G-protein-coupled receptor kinase 5 polymorphism and Takotsubo cardiomyopathy
Giuseppina Novo\textsuperscript{a,c}, Salvatore Giambanco\textsuperscript{a}, Marco Guglielmo\textsuperscript{a}, Luisa Arvigo\textsuperscript{a}, Maria R. Sutera\textsuperscript{a}, Francesco Giambanco\textsuperscript{b}, Salvatore Evola\textsuperscript{a}, Loredana Vaccarino\textsuperscript{c}, Manuela Bova\textsuperscript{c}, Domenico Lio\textsuperscript{c}, Pasquale Assennato\textsuperscript{a} and Salvatore Novo\textsuperscript{a}

\textbf{Background} Takotsubo cardiomyopathy (TTC) is an increasingly reported clinical syndrome that mimics acute myocardial infarction without obstructive coronary artery disease and is characterized by transient systolic dysfunction of the apical and/or mid-segments of the left ventricle. The syndrome mainly occurs in postmenopausal women with high adrenergic state conditions. Nowadays, the pathophysiology of TTC is not yet known and the possibility of a genetic predisposition is controversial.

\textbf{Aims} The purpose of this study was to assess the genetic susceptibility to TTC through analysis of the L41Q polymorphism of the G-protein-coupled receptor kinase 5 (GRK5).

\textbf{Methods and results} In a cohort of 20 patients enrolled in two tertiary Italian centers with diagnosis of TTC, accordingly to the commonly accepted Mayo Clinic criteria and in 22 healthy individuals (control) we have evaluated the polymorphism in GRK5 gene. The TTC patients had a mean age of 65 ± 9 years and 19 of 20 were women. The presence of one or two L41 alleles of GRK5 was significantly more frequent in TTC group than in the control group (40 vs. 8%, \(P = 0.0372\)).

\textbf{Conclusion} In our study, we have found a significant difference in the frequency of GRK5 polymorphism between TTC patients and controls, supporting a genetic predisposition to this cardiac syndrome.

\texttt{J Cardiovasc Med} 2014, 15:000–000

\textbf{Keywords:} genotype, G-protein-coupled receptor kinase 5 gene, polymorphism, Takotsubo cardiomyopathy

\textsuperscript{a}Division of Cardiology, A.O. Policlinico ‘P. Giaccone’, University of Palermo, \textsuperscript{b}Division of Cardiology, ‘G.F. Ingrassia’ Hospital and \textsuperscript{c}Department of Biopathology and Medical and Forensic Biotechnology, University of Palermo, Palermo, Italy

Correspondence to Salvatore Giambanco, MD, Division of Cardiology, A.O. Policlinico ‘P. Giaccone’, University of Palermo, Palermo, Italy
E-mail: g.salvo89@hotmail.it

Received 27 December 2013 Revised 29 March 2014 Accepted 31 March 2014

\textbf{Introduction} Takotsubo cardiomyopathy (TTC), also called ‘stress cardiomyopathy’ or ‘transient left ventricular apical ballooning syndrome’, is an infrequent heart syndrome, accounting for at least 2\% of acute coronary syndrome (ACS) cases.\textsuperscript{1} It has received considerable interest from the scientific community around the world, and in recent years it has been confirmed by the continuous and significant increase in publications related to this cardiomyopathy.\textsuperscript{2–5} TTC is characterized by transient left ventricular dysfunction, symptoms, and ECG changes mimicking acute myocardial infarction (AMI), and modest elevation in cardiac troponin, but in the absence of obstructive coronary disease or angiographic evidence of acute plaque rupture.\textsuperscript{6} Although the syndrome has usually a benign prognosis and a complete recovery occurs in 2–3 weeks,\textsuperscript{7} it occasionally can result in acute complications, including hemodynamic instability, atrial and ventricular arrhythmias,\textsuperscript{8} heart failure, and cardiogenic shock in a minority of patients.\textsuperscript{9,10} The risk of inhospital mortality is low (1–3\%).\textsuperscript{11–13} TTC just over 20 years ago was first described by Japanese investigators,\textsuperscript{9,14} and subsequently was also recognized by American and European populations.\textsuperscript{14–17} It occurs almost exclusively in women, especially in postmenopausal period, and the onset of acute event is often preceded by emotional and/or physical stress.\textsuperscript{18} Classically, left ventriculography shows ‘apical ballooning’ pattern with hyperkinesia of the basal segments.\textsuperscript{19} Recently, new variants have been described with different wall motion anomalies of the left ventricle such as ‘midventricular ballooning’ and sparing or hyperkinesia of the basal and apical segments, or an ‘inverted Takotsubo’ pattern with akinesia of the basal portions and hyperkinesia of the apex.\textsuperscript{20,21} The right ventricular involvement is increasingly recognized and is associated with a worse prognosis.\textsuperscript{22} To date, the pathophysiology of TTC is still unclear,\textsuperscript{23} but the fact that in TTC patients, plasma catecholamines are elevated two to three times higher than in AMI patients, has led to one of the most convincing
hypotheses that it is the result of a transient direct toxic effect of catecholamines on the myocardium. Several studies have provided evidence that high catecholamine levels could be responsible for an increase in the expression and enzymatic activity of G-protein-coupled receptor kinases (GRKs). In physiological conditions, the intracellular signaling, following catecholamine binding to the receptor, is mediated by βARs, activating myocytes by coupling to the Gs subunit of the G protein complex. At the same time, catecholamines promote the activation of GRK that induces phosphorylation of βARs, which promotes β-arrestin binding, and G-protein uncoupling to shut-off signaling. This regulation system is called ‘βARs desensitization’. The upregulation of GRKs could be somewhat connected to the desensitization and downregulation of the β-adrenoceptors (BAR) in the failing heart, suggesting that genetic GRK variants might modify outcomes in cardiac failure and ischemia.

Of the seven human GRKs, GRK2 and GRK5 predominate in myocardium, even if GRK2 seems to be the most relevant isoform at the cardiovascular level. Recently, several studies evaluating genetic polymorphisms potentially involved in the pathogenesis of TTC have been published; however, the findings have been contradictory. The aim of our study was to further investigate the genetic susceptibility to TTC through analysis of the L41Q polymorphism of the G-protein-coupled receptor kinase 5 (GRK5), one of the protein kinases most expressed in the heart and stress-induced acute ventricular dysfunction.

Methods
From May 2007 to April 2013, we have enrolled consecutive patients with TTC from two Italian tertiary hospitals. TTC was diagnosed according to the diagnostic criteria of Mayo Clinic, which includes the following. First criterion is transient hypokinesis, akinesis, or dyskinesis of the left ventricular mid-segments with or without apical involvement. The regional wall motion abnormalities typically extend beyond a single epicardial coronary distribution. Second criterion is the absence of obstructive coronary disease or angiographic evidence of acute plaque rupture. Third criterion is new ECG abnormalities (either ST-segment elevation and/or T-wave inversion) or modest elevation in cardiac troponin. Finally, the fourth criterion is the absence of pheochromocytoma and myocarditis.

Peripheral blood samples in EDTA have been collected from each patient of the study group and control group (healthy individuals). DNA has been extracted using the ‘salting out technique’ and the polymorphism rs17098707A/T L41Q for GRK5 was identified using PCR followed by a Restriction Fragment Length Polymorphism (RFLP) analysis as previously described. Written informed consent was given by each participant. Statistical analysis of data was performed using the Statgraphics software.

Continuous data were reported as mean ± standard deviation and categorical data were expressed in number and percentage. The differences between the groups were analyzed using the χ2 test for categorical variables, whereas the association between multiple variables was analyzed using the multivariate linear regression model. A value of P < 0.05 was considered statistically significant.

Results
Of the 43 consecutive TTC patients recruited overall, 20 gave consent for genetic analysis. The main clinical features of the study population are shown in Table 1. The analysis of polymorphism rs17098707A/T L41Q for GRK5 was performed in 20 TTC patients and 22 healthy individuals were used as control group (Table 2). In the TTC group, the ‘wild-type’ genotype (AA) was found in 12 patients (60%), whereas the ‘variant’ condition was found in five patients (25%) in the heterozygosity (AT) and three patients (15%) in homozygosity state (TT). In the control group, the expression of the AA genotype was observed in almost all of the analyzed population (92%) as the presence of polymorphism for GRK5 was detected only in one patient (4%) in heterozygosity (AT) and in one patient (4%) in homozygosity state (TT). Both groups seem to be in ‘Hardy–Weinberg equilibrium’. The distribution of genotypes between the two populations is significant (P = 0.0372) with an increased frequency of genotypes positive for the T allele in TTC patients. The analysis of the significance of the frequency distribution of genotypes between patients and control group shows that homozygous genotype AA has a high probability of being protective of TTC (odds ratio = 0.150; 95% confidence interval = 0.027–0.827) and the difference in distribution between the two populations is significant (P = 0.0296). The AT (heterozygous) and TT (homozygous) genotypes do not seem to have a clear predisposing effect for TTC and the difference in the genotype distribution between both groups is not significant (Table 3). About the frequency distribution of the alleles between patients and controls (Table 4), it has been found that the T allele, mainly represented in the TTC group (P = 0.0174), seems to be one of the genetic risk factors.
Moreover, value $P$ evaluated, in a cohort of 41 patients with a based on evidence from animal studies, Ser49Gly given the known higher frequency of L41Q polymorphism of the GRK5 in Thr164Ile. Given the known higher frequency.

The catecholamines, produced by the adrenal glands, are released in response to sympathetic nervous system stimulation, and increased cardiac function. However, when catecholamine levels remain chronically elevated, $\beta$ARs desensitization occurs by a process which involves proteins, including GRKs and $\beta$-arrestins, both of which have been associated with cardiac dysfunction. In detail, the $\beta$ARs are phosphorylated by GRKs, translocated, and subsequently internalized after binding to $\beta$-arrestins. $\beta$-Arrestin 1 prevents the inhibition of catecholamine release from the adrenal glands, enhances the secretion of aldosterone, and may contribute to the pathogenesis of TTC. Furthermore, it inhibits the inotropic effects of cardiac $\beta$ARs, resulting in decreased cardiac function. In addition, increased cardiac GRK2 levels have been described in chronic heart failure and are associated with elevated sympathetic nervous system activity. As recently proposed by Santulli et al., GRK2 could be predictive of ventricular remodeling after myocardial infarction and could facilitate the tailoring of appropriate therapy for high-risk patients. Fusco et al. reported that ischemia causes acute cellular and mitochondrial accumulation of GRK2, and induces mitochondria biogenesis after ischemia/reperfusion, indicating a protective effect of GRK2 for mitochondria after acute stress. On the other hand, it has been reported that GRK5-mediated $\beta$AR desensitization could provide adaptive, beneficial effects during early ventricular decompensation, and prior to frank failure. Moreover, GRKs may reflect other pathophysiologic processes. Indeed, the ability of the amino terminus of GRK5 (GRK5-NT) in reducing myocardial transcription factor NF-kB activity and left cardiac hypertrophy has been demonstrated. In recent years, several experimental and clinical studies analyzing polymorphisms potentially involved in the pathogenesis of TTC have been published. However, the data reported by various studies are rather controversial. Given the known higher frequency of some polymorphism in the African-American than white population, racial differences may partly justify this discrepancy. Specifically, the genetic variants most extensively investigated have been those affecting cardiac adrenergic receptor (AR) subtypes ($\alpha$, $\beta_1$, and $\beta_2$), Gs-protein alpha subunit (GNAS), and GRK5.

### Table 2

<table>
<thead>
<tr>
<th>Gene (SNP)</th>
<th>Group</th>
<th>WT (%)</th>
<th>Het (%)</th>
<th>Poly (%)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRK5 (rs17098707)</td>
<td>Control</td>
<td>92.0</td>
<td>4.0</td>
<td>4.0</td>
<td>0.0372</td>
</tr>
<tr>
<td></td>
<td>TTC</td>
<td>60.0</td>
<td>25.0</td>
<td>15.0</td>
<td>0.0372</td>
</tr>
</tbody>
</table>

GRK5, G-protein-coupled receptor kinase 5; Het, heterozygote; Poly, homozygous for polymorphism; SNP, single nucleotide polymorphism; TTC, Takotsubo cardiomyopathy; WT, homozygous for the wild-type.

for Takotsubo syndrome (Relative Risk RR = 4.033; 95% confidence interval = 1.211–13.431).

### Discussion

The stimuli promoting the imbalance of the autonomic system and triggering TTC are multiple, and the real challenge for cardiologists is not their determination, but rather to understand why only some individuals develop stress cardiomyopathy. The identification of a genetic susceptibility in TTC, favoring a better understanding of the pathogenesis of this peculiar syndrome, could allow the development of more appropriate preventive strategies and tailored treatment.

The adrenal-cardiac axis has been increasingly identified as an important contributory factor to the pathogenesis of TTC. The catecholamines, produced by the adrenal glands, are released in response to sympathetic nervous system stimulation, and increased cardiac function. However, when catecholamine levels remain chronically elevated, $\beta$ARs desensitization occurs by a process which involves proteins, including GRKs and $\beta$-arrestins, both of which have been associated with cardiac dysfunction.

### Table 3

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>OR</th>
<th>95% CI</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0.150</td>
<td>0.027–0.827</td>
<td>0.0296</td>
</tr>
<tr>
<td>Het</td>
<td>7.000</td>
<td>0.739–66.249</td>
<td>0.0866</td>
</tr>
<tr>
<td>Poly</td>
<td>3.500</td>
<td>0.334–96.688</td>
<td>0.345</td>
</tr>
</tbody>
</table>

Table 3 Analysis of the significance of the frequency distribution of genotypes between patients and control group

GRK5 polymorphism and Takotsubo cardiomyopathy Novo et al.

zaroff et al. have shown an association between polymorphisms of the genes encoding the $\beta_1$-adrenergic receptor (ADRB1 Arg389Gly and Ser49Gly), $\beta_2$ (ADRB2 Gly16Arg, Gln27Glu, and Thr164Ile), and $\alpha_2$C (ADRA2C del322–325) and cardiac dysfunction attributed to the release of catecholamines after subarachnoid hemorrhage (SAH). In particular, the authors conclude that ADRB1 and ADRA2C polymorphisms are associated with an increased risk of cardiac abnormalities after SAH. These data support the hypothesis that cardiac dysfunction after SAH is a form of neurocardiogenic injury. Note that, the regional dysfunction reversible left ventricular view in SAH patients is phenotypically similar to that of TTC patients.

Sharkey et al. evaluated, in a cohort of 41 patients with stress cardiomyopathy, the functional polymorphisms of adrenergic receptors $\beta_1$ and $\alpha_2$C already implicated in the increased activation of the sympathetic nervous system, but have not found significant differences between TTC patients and controls.

Handy et al. based on evidence from animal studies, have also included in their study the evaluation of the ADRB2, over that of ADRB1 and ADRA2C, assuming that during stress, when epinephrine is the main circulating catecholamine, regional differences in epinephrine-sensitive $\beta_2$-receptors could explain the myocardial

Copyright © Italian Federation of Cardiology. Unauthorized reproduction of this article is prohibited.
response to catecholamine surge seen in TTC. According to their study, the authors conclude that while a molecular defect in adrenergic signaling remains a plausible pathogenic mechanism, their data, as well as those of Sharkey et al., indicate that the TTC is not probably based on genetic variations in adrenergic receptors.

Spinelli et al. have analyzed the genetic polymorphisms in ADRB1, ADRB2, GNAS, and GRK5 genes in 22 TTC patients. The prevalence of most of the polymorphisms was similar between patients and controls, but for L41Q polymorphism of the GRK5, wherein the glutamine (wild-type) at position 41 is replaced by leucine, was significantly more frequent in the TTC group. Specifically, the authors have hypothesized that this polymorphism could attenuate the inotropic effect of catecholamines on cardiomyocytes in confirmation of what has been shown in isolated cells and in transgenic mice, in which the GRK5 L41 variant causes a negative inotropic effect under conditions of acute massive catecholamine release. Moreover, the same polymorphism is associated with an increase in βAR desensitization, which may predispose to TTC.

Recently, Figtree et al., in a large Australian cohort of 92 TTC patients, did not find association of genetic variants in the estrogen receptor-α (ERα), β1AR, β2AR, and catechol-o-methyl transferase (COMT) genes, or with the previously implicated GRK5, with occurrence of TTC. Although their data showed no evidence for specific genetic variants in GRK5 or βAR playing a role in susceptibility of an individual to TTC, the authors believe that this interesting hypothesis cannot be disputed and further research is needed.

In our study, consistent with the data of Spinelli et al., the percentage of TTC patients who presented the rs17098707 polymorphism of the GRK5 gene was significantly higher than that of controls (P = 0.0372). Furthermore, the analysis of data showed that 'wild-type' genotype (AA) could be protective against TTC. In contrast, GRK5 L41 variant, in heterozygous (AT) and homozygous (TT) genotypes, did not seem to be predisposing to TTC, although the T allele could represent one of the genetic risk factors for this cardiomyopathy (P = 0.0174).

Study limitation
The main limitation of our study is the small sample size; however, our results do not pretend to clarify the complicated issue concerning the genetic mechanisms that might modulate the pathogenesis of TTC, rather to provide the cardiological community with a further observation.

Conclusion
We found a significant difference in the frequency of GRK5 polymorphism between TTC patients and controls. These findings reinforce the hypothesis of the role of genetic predisposition in the pathogenesis of TTC. Thus, a defect in the adrenergic signal remains one of the most compelling pathogenetic hypotheses in this field. Further research using larger multicenter studies is needed to better understand the role of genetics in the pathophysiology of this syndrome.

References


