into this database.

Finally, 68 SNPs, detailed in Table S1, were selected for analysis.

The technology used was iPLEX Gold. The script was created in SNPassoc to evaluate whether the entire population met the H-W equilibrium condition.

The association between these two phenotypes was evaluated as binomial variables coded as YES=1 and NO=0, with the SNPs coded as an ordinal variable according to the following convention: 0 for homozygotes of the wild-type allele, 2 for homozygotes of the alternative allele and 1 for heterozygotes.

To evaluate the relationship between the different SNPs under study and the presence of reduced LVEF, various artificial intelligence models for supervised learning were used. The performance of 1) Logistic Regression, 2) Support Vector Machines, 3) Artificial Neural Networks, 4) Naive Bayes, 5) Decision Trees and 6) Random Forest was evaluated, with the collaboration of the genomIT Artificial Intelligence team.

The performance evaluation of the different models for the prediction of rEF was carried out using the cross-validation technique, using F1 score as a measure of precision to select the best model.

To evaluate the relationship between each SNP and the presence of IVCD, a univariate logistic regression model with the presence of reduced LVEF and IVCD as outcomes and each SNP as a predictor was evaluated. SNPs with an associated p value  $<0.05$  were selected for evaluation of their effect using a multivariate logistic regression model, selecting those SNPs with a p value  $<0.05$  using the backward selection strategy with stepwise regression method. The magnitude of the association between each SNP and the outcome under study was expressed as the Odds Ratio and its respective 95% confidence interval (95% CI). The predictive performance of the model was evaluated using the area under the curve (AUC). The same procedure was applied using logistic regression of the SNPs with reduced LVEF.

The analyses were performed with the R ® software (version 4.1.1, R Development Core Team/R Foundation for Statistical Computing, Vienna, Austria).

A Manhattan plot was also generated with different inheritance models for all SNPs, analyzing the nominal level of significance and the Bonferroni level.

#### Ethical considerations

The study was developed in accordance with the ethical principles of the Declaration of Helsinki and approved by the Teaching and Research Committee of the institution.

#### RESULTS

One hundred and eighty-two patients with positive serology for Chagas disease from southern Bolivia and northwestern Argentina were studied.

The median age of the population was 62 years, 39.6% were men. Fifty-seven of them (31%) had rEF and 95 (53%) had some IVCD (Table 1).

Among the different supervised classification models evaluated, the model that uses 7 K folds and a logistic regression classifier obtained a score of  $F1=0.85$ , being the best model obtained; therefore its results are reported.

The univariate analysis of the relationship between each SNP (on the horizontal axis) and the presence of a reduced LVEF and IVCD respectively performed by logistic regression is presented in Figures 1 A and 1 B. A multivariate analysis that revealed the association of reduced LVEF phenotype and IVCD was performed. (Figures 2 A and B, Tables 2 and 3).

It was observed that the variants rs2076300, rs61772962 and rs7071853 were independent predictors of reduced LVEF; whereas, for the presence of blocks, the only predictor was the SNP rs72840788, as is seen in the univariate analysis.

In each of the three SNPs predicting DCM, the proportion of homozygous wild-type, heterozygous and each homozygous variant of the alternative allele was analyzed, according to the presence or not of a reduced LVEF and IVCD. (Figures 3 A, B and C, Figure 4)

The predictive performance of the model for the presence of reduced LVEF is evidenced by the area under the ROC curve (0.662) and its corresponding 95% CI (0.580-0.745) (Figure 5)

Regarding the performance of the neural network for the prediction of reduced LVEF with the three identified SNPs, the best model was that of a neural network with 2 hidden layers of 6 and 3 neurons respectively, with an error rate (proportion of prediction failures) of 0.33 and an accuracy rate (proportion of prediction successes) of 0.67, (Figure 6).

**DISCUSSION**

Heart failure (HF) is a disease of epidemic proportions, representing the final stage of various pathologies. Its prevalence increases two to three times when including patients with asymptomatic systolic dysfunction. (12-14)

Initially, 60-70% of patients with HF had a high mortality within five years after diagnosis, accompanied by a high rate of hospitalization for decompensated HF. However, with the advent of new drugs, a

significant decrease in morbidity and mortality has been seen. (15-21)

Heart failure with reduced LVEF is a progressive disorder that usually begins after a noxa or damaging event that affects the heart muscle, resulting in the loss of functional myocytes or a decrease in the contractile capacity of the myocardium. In most cases, an asymptomatic phase initially occurs due to compensatory mechanisms that are activated and modulate LV function within physiological limits for a certain period of time. However, the sustained activation of the neurohormonal and cytokine systems causes a series of changes in the myocardium, leading to ventricular remodeling and the eventual appearance of symptomatic HF.

Neurohumoral systems include the sympathetic nervous system (SNS), through the activation of the Beta 1 receptor, and the renin-angiotensin system (RAS), mediated by the persistent action of angiotensin II (AT2) and aldosterone on the myocardium.

Parasympathetic inhibition also contributes to HF pathogenesis by reducing nitric oxide (NO) concentrations with the consequent increase in inflammation and ventricular remodeling. (22)

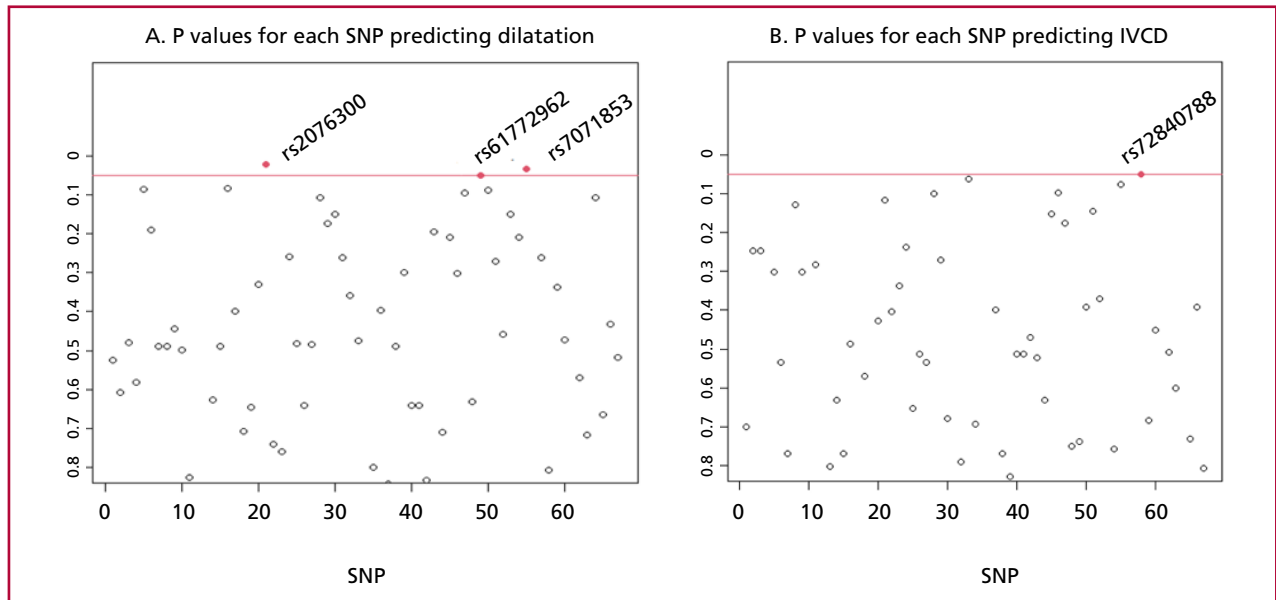
Most of the genes involved in the development of DCM and HF are involved in energy production and regulation processes, as well as calcium signaling and transcription regulation. The genes described are mainly those of sarcomere proteins (MYH7, TNNT2, TNNI3, TNNC, TPM1, MYBPC3, TTN, ACTC, MYL3, MYL2), Z-disc proteins (VCL, LDB3, TCAP, MYOZ2), cytoskeletal or cell membrane proteins (DES, DAG1, SGCA, LMNA, FLNC), desmosomal proteins (JUP, DSP, DSG2, DSC2, PKP2, RYR2, PLN), sodium channel (SCN5A), metabolic proteins (PRKAG2, GLA, LAMP2, GAA) and regulatory proteins (RMB20, BAG3, TGFB3) among others.

Through genome-wide association studies (GWAS), certain SNPs associated with the risk of developing HF have been described.

	Preserved LVEF	Reduced LVEF
N	125	57
Age (years)	59.44 (11.25)	64.68 (10.09)
Male gender	39 (31.2)	33 (57.9)
HTN	38 (30.4)	13 (22.8)
DM		
• No	116 (92.8)	51 (89.5)
• Type I	3 (2.4)	0 (0.0)
• Type II	6 (4.8)	6 (10.5)
HF	4 (3.2)	34 (59.6)
Arrhythmias	21 (16.9)	28 (50.0)

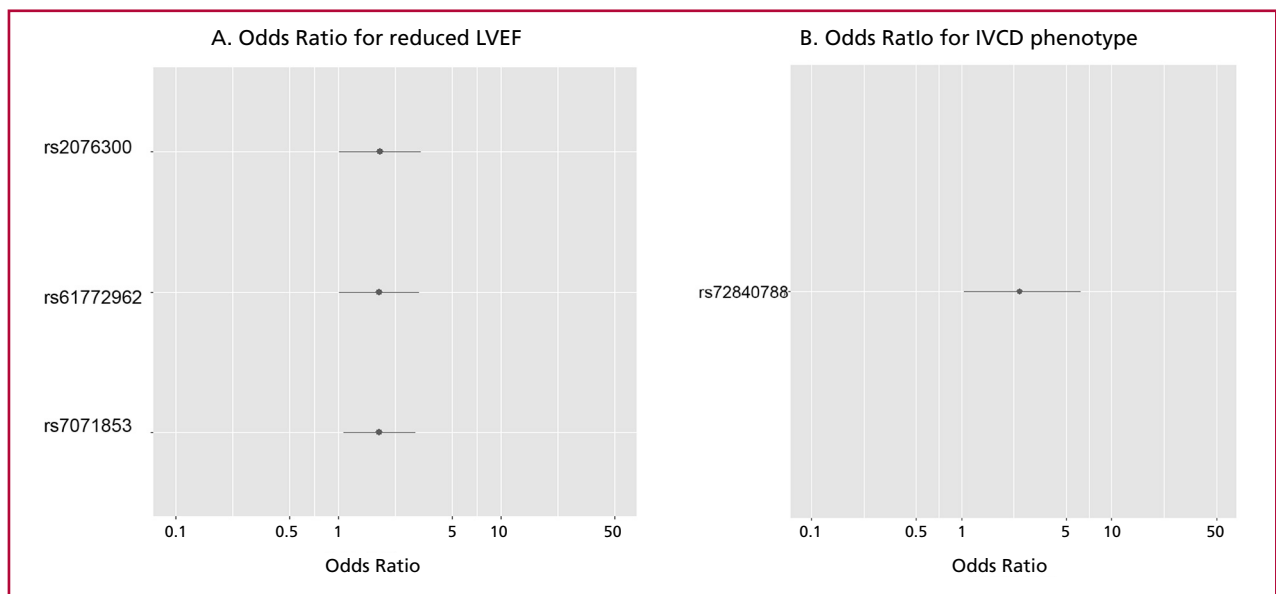
**Table 1.** Baseline characteristics

DM: diabetes mellitus; HF: heart failure; HTN: hypertension; LVEF: left ventricular ejection fraction  
Quantitative variables are presented as mean and standard deviation, qualitative variables as frequency and percentage.



IVCD: intraventricular conduction disorder; SNP: single nucleotide polymorphism

**Fig. 1. A and B:** The vertical axis expresses the p values for each SNP, the red line marks a p = 0.05 significance level, and the red dots correspond to the SNPs that presented a p value < 0.05



95% CI: 95% confidence interval; IVCD: intraventricular conduction disorder; LVEF: left ventricular ejection fraction; SNP: single nucleotide polymorphism

**Fig. 2. A and B.** Odds ratio and 95% CI for SNPs predicting A) reduced LVEF and B) IVCD phenotype

**Table 2.** Multivariate analysis. Odds ratio and 95% CI and p-value for each of the three SNPs independently associated with reduced LVEF

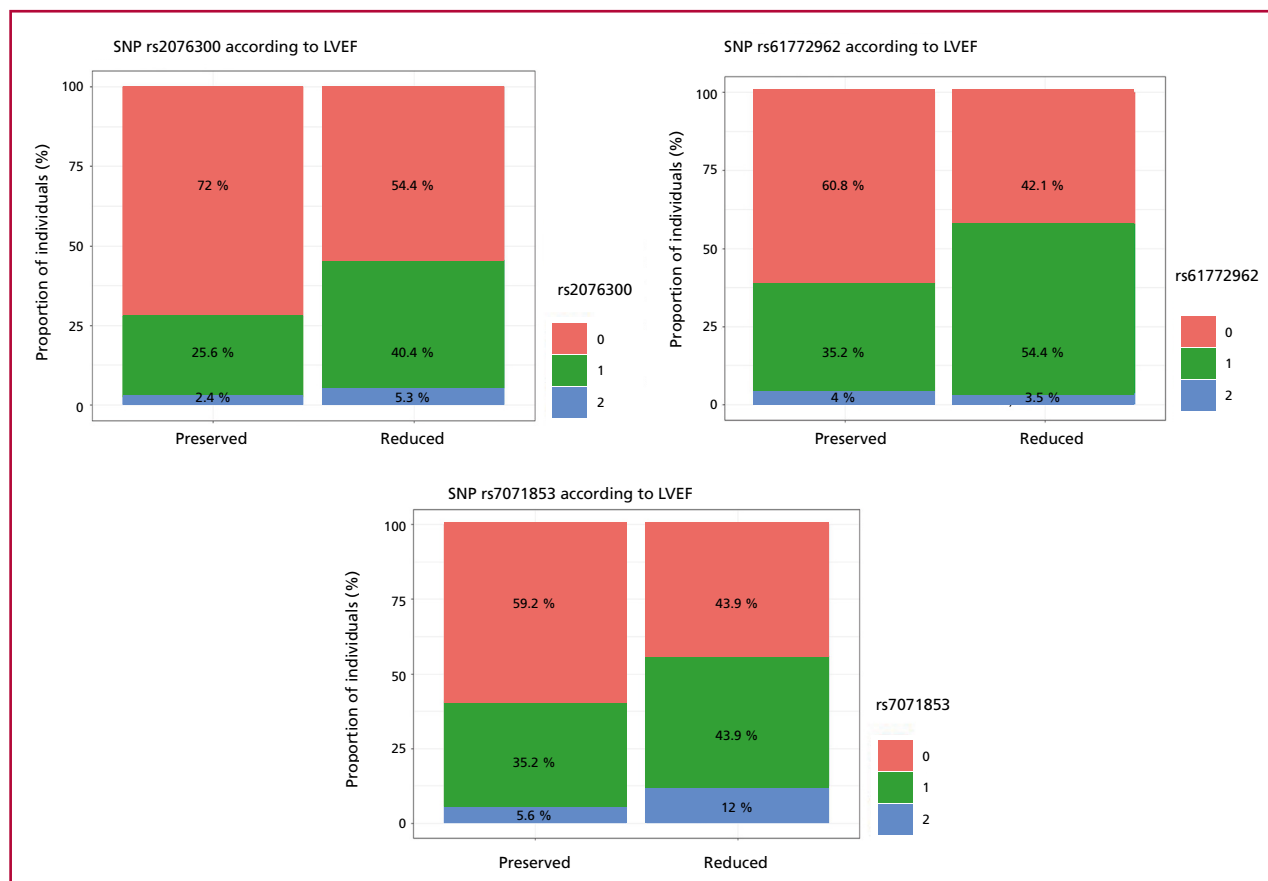
Predictor	Reduced LVEF		
	Odds ratio	95% CI	p value
rs2076300	1.79	1.01 – 3.21	0.046
rs61772962	1.77	1.00 – 3.14	0.049
rs7071853	1.77	1.07 – 2.96	0.026

95% CI: 95% confidence interval; LVEF: left ventricular ejection fraction; SNP: single nucleotide polymorphism

**Table 3.** Odds Ratio, 95% CI and p value for the only SNP associated with the presence of intraventricular conduction disorders

Predictor	Reduced LVEF		
	Odds ratio	95% CI	p value
rs72840788	2.44	1.04 – 6.22	0.048

95% CI: 95% confidence interval; SNP: single nucleotide polymorphism



LVEF: left ventricular ejection fraction; SNP: single nucleotide polymorphism  
 0: homozygous for the wild-type allele 1: heterozygous 2: homozygous for the alternative allele.

**Fig. 3.** Proportion of homozygous wild-type (0), heterozygous (1) and homozygous alternative allele (2) variants of SNP rs2076300 (3 A), rs61772962 (3 B) and rs7071853 (3 C), according to the presence of reduced or preserved LVEF

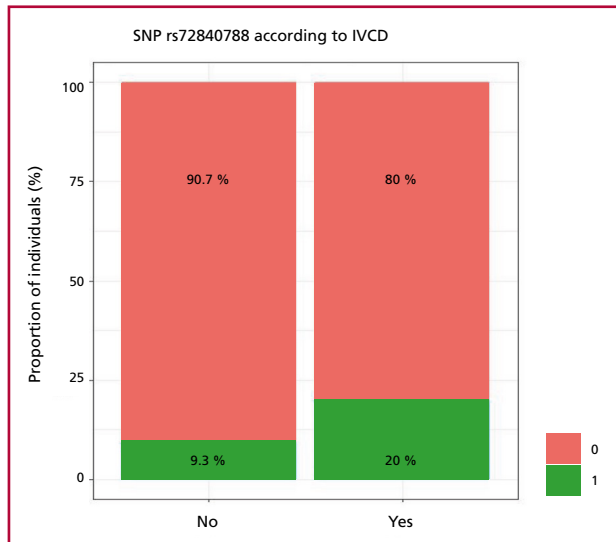
One of the most widely studied SNPs is the variant responsible for the change of arginine to glycine (Arg-389Gly) in the Beta 1 receptor. In the general population, Arg389Gly polymorphism was not significantly associated with HF, but in the subgroup analysis according to ethnicity, the presence of Gly389 in Asian patients was shown to increase the risk of HF by 35% compared to Arg389 carriers (RR 1.35; 95% CI: 1.16-1.57;  $p < 0.001$ ). In a more detailed subgroup analysis, Arg389 homozygotes were associated with an improvement in LVEF in East Asians (95% CI 1.85-3.40;  $p < 0.001$ ) and mixed population (95% CI 0.72-2.91;  $p < 0.001$ ); while among white patients, Arg389 homozygotes had an improvement in LV systolic diameter. The improvement was significantly greater than

that of Gly389 (95% CI 0.04-0.36,  $p=0.001$ ). (23-26)

In the case of renin genes, G/AI 9-83 polymorphisms were analyzed in patients with DCM. The heterozygous form was found in only 37.5% of control subjects. This study did not show involvement of renin A/G polymorphisms in HF pathogenesis. (27)

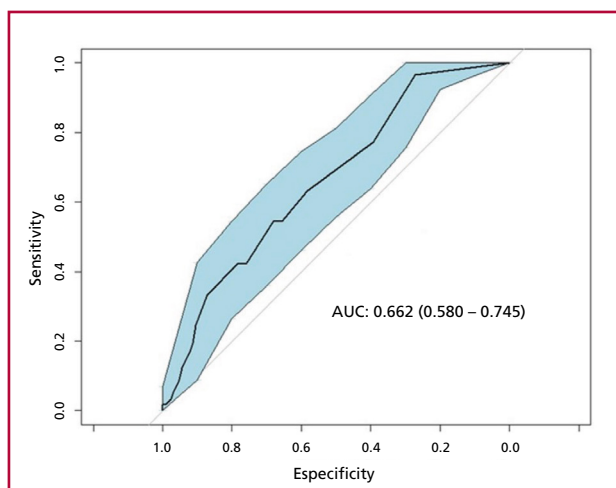
Patients with DCM may present hypertrophy and fibrosis. An association of the angiotensin II type 1 receptor and gene polymorphisms (AGTR1, A1166C) was observed in angiotensin converting enzyme (ACE)-mediated left ventricular hypertrophy (LVH) in endurance athletes, that would be a variant for future study in DCM. (28)

On the other hand, in several studies, two SNPs (rs763361 and rs727088) in the last exon of CD226



IVCD: intraventricular conduction disorder; SNP: single nucleotide polymorphism  
 0: homozygous for the wild-type allele 1: heterozygous

**Fig. 4.** Proportion of homozygous wild-type, heterozygous and homozygous variants of the alternative allele of the SNPrs72840788, according to the presence of IVCD



AUC: area under the curve; LVEF: left ventricular ejection fraction; 95% CI: 95% confidence interval

**Fig. 5.** Predictive performance of the model for the presence of reduced LVEF: area under the ROC curve (0.662) and its 95% CI (0.580-0.745)

have been reported in the Titin gene (TTN), as associated with an increased risk of DCM. (29)

Another gene to consider is MMP2. This gene SNPs were analyzed to evaluate their association with the development of DCM and three of them were found to be related both to the risk of suffering from it as to its worse prognosis. (30)

Since there is no consensus on the variants that could predispose to the development of DCM in response to harmful factors affecting the myocardium, we decided to establish an analysis panel. This in-

cludes structures related both to neurohumoral systems as to aspects of energy metabolism that could be involved in the development of DCM. The international database TheGenomeAggregationDatabase (GnomAD) choosing SNPs with a frequency of 10% to 40% in the world population. The SNPs used are detailed in Table S1.

In our analysis we were able to observe that the variants rs61772962, rs7071853 and rs2076300 were independent predictors of reduced LVEF in our population of patients with chronic Chagas cardiomyopathy.

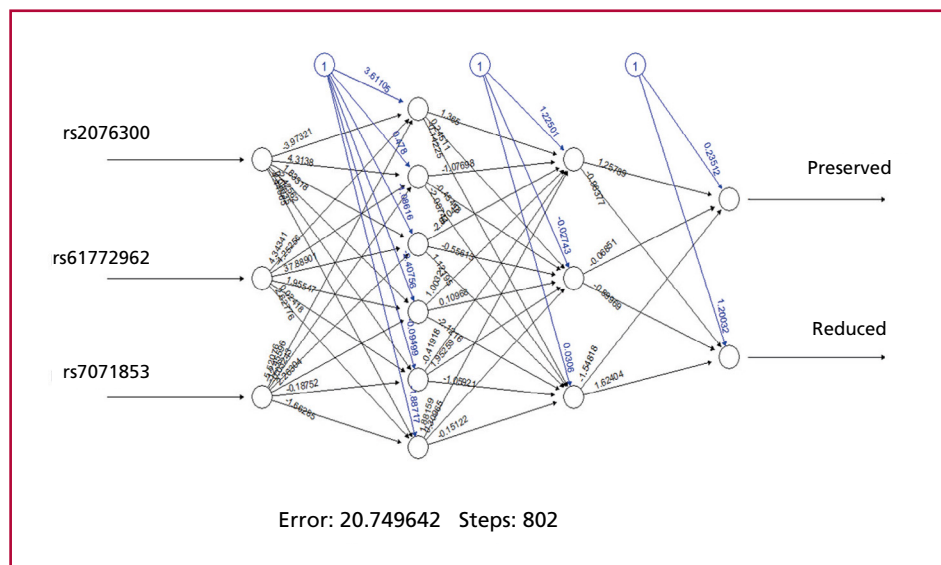
The SNP rs61772962 corresponds to an intronic variant of the PRKAA2 gene. This gene encodes the alpha-2 catalytic subunit of the 5'-AMP-activated protein kinase. This AMPK protein, is an important cellular energy-sensing enzyme. In response to cellular metabolic stress, AMPK is activated and thus phosphorylates and inactivates acetyl-CoAcarboxylase (ACC) and beta-hydroxy beta-methylglutaryl-CoA reductase (HMGCR) key enzymes involved in the regulation of de novo biosynthesis of fatty acids and cholesterol. (31) The AMPK $\alpha$ 2 variant is required for energy metabolism in cardiomyocytes. (32)

The RNF207 and PRKAA2 genes, known for their involvement in cardiac action potentials, energy homeostasis and morphology, have been postulated as candidates for DCM models after study in dogs and follow-up in humans. (33)

Dominant mutations in the  $\gamma$ 2 regulatory subunit of AMP-activated protein kinase (AMPK), encoded by the PRKAG2 gene, cause glycogen storage cardiomyopathy in mutant mice. (34)

The rs7071853 corresponds to the gene for the BAG3 regulatory protein (position chr10:119552094 (GRCh38.p14). On the other hand, rs72840788 was associated with the prediction of conduction disorders and corresponds to the intronic position of that protein. The BAG3 protein plays an important role in maintaining myocardial homeostasis and excitation-contraction coupling and is an adaptive mechanism to maintain cellular homeostasis under stress. It is most prominently expressed in the heart, skeletal muscle, and in many forms of cancer. In the heart, it acts as a chaperone for heat shock proteins to facilitate autophagy. Mutations in BAG3 have been associated with the development of a variety of phenotypes, including hypertrophic/restrictive and dilated cardiomyopathy. (35-40)

The rs2076300 is part of the DSP gene that encodes for desmoplakin, a calcium-dependent protein of the cadherin family essential for desmosomes, multiprotein structures involved in signal communication between cells and in the coordination of cardiac muscle contractions. Desmosomes are critical intercellular junctions for the mechanical and electrical integrity of tissues, specially in the myocardium and epithelial tissues. Mutations in the DSP gene are typically associated with arrhythmogenic cardiomyopathy. However,



**Fig. 6.** Neural network to assess the predictive power of reduced LVEF of the three identified SNPs

LVEF: left ventricular ejection fraction SNP: single nucleotide polymorphism

they can also cause dilated cardiomyopathy, which presents with a higher incidence of ventricular arrhythmias and an increased risk of sudden death. This finding is significant, as it demonstrates that a proportion of patients with a clinical diagnosis of dilated cardiomyopathy harbor mutations in genes encoding intercalated disc proteins. (41-45)

Attempting to confirm these results with other statistical methods using the “Bonferroni threshold” did not yield reproducible results. Since most effects of a single SNP are in the range of a 10 to 15% modification of the baseline risk of presenting a certain phenotype, the Bonferroni adjustment for the evaluation of 70 SNPs, maintaining the global alpha level of 0.05 and a power of 80%, determines that the rejection of the null hypothesis for each test could be carried out only with a p value  $<0.0007$ , which implies, under the most optimistic effect of a 15% increase in risk (RR = 1.15), the need to recruit 6280 individuals. This is why we believe that the results could not be confirmed with other tests, due to the sample size used.

## CONCLUSION

In this derivation model, in a group of patients with positive serology for Chagas, 3 SNPs were identified as predictors of reduced LVEF (rs2076300, rs61772962 and rs7071853) and one predictor of conduction disorders (SNP rs72840788).

When trying to confirm these results with other statistical methods using the “Bonferroni threshold” the results could not be reproduced, due to the sample size used. This reinforces the idea that we must continue in this line of analysis, increasing the number of patients. It is essential to continue exploring the hypothesis that, even in the absence of a family history

of DCM and pathogenic variants in genetic studies, there may be polymorphisms that predispose to the development of ventricular dilatation.

This approach would be very useful in the future, not only for early identification of patients predisposed to present DCM and HF, but also for anticipating treatment, thus improving both morbidity and mortality in our patients. We believe that this could open new perspectives in pharmacogenomics applied to HF.

This study aims to be a starting point to promote future research and strategies. Its intention is to establish a line of research that, by incorporating a larger number of patients and continuing with the analysis, will make it possible to achieve greater statistical power.

## Conflicts of interest

None declared.

(See authors' conflict of interests forms on the web).

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## Policy on the use of artificial intelligence

Artificial intelligence resources have not been used to write the text or to create the tables or graphs.

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## SUPPLEMENT 1

Table S1. Analyzed SNPs

GENE	SNP		GENE	SNP	
ADRB1	rs12414657	Upstream variant	BAG3	rs1831018	BAG cochaperone 3
	rs1801252	Missense variant		rs72840788	Intron
	rs1801253	Missense variant		rs7071853	
	rs3813719	Downstream variant	ATP2A2	rs1860561	Intron
	rs3813720	Downstream variant	DSP	rs2076300	Synonymous variant
CHRM2	rs13247260	Upstream intron		rs926411	Intron
	rs6962027	Prime UTR variant	SIRT1	rs2236318	Intron
	rs6967953	Prime UTR variant		rs2273773	Synonymous variant
NOS1	rs12811583	intron	PPARGC1A	rs2290604	Intron
	rs1875140	intron		rs3755863	Synonymous variant
	rs3741475	Synonymous variant	AGTR1	rs275653	Upstream variant
TTN	rs2042995	Missense variant		rs5186	3 Prime UTR variant
	rs2255167	intron		rs387967	Upstream variant
PRKAA2	rs17848595	Synonymous variant		rs422858	Upstream variant
	rs61772962	Intron	SOD2	rs2758332	Intron
	rs17848596	Missense variant		rs5746094	Intron
MTOR	rs1034528	Intron	PRKAB1	rs278145	intron
	rs11581010	Intron		rs278149	intron
	rs17036350	Intron	PPARGC1A	rs2946385	Stop gained
	rs74225573	Intron		rs8192678	Missense variant
RYR2	rs10802607	Intron	PRKAB2	rs34838459	Synonymous variant
	rs67622164	Intron	LOC105375743	rs34866937	intron
	rs10925391	Intron	AKT1	rs3730346	intron
	rs16835818	Intron		rs3730358	intron
JUP	rs1126821	Missense		rs3803304	intron
	rs8067890	Intron	HIF1A	rs373909145	intron
	rs7405731	Missense variant	NPPA	rs5063	Missensevariant
	rs7216034	Intron		rs5064	Intron
SOD2	rs11752345	Intron		rs5065	stop loss
	rs4880	Missense variant	DERL32KB	rs6003909	Upstream variant
PRKAB2	rs1348316	Intron	NOS2P3	rs62066941	Intron
	rs72708505	Intron	LDHA	rs6498	Synonymous variant
CDKN1A	rs146170154	Intron	SH2B3	rs7310615	Intron
ZNF592	rs149369954	non coding transcrip tvariant	SIRT1	rs7896005	Intron
			CHRM2	rs8191992	3 Prime UTR variant
			CLCNKA2KB	rs945425	Upstream variant

SNP: single nucleotide polymorphism



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