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ORIGINAL ARTICLE

Obesity-Related Genetic Determinants of Heart Failure Prognosis

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Abstract

Purpose Recent advances in genomics offer a smart option for predicting future risk of disease and prognosis. The objective of this study was to examine the prognostic value in heart failure (HF) patients, of a series of single nucleotide polymorphisms (SNPs).

Methods A selection of 192 SNPs found to be related with obesity, body mass index, circulating lipids or cardiovascular diseases were genotyped in 191 patients with HF. Anthropometrical and clinical variables were collected for each patient, and death and readmission by HF were registered as the primary endpoint.

Results A total of 53 events were registered during a follow-up period of 438 (263–1077) days (median (IQR)). Eight SNPs strongly related to obesity and HF prognosis were selected as possible prognostic variables. From these, rs10189761 and rs737337 variants were independently associated with HF prognosis (HR 2.295 (1.287–4.089, 95% CI); $p = 0.005$), whereas rs10423928, rs1800437, rs737337 and rs9351814 were related with bad prognosis only in obese patients (HR 2.142 (1.438–3.192, 95% CI); $p = 0.00018$). Combined scores of the genomic variants were highly predictive of poor prognosis.

Conclusions SNPs rs10189761 and rs737337 were identified, for the first time, as independent predictors of major clinical outcomes in patients with HF. The data suggests an additive predictive value of these SNPs for a HF prognosis. In particular for obese patients, SNPs rs10423928, rs1800437, rs737337 and rs9351814 were related with a bad prognosis. Combined scores weighting the risk of each genomic variant could effect interesting new tools to stratify the prognostic risk of HF patients.

Keywords Heart failure prognosis · Genomic cardiovascular predictors · Cardiometabolic disorders · Single nucleotide polymorphisms

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Introduction

Heart failure (HF) is a complex cardiometabolic disorder resulting from the combination of genetic and environmental factors. However, the current definition of HF restricts itself to stages at which clinical symptoms are apparent [1], and subsequently, therapy is mostly directed to the underlying cardiac problem, which will determine the specific treatment to use. Consequently, the genetic component of the disease is usually not addressed in clinical management.

Considering that HF remains the leading cause of hospitalisation in elderly people in the USA [2], and that the incidence of the disease is predicted to rise over the next years [3], it becomes important and urgent to understand the genetic basis of the disease. Some common gene variants could increase the risk of developing HF and others could modify the disease progression or the response to pharmacological therapy [4]. Thus, recent advances in genomics offer a smart option for predicting the future risk of disease early in life or prognosis. However, the complexity and heterogeneity of HF with many aetiological roots have made the identification of genes difficult. This obligates researchers to conceive new approaches to deal with the problem. The common idea is to look for a direct underlying cardiac cause of HF, that is, a myocardial abnormality causing systolic and/or diastolic ventricular dysfunction. Therefore, we moved to more indirect causes of HF based on its metabolic component. In this sense, a series of gene variants in the form of single nucleotide polymorphisms (SNPs) have been characterised for complex cardiometabolic disorders [5] that could be related to HF. The main players in this scenario are circulating lipids and obesity. Many of these variants have been for blood lipids, with a total of 164 common SNPs identified to date at a genome-wide significance level [6, 7].

Extensive evidence demonstrates the adverse effects of obesity on central and peripheral haemodynamics, as well as on cardiac structure and function [8]. This explains the positive relationship between body mass index (BMI) and the risk of incidence of HF [9] or cardiovascular disease [10]. However, despite the adverse effects of obesity on cardiac structure and function, numerous studies have suggested that obese patients with HF have a better prognosis than non-obese patients, considering obesity as a category of BMI [11, 12], a high percentage of body fat [13] or a high waist circumference [14], the so-called obesity paradox. These data have suggested a possible protective role of body fat on HF that can be related to the fact that cardiac cachexia is related to cardiac dysfunction. More recently, it has been observed that patients who were overweight or obese before HF development have a lower mortality rate after HF diagnosis compared with normal BMI patients [15]. All these together suggest that even the weight loss recommendation usually given to obese patients with HF should be taken with care.

Taking all these information into account, the objective of this study was to select a series of SNPs found to be related with obesity, BMI or circulating lipids or directly with HF to be genotyped in a sample of patients with an HF diagnosis. The prognostic value of those SNPs on their clinical outcome was examined during a mid-time follow-up.

Materials and Methods

Subjects

Patients admitted to our tertiary hospital with a HF diagnosis—in accordance with the current European guidelines of clinical practice—were invited to participate in the study. The main exclusion criteria were invasive interventions within the last 6 months, acute coronary syndrome in the 3 months preceding admission, chronic inflammatory diseases, autoimmune diseases, malignant diseases, active infection in the last 3 weeks, pregnancy and serious kidney or liver disease. Anthropometrical and clinical variables were collected for each patient, and a follow-up period was established for registry of mortality and readmission due to HF as the primary endpoint. All patients were included in the study under a signed written informed consent. The entire study and protocols were approved by the Ethics Committee for Human Studies at Galicia (Spanish region) in accordance with the 1975 Declaration of Helsinki.

Measurements and Laboratory Data

Venous peripheral blood samples were obtained for the measurements, at the same time (8:00 am in the morning), after overnight fasting. Serum biochemistry was obtained with Cobas Integra model 700 multichannel analyser (Roche Diagnostics, Indianapolis, USA) and measurements such as triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and N-terminal pro brain natriuretic peptide (NT-proBNP) were calculated. BMI was calculated upon acceptance into the study and categorised as normal weight (< 25 kg/m^2), overweight ($25 \leq$ BMI < 30 kg/m^2) and obese (BMI ≥ 30 kg/m^2). The diagnosis of diabetes mellitus was based on the latest criteria established by the American Diabetes Association [16]. Hypertension was defined as systolic/diastolic blood pressure > 140/90 mmHg or the current use of any antihypertensive medication. Dyslipidaemia was defined by TC ≥ 5.69 mmol/L, TG ≥ 1.69 mmol/L and HDL-C < 1.03 mmol/L, or current use of antihyperlipidaemic drugs. Therapeutic strategy and pharmacological treatment were prescribed according to Clinical Practice Guidelines published by the European Society of Cardiology [1].

SNP Selection

The objective of this study was to assess the independent and interactive roles of genetic variants—identified from genome-wide association studies (GWAS) involved in metabolic traits, such as obesity, BMI, circulating lipids and HF (listing of SNPs in Supplementary Table S1) [6, 17–24]—on the clinical outcome of patients with HF. Selected SNPs included common genetic variants influencing obesity at two loci, FTO and MC4R, which have been reproducibly associated with obesity measured by BMI. Also, BMI-associated loci found in SH2B1, TMEM18, NEGR1, KCTD15, BDNF, ETV5/DGKG, SEC16B/RASAL2, BCDIN3D/FAIM2, SH2B1 and MTCH2 genes were included. Additionally, three loci in NPC1, near MAF and near PTER, as BMI-related alleles on the risk of obesity-related diseases, such as type 2 diabetes, were included. Recent GWAS studies have also localised common DNA variants affecting circulating serum HDL-C, LDL-C, TC and TG. SNPs at approximately 38 loci have been associated with one or more of the three traits at the genome-wide significance level (LCAT, APOB, APOE, PCSK9, LDLR, HMGCR, CETP, MLXIPL, GCKR, TRIB1, GALNT2). SNPs from GWAS associated with type 2 diabetes, related to obesity, and from other metabolic traits also obesity-related, such as glucose, insulin/insulin response and C-reactive protein, have been identified and were also included. We also included 25 SNPs identified from GWAS that increase the risk of breast cancer, a cancer for which obesity is an established risk factor and that has been shown to exhibit potential interactions with obesity [25].

Genotyping

DNA was extracted from the buffy coat by using the chemagic DNA Buffy Coat Kit special with the Chemagic MSM I system (Perkin Elmer, Waltham, MA), based on magnetic beads. After quantification of dsDNA using PicoGreen (Thermo Fisher, Waltham, MA, USA), the DNA was diluted to a final concentration of 50 ng/μL in water.

Genotyping of 200 SNPs was performed by the CEGEN-PRB2 USC node using the iPlex® Gold chemistry and MassARRAY platform, according to the manufacturer's instructions (Agena Bioscience, San Diego, CA, USA). All assays were performed in 384-well plates, including negative controls and a trio of Coriell samples (NA10860, NA10861 and NA11984) for quality control.

Follow-up Period

The primary endpoint was the combination of cardiac death and readmission due to HF. Cardiac death was confirmed by the review of the death certificate and hospital chart or the physician's records. Readmission due to HF was confirmed by the appropriate combination of symptoms, an electrocardiogram study and analytical changes.

Data Analysis

The statistical analyses were performed with Statistical Package for the Social Sciences (SPSS), version 17.0, and R version 3.2.3 [26]. The categorical or dichotomous variables were expressed as absolute values and percentages and were compared with the Pearson chi-squared (^{2}) test. The continuous variables were described as the mean ± standard deviation (SD) when normally distributed or as the median and interquartile range for non-parametric data. For the comparison of quantitative continuous variables, Student's *t* test or the analysis of variance (ANOVA) was used when the variables were normal, and the Wilcoxon signed-rank or the Kruskal–Wallis test was used otherwise. Pearson's or Spearman correlation tests were used with normal or non-normal data, respectively.

Due to the very similar number of variables and samples, prior to the analysis of each SNP with Cox regression checking for the ability of genotypes to predict mortality and readmission caused by HF, we carried out an unsupervised Lasso procedure for SNP selection. We used a cross-validation strategy as implemented in the function cv.glmnet() in the R package *glmnet*, with default parameters. Previously, we implemented a strategy to avoid missing data in the set analysed with *glmnet* (missing data is not allowed in *glmnet*). We considered different approaches with the Lasso Cox procedure: all samples, only obese samples, only non-obese samples and all samples but with SNP interaction with obese status. With this approach, we found a consensus list of SNPs to further evaluate with standard Cox regression with strong possibilities that this analysis would show any difference in relation to obesity. Since the Lasso selection of SNPs was based on a cross-evaluation and free of statistical tests, the reduced number of SNPs on this new consensus list of SNPs was used to establish a new significance threshold for standard Cox regression ($p < 0.00625$). Different Cox proportional hazard analyses were carried out to assess the independence of the clinical variables (including BMI, diabetes mellitus, hypertension, hyperlipidaemia and circulating lipid concentrations) to predict mortality and readmission due to HF and data were presented as hazard ratios (HR) with 95% confidence intervals (CI). Those clinical variables that were in this univariate analysis reached a p value of < 0.05 and additional general variables, such as age and gender, were included in the multivariate Cox regression analysis of the SNPs-HF association. Kaplan–Meier curves (analysed with a log-rank test) were performed to evaluate the prognostic value of genotypes during follow-up. In the quality control of genotyping, we asked that SNPs have no more than a 5% missing genotype rate and a strong Hardy–Weinberg disequilibrium ($p < 0.000001$). In the clinical records, we asked for the samples to have records

of HF, time of follow-up, gender, BMI, age, blood lipid concentrations, NT-proBNP, hypertension, hyperlipidaemia, diabetes mellitus, alcohol consumption and smoking.

Results

Data Quality Control

In the quality control for genotyping, some SNPs were discarded for analysis. rs4836133 was not biallelic; three other SNPs (rs13281615, rs2642442 and rs6602024) had a Hardy–Weinberg equilibrium, with an extremely low p value; and four other SNPs (rs1412239, rs2068888, rs206936 and rs8028313) had a missing genotype rate greater than 5%. Thus, we ultimately analysed 192 SNPs.

From the total patients initially included in the study, nine samples were discarded because they had a missing genotype rate greater than 5%. Twenty-seven other samples were discarded because of incomplete records (missing data) in the patient's database. Therefore, 191 samples with both good-quality genotypes and complete clinical records were ultimately analysed.

Patients and Outcomes

Initially, the cohort for this study included 227 patients with HF. After the quality control procedure detailed above, the final cohort analysed included 191 patients. The clinical characteristics of this final group of patients are shown in Table 1. The mean age of the population was 70.1 years, 67% of the patients were male, 34% were diabetic and 72.8% were hypertensive.

After a median follow-up of 438 (263–1077) days (median (IQR)), 31 patients died from cardiovascular deaths (16.2%) and 34 (17.8%) had HF readmission. In total, 53 cardiac events (27.7%) occurred considering the combined endpoint of death and hospital readmission due to HF. Patients that suffered events were older and had lower levels of LDL-C and TC and higher NT-proBNP levels. (Table 1).

SNP Selection by Lasso Regression

We carried out a Lasso procedure for SNP selection. We used the function cv.glmnet() in the R package, *glmnet*, with default parameters. As we described in the "Material and Methods" section, we tried different approaches in relation to obesity. The aim of this study was the "obesity paradox"; therefore, we tried to preselect any genetic marker with differences in their ability for an HF prognosis in relation to the exposure of interest, obesity. With COX regression, we obtained a consensus list of eight SNPs for further analysis with COX regression: rs10189761, rs10423928, rs10761731, rs1260326, rs1465330, rs1800437, rs737337 and rs9351814.

Univariate and Multivariate Analyses Revealed Predictors of Cardiovascular Events

After the SNP selection, using the above criteria, the consensus list of eight SNPs was analysed for their possible relationship with cardiovascular outcomes in a univariate analysis. The genotypes were categorised to transform each SNP in a dichotomous variable. Clinical variables and anthropometric parameters were also analysed, and the relevant results are shown in Table 2. Age, diabetes mellitus, TC and LDL-C were found to be significantly related with cardiovascular events.

Therefore, those baseline variables and gender were used for the adjusted multivariate analysis of each of the eight SNPs. Statistically significant characteristics and SNPs were identified by this method. We found that rs10189761 and rs737337 variants were independently associated with an HF prognosis. Age was also a bad prognostic variable whereas higher TC was related with better prognosis, as expected in HF patients. These results are shown in Table 3. Figure 1 shows the Kaplan–Meier curves for the prediction of cardiovascular events-free survival by the variants of the two SNPs described above.

A possible relationship between the SNP variants and BMI can influence the prognosis of cardiovascular disease. Therefore, this relationship was analysed for each of the two SNPs that were examined further. However, the Kaplan–Meier analysis of each SNP for the prognosis of HF, stratifying the subjects based on being obese (BMI $\geq$ 30 kg/m^2) and non-obese (BMI < 30 kg/m^2), showed that rs737337 (log rank = 0.382 or 0.021, for non-obese and obese subjects, respectively), but not rs10189761 (log rank = 0.079 or 0.162, for non-obese and obese subjects, respectively), could show a relationship with obesity and HF prognosis. The dependence on obesity of the prognostic value of the eight selected SNPs was analysed by Cox regression. rs10423928, rs1800437, rs737337 and rs9351814 were better predictors of cardiovascular events in obese than in non-obese patients (Table 3), and virtually no effect was detected among non-obese patients.

Additive Value of Different SNPs

Because the different variants of the SNPs are located on different chromosomes and may have different non-interactive functions, we tried to test whether the prediction of cardiovascular outcomes in patients with HF could be improved by considering the findings for multiple loci. We suggested a combined score for rs10189761 and rs737337 (Score I; Supplementary Table S2) to stratify the risk of death or readmission by HF. The risk alleles AA of rs10189761 and TT of rs737337 were given a weighted score of 1 and the risk

Table 1 Baseline patient characteristics of total population and regarding the appearance of a cardiovascular event

Variable	Total population ($n = 191$)	Free events ($n = 138$)	Events ($n = 53$)	p value
Age (years)	70.1 ± 12.5	68.4 ± 12.7	74.9 ± 10.4	*0.002*
Male (%)	67.0	65.0	71.7	0.377
DM (%)	34.0	32.1	39.6	0.328
HT (%)	72.8	71.5	75.5	0.585
HLD (%)	55.5	53.3	60.4	0.378
LVEF (%)	41.8 ± 14.9	42.2 ± 14.3	40.6 ± 16.3	0.495
BMI (kg/m^2)	30.0 (29.2–31.0)	30.0 (28.9–31.1)	29.9 (28.6–31.3)	0.517
TG (mg/dL)	110.0 (102.-119)	114.(104–124)	97.5(83.0–111.0)	0.069
TC (mg/dL)	159.1 ± 43.9	166.1 ± 44.1	139.5 ± 36.5	*< 0.001*
HDL-C (mg/dL)	43.9 (41.5–46.3)	45.6 (42.6.-48.6)	40.0 (36.3–43.3)	0.038
LDL-C (mg/dL)	90.3 ± 37.0	95.3 ± 38.7	76.9 ± 28.5	*0.002*
NT-ProBNP (pg/mL)	4166 (3256–5076)	2797 (2145–3448)	7740 (5111–10,368)	*< 0.001*
ACEI (%)	71.7	72.3	69.8	0.737
BB (%)	79.6	81.0	75.5	0.396
Diuretics (%)	84.8	81.8	92.5	0.166
Mineralocorticoid antagonists (%)	76.4	78.8	69.8	0.190

Statistical difference ($p < 0.05$) indicated in italics, with respect to the presence of events (death or readmission by HF)

Abbreviations: *ACEI*, angiotensin-converting enzyme inhibitors; *BB*, beta-blockers; *BMI*, body mass index; *DM*, diabetes mellitus; *HDL-C*, high-density lipoprotein cholesterol; *HLD*, hyperlipidaemia; *HT*, hypertension; *LDL-C*, low-density lipoprotein cholesterol; *LVEF*, left ventricle ejection fraction; *NT-proBNP*, N-terminal pro brain natriuretic peptide; *TC*, total cholesterol; *TG*, triglycerides

alleles AT or TT of rs10189761 and CC or CT of rs737337 were given weighted scores of 0. In each patient, the sum of the weighted scores from rs10189761 and rs737337 was used to predict major clinical events-free survival. The combined Score I was highly predictive of a bad prognosis and was independently associated with death and readmission by HF even after adjusting for baseline characteristics (HR 2.295 (1.287–4.089, 95% CI); $p = 0.005$). Figure 2 shows the Kaplan–Meier curves for the prediction of cardiovascular events-free survival by the suggested combined Score I.

Table 2 Univariate Cox analyses of cardiovascular events (cardiovascular death + readmission by HF) for clinical variables

Variable	HR (95% CI)	p value
Age	1.036 (1.009–1.064)	*0.009*
Gender (male)	0.906 (0.494–1.663)	0.751
DM	2.256 (1.256–4.050)	*0.006*
HT	1.746 (0.925–3.296)	0.085
HLP	1.475 (0.849–2.561)	0.168
Smoker	1.009 (0.687–1.482)	0.964
Alcohol	0.520 (0.234–1.152)	0.107
BMI	1.007 (0.961–1.055)	0.778
TC	0.981 (0.974–0.989)	*< 0.001*
LDL-C	0.983 (0.975–0.992)	*< 0.001*

Statistically difference ($p < 0.00625$) indicated in italics

Abbreviations: *BMI*, body mass index; *DM*, diabetes mellitus; *HDL-C*, high-density lipoprotein cholesterol; *HLD*, hyperlipidaemia; *HT*, hypertension; *LDL-C*, low-density lipoprotein cholesterol; *LVEF*, left ventricle ejection fraction; *NT-proBNP*, N-terminal pro brain natriuretic peptide; *TC*, total cholesterol; *TG*, triglycerides

Considering the results of the SNP variants to predict cardiovascular events in obese and non-obese patients (Table 3), a new score (Score II; Supplementary Table S3) was calculated to evaluate the risk of new events in obese and non-obese patients. This new combined Score II for rs10423928, rs1800437, rs737337 and rs9351814 was highly predictive of a bad prognosis. It was independently associated with death and readmission caused by HF after adjusting for baseline characteristics only in obese patients with BMI ≥ 30 kg/m^2 (HR 2.142 (1.438–3.192, 95% CI); $p = 0.00018$), but not in non-obese patients. Figure 3 shows the Kaplan–Meier curves for the prediction of cardiovascular events-free survival by the combined Score II for obese and non-obese patients.

Discussion

Two SNPs (rs10189761 and rs737337) were identified as predictors of major clinical outcomes in patients with HF,

Table 3 The association between genetic variants and cardiovascular outcomes

	Multivariate*					
	Total population		Obese		Non-obese	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
rs10189761 (AA)	2.099 (1.028–4.289)	*0.042*	1.904 (0.713–5.088)	0.199	2.664 (0.879–8.077)	0.083
rs10423928 (AT)	1.683 (0.923–3.070)	0.089	3.281 (1.333–8.077)	*0.010*	0.937 (0.381–2.299)	0.886
rs10761731 (AA)	1.177 (0.616–2.248)	0.622	1.918 (0.779–4.719)	0.156	0.785 (0.283–2.178)	0.642
rs1260326 (CT/TT)	1.670 (0.824–3.385)	0.155	6.735 (0.891–50.894)	0.065	1.041 (0.449–2.411)	0.926
rs1465330 (TT)	1.283 (0.612–2.691)	0.509	2.555 (0.981–6.657)	0.055	0.493 (0.137–1.778)	0.280
rs1800437 (CG)	1.704 (0.929–3.123)	0.085	3.242 (1.316–7.988)	*0.011*	0.941 (0.381–2.323)	0.894
rs737337 (TT)	3.682 (1.133–11.962)	*0.030*	9.465 (1.236–72.500)	*0.030*	1.615 (0.369–7.078)	0.525
rs9351814 (AC/CC)	1.750 (0.950–3.224)	0.073	3.245 (1.239–8.493)	*0.017*	0.857 (0.358–2.052)	0.729

Italicised values indicate p values < 0.05

*Adjusted by age, gender, diabetes mellitus and total cholesterol

independent of age, diabetes mellitus, blood lipid concentrations and obesity. To the best of our knowledge, this is the first time that this finding has been reported for these SNPs. Four SNPs (rs10423928, rs1800437, rs737337 and rs9351814) were also predictors of cardiovascular events, but only in obese patients with HF, not in non-obese patients. Combined scores weighting the risk of each allele of these genomic variants were created. Score I, which consisted of the two SNPs affecting the whole population, was also a good independent predictor of a bad prognosis in this clinical context. This revealed an additive value of each of these SNPs over the total outcome, suggesting that the combination of these variants increased the predictive value. Score II, which involved the four SNPs affecting obese patients, was also a predictor of cardiovascular events in this subpopulation—revealing its particular value for obese patients with HF.

A selection of 200 SNPs, with a possible relevant role in cardiovascular disease, was made based on a previously reported association of these genomic variants with cardiometabolic disorders present in the pathophysiology of HF, mainly lipid disorders and obesity, as well as a direct relation with HF. After a quality control analysis of the genotyping data, only 192 SNPs could be included in the final study. To select the SNPs most clearly associated with obesity, a Lasso procedure was carried out, from which a consensus list of eight SNPs was obtained. However, only two of the eight SNPs, rs10189761 and rs737337, were independently associated with the primary endpoint (the combination of cardiovascular death and readmission by HF) after an adjusted analysis for baseline characteristics. Interestingly, there was no direct relationship between the SNP variants and BMI. Also, this parameter did not influence the association of SNPs with prognosis. Patients who underwent cardiovascular events were older and presented lower levels of TC and LDL-C. This result is not new, as low serum TC and LDL-C levels predicted poor outcomes in patients with chronic HF [27], regardless of aetiology and systolic function [28]. However, obesity seems to influence the relationship of the SNPs with the prognosis of HF since four SNPs, rs10423928, rs1800437, rs737337 and rs9351814, were independently associated with a bad prognosis in obese patients, but not in non-obese patients. These data enhanced the relationship of these SNPs with obesity, and revealed their value for stratifying the cardiovascular risk in this subpopulation.

Little is known about the genes and proteins related with the genetic variants revealed by the study. SNP rs10189761 is in the transmembrane protein 18 gene (TMEM18) in region 2p25.3. This SNP, to our knowledge, has not been previously related to HF or directly to cardiovascular diseases. However, TMEM18 is one of the most conserved human obesity genes confirmed by genome-wide association studies [24, 29]. Its role as an obesity risk gene has been confirmed in adult Europeans and extended to childhood and adolescent obesity [30, 31]. TMEM18 encodes a small transmembrane protein, which localises to the nuclear membrane in a wide variety of tissues, including the brain—particularly in the hypothalamus [32]—which allows for the speculation that this protein (or gene) might play a role in appetite regulation, and thus eating behaviours. Although the exact function of TMEM18 has not been clearly elucidated, it is hypothesised that TMEM18 might be involved in gene silencing, as a consequence of its ability for DNA binding and restraining the chromatin very close to the nuclear membrane [33].

Interestingly, the other SNPs related with prognosis have been previously associated with the regulation of

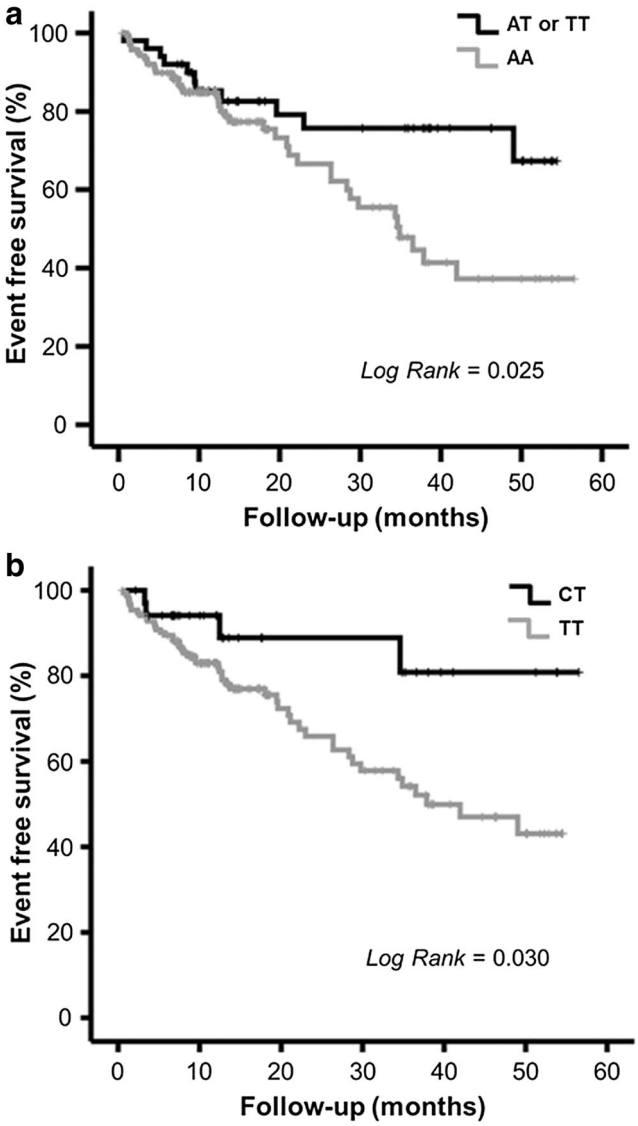

Fig. 1 The SNP predictive model of cardiac death and readmission by HF-free survival in patients with HF. The prognosis prediction of rs10189761 (**a**) and rs737337 (**b**)

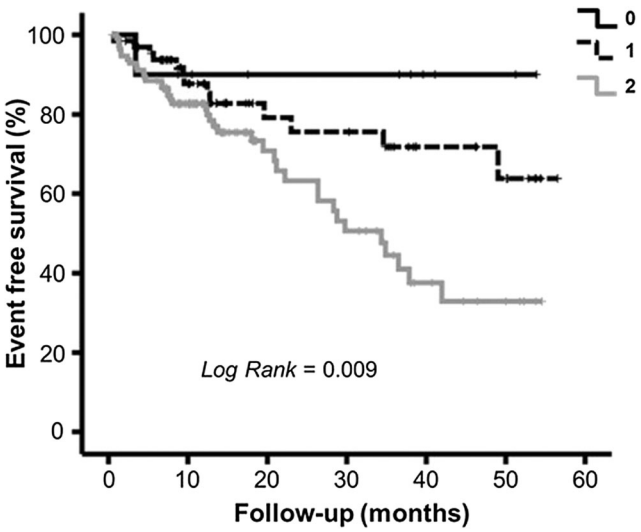

Fig. 2 The genomic combined score of rs10189761 and rs737337 for the prediction of cardiac death and readmission by HF-free survival in patients with HF

blood lipids. SNP rs737337 is in the locus LOC55908 of the chromosome 19, near the gene angiopoietin-like 8 (ANGPTL8), also known as betatrophin, and has been associated with circulating HDL-C [6]. SNP rs737337, encoding p.Thr712Thr, was also associated with total cholesterol levels in the Chinese population [34]. ANGPTL8 is a liver and adipose tissue–produced protein that regulates triglyceride levels in plasma. In accordance with the suggested ANGPTL3-4-8 model, upon refeeding, circulating ANGPTL3 and ANGPTL8 promote the replenishment of white adipose tissue depots by specifically inhibiting lipoprotein lipase activity in oxidative tissues (cardiac and skeletal muscles). This correlates with the increase of glucose levels. During fasting or exercise and cold exposure, ANGPTL4 represses white adipose tissue lipoprotein lipase activity to assure that plasma triglycerides are specifically shuttled to exercising muscle and brown adipose tissue, respectively [35]. Interrupting this balance may lead to obesity, lipotoxicity or hypertriglyceridaemia, representing excess triglycerides in white adipose tissue, non-adipose tissues and plasma, respectively [36]. In fact, circulating concentrations of ANGPTL8 were significantly lower in individuals with dyslipidaemia [37] and were increased in obesity and reduced after exercise training, supporting the ANGPTL3-4-8 model [38]. Obese subjects are characterised by lower fasting ANGPTL8 and a more pronounced ANGPTL8 suppression during the glucose challenge. The impaired ANGPTL8 response in obese subjects is restored after weight loss and is comparable with lean individuals [38]. These interesting functions of the ANGPTL pathways have made it possible to focus on possible new targets for metabolic disorders, and they could help elucidate the relationship observed between rs737337 and an HF prognosis and obese patients with HF.

Regarding the four obesity-related predicting SNPs, apart from rs737337 (explained previously), rs9351814 has not been related with a gene. Interestingly, rs10423928 and rs1800437 are both located in the glucose-dependent insulinotropic polypeptide receptor (GIPR) gene. This receptor signals for GIP, an incretin hormone secreted from gastrointestinal cells in response to food intake to control whole-body metabolism. However, it has extrapancreatic effects [39]. Regarding cardiovascular problems, GIPR expression levels are higher in carotid endarterectomies from patients with symptoms than in asymptomatic patients, and the expression is associated with unstable and inflammatory plaques [40]. On the contrary, inactivation of GIPR improves

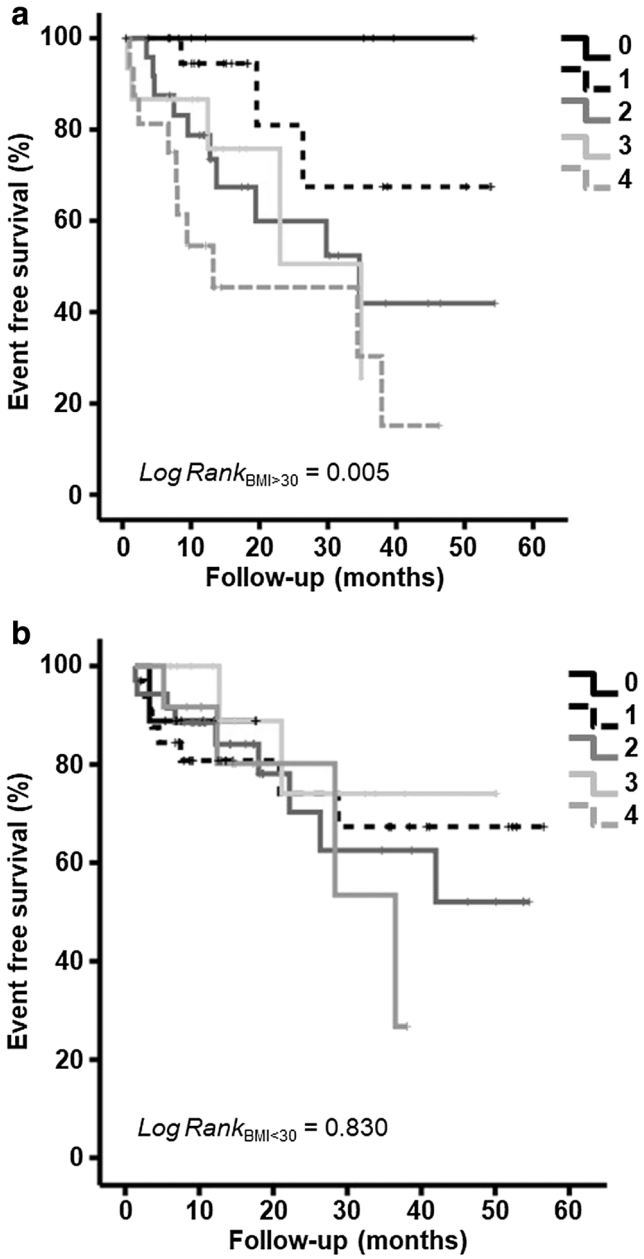

Fig. 3 Kaplan–Meier curves for the prediction of cardiovascular events-free survival by the combined score 2 considering obese patients (BMI ≥ 30 kg/m^2) (**a**), and considering non-obese patients (BMI < 30 kg/m^2) (**b**)

outcomes following experimental myocardial infarction [41]. Therefore, the GIPR gene seems to have a role in the evolution of cardiovascular disease directly or indirectly associated to its metabolic role. Our results, through the SNP variants on this gene, link the association of GIPR to obesity, with the outcomes of patients with HF, for the first time to our knowledge.

Given the multigenic and multifactorial aetiology of HF, it could be even more useful than a single marker, using a combination or score of different independent or related markers. This was the purpose of the suggested combined Score I in this study. It was found that the combination of the risks weighting for each SNP (rs10189761 and rs737337) showed an additive predicting value for HF outcomes. Something similar occurred for Score II in the obese subpopulation. As Score II increased, the incidence of cardiovascular events increased. This suggests the independent contribution of each one of the four SNPs included in Score II to the prediction of adverse outcomes. Therefore, even without knowing the molecular mechanism associated with each variant, and the possible causal relation of this modification with the evolution of HF, our finding would serve to better stratify the future risk of complications regarding HF, particularly in obese patients. Further studies in larger populations should be conducted to confirm our results.

Limitations

The main limitation of our study was the reduced number of patients recruited and that they were non-consecutive. Although the results were statistically significant, additional larger studies would help confirm our findings. Our study population was almost all Caucasian, so possible variations of our findings in different ethnicities would be possible.

Conclusions

SNPs rs10189761 and rs737337 were identified, for the first time, as independent predictors of major clinical outcomes in patients with HF. The data suggests an additive predictive value of these particular SNPs for HF prognosis. In particular for obese patients, SNPs rs10423928, rs1800437, rs737337 and rs9351814 were observed to be related with a bad prognosis. Combined scores weighting the risk of each allele of these genomic variants were shown to be good independent tools for the prediction of a bad prognosis in the clinical context of HF. These findings would effect very interesting new tools to stratify the prognostic risk of HF patients.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and the Ethics Committee for Human Studies at Galicia (Spanish region) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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