Association between beta$_1$-adrenergic receptor polymorphism and risk of ICD shock in heart failure patients

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ABSTRACT

Background

Sympathetic activation in heart failure patients favors the development of ventricular arrhythmias, thus leading to an increased risk of sudden cardiac death. β_1 and β_2 adrenergic receptor polymorphisms have been linked to the risk of sudden death. Implantable cardioverter-defibrillators (ICD) are implanted in a large percentage of heart failure patients, and beyond preventing sudden cardiac death they provide a continuous monitoring of major ventricular arrhythmias and of their own interventions. We investigated whether functionally relevant β_1 and β_2-adrenergic receptor polymorphisms are associated with risk of ICD shocks, as evidenced in ICD memory.

Methods

311 patients with systolic heart failure were enrolled, and number and timing of shocks in ICD memory were recorded.

Four selected polymorphisms were determined: β_1 adrenergic receptor polymorphisms $Ser^{49}Gly$ and $Arg^{389}Gly$ and β_2 adrenergic receptor polymorphisms $Arg^{16}Gly$ and $Gln^{27}Glu$.

Results

Only $Ser^{49}Gly$ was significantly correlated with time free from ICD shocks, both considering time to the first event in a Cox model (hazard ratio 2.117), and modeling repeated events with the Andersen-Gill method (hazard ratio 2.088). Gly allele

carriers had a higher probability of ICD shock. The relationship remained significant even after adjusting for ejection fraction and beta-blocker dosage (hazard ratio 1.910).

Conclusions

Data from our study suggest that the β adrenoreceptor Gly 49 allele of the β_1 adrenergic receptor Ser^{49}Gly polymorphisms may increase the risk of ICD shock in patients with heart failure, independently of beta-blocker dosage.

Introduction

In heart failure (HF) patients with reduced ejection fraction (EF), sympathetic activation, mediated by the β_1-adrenergic and β_2-adrenergic receptors, favors the development of ventricular arrhythmias, thus leading to an increased risk of sudden cardiac death. Implantable cardioverter-defibrillator (ICD) is therefore indicated in a large percentage of HF patients; beyond preventing sudden cardiac death, ICD provides a continuous monitoring of major ventricular arrhythmias and of its own interventions. ICD shocks represent an event with the peculiar characteristic that it can repeat several times.

In the present study, in order to assess the role of functionally relevant β_1 and β_2 adrenergic receptor polymorphisms, we investigated whether they are associated with the risk of ICD shocks.

Two β_1-adrenergic receptor functionally relevant single nucleotide polymorphisms (SNPs) have been identified, with amino acid substitutions $Arg^{389}Gly$ (1) and $Ser^{49}Gly$, and widely studied, associated with better prognosis in patients with HF in some, but not all, studies.

For the β_2-adrenergic receptor thirteen SNPs have been described; two common SNPs result in the amino acid substitutions $Gly^{16}Arg$ and $Gln^{27}Glu$. There are only a few reports suggesting a prognostic effect of these polymorphisms, both in general population (2) and in HF patients (3,4), while there are not studies on their effect on ventricular arrhythmias.

The role of these four polymorphisms was investigated in the present study in 311 patients with HF, implanted with an ICD according to current guidelines (5).

Methods

Study population

Consecutive eligible patients were enrolled from the HF outpatient clinic of two Hospitals (Verona and Mantova). Diagnosis of heart failure with reduced EF was based on criteria defined by European guidelines (5).

Eligibility criteria required that patients had to be implanted with ICD at least one month before, either for primary or for secondary prevention, according to current guidelines. Further eligibility criteria were age >18 years and reduced left ventricular EF at the time of ICD implantation, irrespective of functional class. All patients had to be of Caucasian ethnicity, as in the area non-Caucasian patients are very few, and their inclusion would have added heterogeneity, without reaching the statistical power to allow any comparison.

Patients with a documented history of myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass graft or >50% diameter stenosis of any of the major coronary epicardial arteries were classified as having ischemic HF. Other patients were classified as having nonischemic HF. Patients with HF caused by primary valvular disease, myocarditis, obstructive or hypertrophic cardiomyopathies were excluded from the present evaluation.

Patients were followed by the outpatient HF clinic of the two Hospitals. All patients had to be on optimal medical therapy on enrolment, according to European guidelines (5).

The study was approved by the Institutional Review Board of both participating hospitals; it was conducted in accordance with the Helsinki Declaration, and all patients provided written informed consent.

Demographic and clinical data

Demographic variables included sex and age. Clinical variables included ICD indication (primary vs. secondary prevention), the presence of right ventricular stimulation or biventricular stimulation, etiology (ischemic vs. nonischemic), and the presence of diabetes. These variables were obtained from medical records at baseline ICD assessment.

For any ICD intervention the dosage of β-blocker, titrated over time, was recorded; to keep into account different molecules, the dosage was expressed as the percentage of the target dosage in the European Guidelines (5). It was also recorded whether patient was on amiodarone, and if the rhythm before the shock was sinus rhythm or atrial fibrillation. For each ICD intervention, EF and diastolic filling pattern were recorded from the most recent routine echocardiogram, when available.

ICD therapies

All ICDs were programmed on an individual basis, without a standard protocol for the study. However each device had a “shock only" window for the treatment of high frequency ventricular arrhythmias (≥190 bpm). Arrhythmias in lower range of frequencies were only monitored (in patients implanted in primary prevention), or treated in the first instance with Anti-Tachycardia Pacing (ATP) (in patients implanted in secondary prevention).

The study end-point was appropriate ICD shock, delivered either for ventricular tachycardia or ventricular fibrillation in the high frequency range. Patients were seen at the center of implantation at regular intervals. Each subject’s ICD was interrogated during clinical follow-up visits. Arrhythmic events were recorded from the ICD and stored, or retrieved from archive storage. All therapy EGM recordings were reviewed by an expert electrophysiologist in order to rule out inappropriate ICD therapies.

Therapies delivered to treat rhythm other than ventricular tachyarrhythmias (e.g. atrial fibrillation) were considered inappropriate. Only appropriate therapies were included in the analysis. It was recorded also if the ICD intervention took place during an electrical storm, defined as the occurrence of 3 or more shocks during a single 24-h period (6).

The choice to include in the analysis only shocks and to exclude arrhythmias terminated by fast pacing, i.e. ATP, was based on considering that the time interval during which our data were collected was wide, and in the first years ATP was used mainly to treat slower tachycardias, with heart rate below 190-200; however, these arrhythmias do not always cause or proceed to cardiac arrest and cannot thus be considered a surrogate variable for fatal arrhythmias. Shock programming, on the contrary, was more homogenously applied over time in faster, not electrically organized arrhythmias, which are easily interpreted as a surrogate of sudden cardiac death (7,8).

Sample preparation and DNA Genotyping

Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) and stored frozen at -80° C prior to DNA extraction. Genomic DNA was extracted from whole blood by an automated “on-column” DNA purification method on a QIAsymphony SP instrument (QIAGEN GmbH, Germany), according to manufacturer’s protocols. DNA quality and concentration was assessed on a NanoDrop 8000 spectrometer (ThermoScientific Inc.). A 5’ nuclease assay with MGB TaqMan Probes (TaqMan® SNP Genotyping Assays, Life Technologies) on a ABI PRISM® 7900HT Sequence Detection System instrument (Life Technologies) was used to genotype the 5 selected polymorphisms, namely ADRB1 rs1801252 ($Ser^{49}Gly$), ADRB1 rs1801253 ($Arg^{389}Gly$), ADRB2 rs1042713 ($Arg^{16}Gly$), and ADRB2 rs1042714

(Gln^{27}Glu). Assay results were analyzed with the dedicated SDS software; all the automatic genotype calls were inspected by an operator to check for clusters quality and manually edited or removed when appropriate.

Statistical analysis

Continuous variables are presented as mean ± standard deviation or median and interquartile range when a Gaussian distribution could not be assumed. Categorical variables are presented as absolute numbers and percentages. All statistical analyses were performed using Intercooled Stata version 8.0.

A Cox proportional hazard model was applied in order to determine the independent role of genetic polymorphisms as predictors of appropriate ICD shocks, both as univariate predictors and adjusting for covariates. The hazard ratio (HR) is reported along with its confidence interval (c.i.). The role of genetic polymorphisms was first assessed on all 3 genotypes, then analyzing heterozygotes in combination with homozygotes for the variant allele. The Andersen-Gill proportional-intensity model (9) was used to identify the independent predictors of ICD shock. This technique allows all the events to be analyzed, in contrast to Cox modeling used in most studies, which only consider the first event. Coefficients are reported with their c.i. The model was applied using Intercooled Stata 8.0, for which the Andersen-Gill model algorithm had been published (10).

Results

Study population

A total of 311 patients were enrolled in the study. Demographics are reported in Table 1.

All patients were Caucasians, born in Italy. Male subjects were 263 (84.6%) and the average age was 64.2±10.6 years. The etiology was ischemic in 188 patients (60.5%). Sinus rhythm was observed in 221 patients (71.1%). The ICD had been implanted in primary prevention in 244 patients (78.5%); biventricular stimulation was applied in 210 patients (67.5%). Median beta-blocker dosage on enrolment was 50% (interquartile range 37.5-100).

Enrolment began on March 2009 and arrhythmia and ICD therapy endpoints were collected from the first record available (October 1998) up to November 2012. The median follow-up was 49.1 months (interquartile range 32.9-67.9).

ICD therapies

During follow-up 236 patients (75.9%) did not experience any shock. The median follow-up of patients without ICD interventions was 42.9 months (interquartile range 29.2-64.7). In 75 patients, 284 shocks were documented. A single ICD shock was recorded in 33 patients (11%). Multiple ICD shocks during follow-up were recorded in 42 patients. A total of 10 patients experienced 12 arrhythmic storms.

Beta adrenergic receptor polymorphisms

The genotype frequencies of the different polymorphisms are presented in Table 2.

The genotype frequencies were in agreement with those predicted by the Hardy-Weinberg equilibrium.

Beta adrenergic receptor polymorphisms and ICD shocks

The univariate relationship at the Cox model between time free from ICD shock and genetic polymorphisms is reported in Table 3. The relationship at the Cox model between time free from shock and homozygotes for the reference allele vs. carriers of the variant allele is reported in Table 4.

The analysis was then performed using the Andersen-Gill method, by which all events are kept into account. The results for ICD shock occurrence according to genetic polymorphism are reported in Table 5.

The influence of clinically relevant covariates was also assessed with the Andersen-Gill method: no effect was evident for gender, age, ICD indication (primary vs. secondary prevention), etiology (ischemic vs. nonischemic), or diabetes. The Andersen-Gill hazard ratio was significant for both β-blocker dosage (HR 0.976 for each 1% increase, c.i. 0.966 – 0.988, p=0.0001) and for ejection fraction (HR 0.948 for each 1% increase; c.i. 0.910 – 0.988; p=0.011). No effect was evident for atrial fibrillation, amiodarone therapy, biventricular stimulation and mitral diastolic filling pattern.

When including in a multivariate model the β_1 $Ser^{49}Gly$ polymorphism, the only one related with ICD shock at univariate analysis, and the two significant covariates (namely beta-blocker dosage and ejection fraction), all of them maintained the statistical significance (Table 6), thus proving their independent predicting value.

An additional sensitivity analysis was conducted: the analysis for repeated ICD shocks was performed excluding ICD storms, in order to avoid an excessive influence of patients with several ICD shocks. Nevertheless, the significance of the effect of β_1

Ser[49]Gly remained, and it was further increased (HR 2.892; c.i. 1.631 – 5.129; p=0.0001].

Discussion

Current guidelines (5) recommend ICD implantation for a significant proportion of the heart failure patients. It is however well known that, particularly in primary prevention, the proposed criteria have a low specificity. Even in our series, over a median 49 month period, 75% of the patients did not experience any ICD shock. Identifying new markers of arrhythmic risk could possibly improve risk stratification and ICD usage. Even potentially more interesting, on the opposite side, could be the identification of patients at higher risk of ICD shock, not only for the impact on the quality of life, but for the effect on long-term mortality of appropriate shocks, as evidenced by a recent meta-analysis (11).

Only in recent years an interest has arisen in the genetic influence on the risk of developing fatal ventricular arrhythmias in HF patients, in particular the presence of β1 and β2-adrenergic receptor polymorphisms.

For the β_1-adrenergic receptor two functionally relevant single nucleotide polymorphisms (SNPs) have been identified: a polymorphism leads to either a Glycine (Gly) or an Arginine (Arg) at amino acid position 389 ($Arg^{389}Gly$) and another polymorphism leads to either a Serine (Ser) or a Glycine (Gly) at amino acid position 49 ($Ser^{49}Gly$). The Arg389 allele has demonstrated higher coupling affinity and hyperactive signaling in experimental heart failure models. It has been reported to be associated with congestive heart failure and ventricular tachycardia (1).

The β_1-adrenergic receptor polymorphism $Arg^{389}Gly$ was significantly related with the presence of ventricular tachycardia on Holter monitoring, in one of the first papers on the topic, published by Iwai *et al.* (1) on 163 patients with idiopathic dilated cardiomyopathy; the Gly389 allele was associated with a lower frequency of ventricular tachycardia. In a paper by Biolo *et al.* (12) in a group of 201 patients with

systolic HF of any etiology, the prevalence of non-sustained ventricular tachycardia, as detected by Holter monitoring, was significantly affected by the β_1-adrenergic receptor polymorphism Arg^{389}Gly, with a lower frequency in homozygous Gly^{389}Gly patients; however, it was not affected by the Ser^{49}Gly polymorphism. In a more recent paper by the same group (13), in seventy-three HF patients implanted with ICD, the time to the first appropriate ICD therapy was significantly shorter in carriers of two variant alleles, defined as "risk" genotypes, namely Arg allele carriers of the β_1 Gly^{389}Arg polymorphism and T allele carriers of the GNB3 C825T polymorphism, a gene coding for the G protein 3 subunit. When only the β_1 Gly^{389}Arg polymorphism was considered, however, there was no statistically significant difference in appropriate ICD shocks in patients with at least one Arg389 allele, compared with Gly^{389}Gly homozygous patients. It should however be reminded that only 24 subjects that underwent therapies, considering both shock and ATP, were considered as appropriated and thus included in the analysis. Moreover, the use of a Cox regression model implies that only the first event of a patients is considered, while a median of 3 episodes per patient was recorded.

The use of the Andersen-Gill proportional-intensity regression model (9), an extension of the Cox proportional-hazards method, allows to take into account the risk of repeated events and not just the first event, thus increasing the statistical power of the design. Its use is becoming more common in recent years, and it has been applied in modeling the risk of recurrent syncope (14), ICD therapies (15) and hospital readmission in HF patients implanted with left ventricular assist device (16). With the use of this method we were able to outline that the Ser^{49}Gly polymorphism of the β_1-adrenergic receptor significantly affects the risk of repeated ICD shocks, with the Gly49 allele carrying an increased risk of ICD shock.

This result is at odds with the absence of effect of the Ser49Gly polymorphism on the prevalence of ventricular tachycardia reported by Biolo *et al.* (11). The difference in sensitivity between a 24-hour Holter monitoring and a prolonged follow-up through ICD memory must however be taken into account; moreover, 3 consecutive ventricular ectopic beats already define a non-sustained ventricular tachycardia, but their significance and their prognostic value is different from an arrhythmia inducing an appropriate ICD shock.

ICD shock could be considered as a surrogate for fatal arrhythmias and thus for sudden cardiac death, the latter being almost always caused by an arrhythmic event. Although this concept has been questioned, mainly for the observation that a reduction in ICD shock is not associated with an improvement in survival (17), one could expect in any case a lower number of ICD shocks to be linked with a better prognosis and better quality of life (11).

The Gly49 allele, however, was associated in previous studies with a better prognosis, and this observation could indirectly conflict with our results. The most frequently quoted studies are two papers published by a Swedish group (18,19); these studies, however, enroll also patients with preserved ejection fraction, which have a different natural history; moreover in the first cohort (17) only 40% of the patients were on beta blockers. In the second cohort (18) with 83% of the patients on beta blockers, the five year transplant-free survival did not differ between Ser 49 homozygotes patients and Gly49 allele carriers.

The only other study to report a significant effect of Ser49Gly polymorphism is the one by Forleo *et al.* (3), which reports a better 33 month transplant and hospitalization-free survival in Ser49Gly heterozygous patients compared to Ser 49 homozygous patients. In synthesis two studies, both on idiopathic dilated

cardiomyopathy patients only, describe a significant protective effect of the Gly49 allele.

A series of other studies, however, did not detect any prognostic effect of Ser49Gly polymorphism; all these studies were conducted on patients with heart failure of any etiology. No influence on all-cause mortality and heart failure-related mortality was found by Biolo *et al.* (11) in 201 patients; no result on transplant-free survival was found by de Groote *et al.* (20) in 444 patients, by Shin *et al.*(4) in 227 patients, by Sehnert *et al.* (21) in 637 patients and by Leineweber *et al.* (22) in 226 end-stage HF patients. The absence of a prognostic influence of Ser49Gly polymorphism is confirmed by the meta-analysis by Liu *et al.* (23). Overall mortality was not affected; the result was probably mainly driven by the study by Wang *et al.* (24), which, opposite to the previously mentioned studies, documented a lower heart-failure related mortality in Ser 49 homozygotes in a population of 430 Chinese patients, but none of the three studies included in the meta-analysis had a better prognosis for Gly49 allele carriers. A larger number of studies was included for the composite end-point of death, hospitalization and transplant, but the result was always not significant. *In vivo* results are conflicting on the role of Ser49Gly polymorphism on prognosis, and thus do not contradict our result on the Gly49 allele being associated with an increased risk of ICD shock.

Another possible conflict between our results and published data is the absence of any effect of the Arg389Gly polymorphism on time free from ICD shock. As already pointed out, the only paper examining the influence of β-adrenoreceptor polymorphisms on ICD interventions (12) did not show any significant effect of Arg389Gly polymorphism when considered alone. As far as prognosis is concerned, in the paper by Biolo *et al.* (11) HF-related mortality was significantly reduced in Gly389Gly patients. Other papers, however, failed to identify any prognostic effect of

Arg^{389}Gly polymorphism in heart failure patients. In a sub-study of the Merit-HF trial (25) on 600 patients, and in the study of de Groote *et al.* (19) on 444 patients, no effect on hospitalization-free survival was documented. In the paper by Sehnert *et al.* (20) no effect on transplant-free survival was evident in 637 patients on beta blocker treatment. In the paper by Forleo *et al.* (3) no effect on hospitalization and transplant-free survival was evident, whereas in the study by Leineweber *et al.* (21) in 226 end-stage HF patients no prognostic effect was reported. So even prognosis, an end-point which obviously does not coincide with time free from ICD shocks, does not have a definite relationship with the Arg^{389}Gly polymorphism.

For the β_2-adrenergic receptor thirteen SNPs have been described; two common SNPs result in the amino acid substitutions Gly^{16}Arg and Gln^{27}Glu. These two variants are in strong linkage disequilibrium; Glu27 almost always is paired with Gly16 in humans. In an epidemiological study Gln27 homozygous individuals have evidenced an increased risk of sudden cardiac death in two different populations without HF (2). In another study on HF patients (3), the presence of the Arg16 allele and the homozigosity Gln^{27}Gln were associated with a better prognosis in patients with idiopathic dilated cardiomyopathy, but only the simultaneous presence of two copies of Arg16 Gln27 was associated with a worse prognosis in another study (4), in patients with HF of all etiologies. However, no prognostic effect in HF patients was found in other studies (19,20,21). We were not able to identify any effect of these β_2-adrenergic receptor polymorphism.

Conclusions

In conclusion, data from our study suggest that the Gly 49 allele of the β_1-adrenergic receptor Ser^{49}Gly polymorphisms may identify patients with heart failure at increased risk of ICD shock and thus of life-threatening arrhythmias.

The main drawback of the current study is the limited number of patients; the hypothesis ought to be verified in a larger study, which could also assess the role of gene haplotypes.

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Took part in:

- *conception and design of the study*
- *substantial contributions to the acquisition of data*
- *analysis and interpretation of data*
- *statistical analysis*
- *drafting of the manuscript*
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