

Genetic and Epigenetic factors of Takotsubo Syndrome: a Systematic Review

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Running title: Takotsubo cardiomyopathy

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Abstract: Takotsubo syndrome (TTS), recognized as stress's cardiomyopathy, or better as left ventricular apical balloon syndrome in the recent years, is a rare pathology, described for the first time by Japanese researchers in 1990. TTS is characterized by an interindividual heterogeneity in onset and progression, and by the strong predominance in postmenopausal women. The clear causes of these TTS features are uncertain, given the reduced understanding of this intriguing syndrome until now. However, the increasing frequency of TTS cases in the last years, and particularly correlated to SARS-CoV-2 pandemic, leads us to imperative necessity both of a complete knowledge of TTS pathophysiology for identifying biomarkers facilitating its management, and targets for specific and effective treatments. The suspect of a genetic basis in TTS pathogenesis has been evidenced. Accordingly, familial form of TTS has been described. But a systematic and comprehensive characterization of the genetic or epigenetic factors significantly associated with TTS is lacking. Thus, we, here, conducted a systematic review of literature before June 2021, to contribute to identify potential genetic and epigenetic factors associated with TTS. Interesting data were evidenced, but of reduced number and with diverse limitations. Consequently, we concluded further work is needed to address the gaps discussed, and probably a clear evidence may arrive using multi-omics investigations.

Key-words: Takotsubo cardiomyopathy (TTS); biomarkers; specific and effective treatments; TTS management; genetic and epigenetic factors; systematic review

1. Introduction

Takotsubo Syndrome (TTS) is a cardiac disease first described in Japan in 1990 by Sato et al. [1], which is far from being completely known. TTS is a form of transient acute cardiac dysfunction, occurring predominantly in postmenopausal women and triggered in most cases by an emotional or physical stress [2]. Accordingly, in Western countries, a female prevalence has been observed, with a female-to-male ratio of 9:1. Up to 90% of all patients with TTS are, indeed, women, mostly postmenopausal, since oestrogen deficiency is considered as a predisposing factor or a prerequisite [3]. In Japan, men are, while, more affected than women, for unknown reasons [4].

The name Takotsubo originates from the Japanese word for an octopus trapping jar, which resembles the shape of the end-systolic heart in TTS on ventricular angiogram [2]. However, other terms (such as broken heart syndrome, apical ballooning, or stress cardiomyopathy and takotsubo cardiomyopathy) have been also used to refer to TTS.

As above-mentioned, the syndrome usually worsens with physical and emotional stress secondary to non-heart disease and surgery. Given the abundance and recurring high level of emotional stress in current everyday life, the incidence of TTS is inadequately low [5]. Accordingly, it is appropriate to underline that only some individuals develop TTS, even if exposed to same stressors. Thus, TTS is an enigmatic disease with a multifactorial and unresolved pathogenesis.

A genetic predisposition has been suggested, even if based on few familial TTS cases [6]. In addition, promising loci, copy number variations, and polymorphisms have already identified by genetic studies in TTS patients, but with inadequate results requiring further investigations for achieving mechanistic conclusions.

Concerning clinical features, Takotsubo is a peculiar syndrome that mimics acute myocardial infarction with sudden onset of chest pain or shortness of breath, echocardiographic

(ECG) variations (ST-elevations, ST-depression, T-inversion, long QTc or even Q-waves) and dynamic troponin elevation. However, no coronary obstructions are found on coronary angiography, but a typical myocardial dysfunction is evident on ventricular angiography [7]. Specifically, TTS is characterized by an acute reversible form of left ventricular systolic dysfunction, extending beyond a coronary artery vascular territory usually due to physical or psychological stressors [8]. Thus, typical clinical TTS characteristics are represented by the following factors: 1) absence of obstructive coronary artery disease (CAD) or angiographic evidence of acute plaque rupture; 2) new ECG abnormalities; 3) absence of recent significant head trauma, intracranial bleeding, or myocarditis [9]. Besides, only some cases show severe acute complications, including a 4% to 5% mortality related to cardiogenic shock and cardiac arrest [10]. Thus, an interindividual heterogeneity in onset and progression characterizes TTS, as well as typical is the abovementioned strong predominance in TTS postmenopausal women. Recently, the European Society of Cardiology (ESC) has also established the International Takotsubo Diagnostic Criteria (InterTAK Diagnostic Criteria), which implement a diagnostic algorithm and assign a score to TTS [7]. However, no treatments have been not established by ESC, because of absent of a precise understanding on molecular and cellular mechanisms involved in TTS pathophysiology until now [11].

In this review, we want to make light on genetic and epigenetic factors that eventually may influence TTS pathogenesis. Familial form of TTS has been described [12], but not a systematic and comprehensive characterization of the genetic or epigenetic factors significantly associated with TTS.

2. Literature search 's strategy for evidencing genetic TTS factors

In order to evidence the genetic factors related to TTS, a comprehensive search from the international web databases PubMed, Medline and Web of Science, including articles published before June 2021, has been conducted. The following terms have been used as searching keywords

without restrictions on language, ethnicity or geographic area: “*Takotsubo gene*”, “*Takotsubo genes*”, “*stress-induced Takotsubo Syndrome*”, “*Takotsubo Syndrome genes*”, “*Takotsubo Syndrome genetic*”, “*Polymorphisms Takotsubo*” and “*stress cardiomyopathy*”.

This strategy has led to the data described in the subsequent paragraphs.

2.1 Genetic studies on TTS

2.1.1 Familial forms

A restricted number of cases affected by familial TTS form has been reported in the literature. A genetic predisposition for the development of TTS has also been suspected based on existing case reports of this disease among siblings and mother–daughters. Multiple cases of TTS in the same family and recurrent cases indicate a genetic component.

The data are described in Table 1[13-20].

Table 1: Familial case of Takotsubo

Authors	Year	Patients
Pison	2004	two sisters
Kumar	2010	mother and daughter
Schultz	2012	In three families, several close relatives developed SIC
Subban	2012	A 68-year-old female and her daughter aged 43 got admitted to our institute simultaneously
Musumeci	2013	At the same time in two sisters
Ikutomi	2014	two sisters
Caretta	2015	two sisters
Ekenback	2019	twin sisters

2.1.2 GWAS and polymorphism studies

Current literature has focused on the possible existence of associations between TTS and single nucleotide polymorphisms (SNPs) in genes associated with sympathetic stress. Several studies analysing polymorphisms potentially involved in the pathogenesis of TTS have been published (Table 2) [21-33].

The genes encoding the B1 (*ADRB1*), B2 (*ADRB2*), and alpha 2c (*ADRA2C*) adrenergic receptors, which harbour functional variants modulating cardiac response to catecholamines, have been examined. Precisely, in a cohort of 41 patients with TTS, *Sharkey* and colleagues [21] found no difference in the frequency of adrenoceptor *ADRA2C* and adrenoceptor *ADRB1* polymorphisms compared with controls. The same polymorphisms were evaluated in another cohort of 61 patients [22], reporting a different distribution of *ADRB1* (rs1801253) p.Arg389Gly SNP. Exactly, homozygous Arg/Arg more frequent in TTS, and *ADRB2* (rs1042714 p.Gln27Glu) homozygous Gln/Gln result to be more frequent in healthy controls. However, no significant differences for B2-adrenergic receptor (rs1042713, p.Arg16Gly) variation, were detected.

Spinelli and co-workers [23] investigated the eventual associations of genetic polymorphisms in *ADRB1*, *ADRB2*, *GNAS*, *GRK5* genes with TTS enrolling 22 cases. The genetic analysis showed a similar distribution between cases and controls for the major number of examined polymorphisms. The unique significant difference was assessed for the rs17098707 polymorphism in the *GRK5* gene, with a higher prevalence of the leucine allele at position 41 in TTS subjects. In contrast with this paper, *Figtree* and colleagues [24], detected not any association. Successively, the *Australian Tako-tsubo Taskforce* enrolled 92 TTS patients [24] to be genotyped

for rs17098707 in *GRK5* gene, rs1801253 in *ADRB1* gene, rs1800888 in *ADRB2* gene, rs4680 in *COMT* gene, rs6915267 and rs71017521 in *ESR1* gene. No associations were found.

In 2015, the rs17098707 in *GRK5* was also studied by *Novo* group in 20 TTS patients and 22 healthy individuals [25]. In the TTS group, the ‘wild-type’ genotype (AA) was found in 12 patients, while the ‘variant’ condition was found in five patients in the heterozygosity state (AT) and three patients in homozygosity state (TT). In the control group, the frequency of the AA genotype was observed in majority of the analysed population (92%).

During the same year, *Vriz* and coworkers [26] analysed β 1- and/or β 2-adrenoceptor polymorphisms (β 1-Arg389Gly polymorphism; rs1801253 *ADRB1*, β 2-Arg16Gly

polymorphism; rs1042713 ADRB2, β 2-Gln27Glu polymorphism; rs1042714 ADRB2) in 97 TTS patients compared with 81 patients with anterior STEMI (acute ST-elevation myocardial infarction) and 101 controls. β 1-Adrenoceptor (rs1801253 p.Gly389Arg), β 2-adrenoceptor (rs1042713, p.Arg16Gly) and β 2-adrenoceptor (rs1042714 p. Gln27Glu) genotype frequencies were significantly different among groups. However, significant statistical differences were not detected, when TTS and STEMI patients were compared. A significant difference in the frequency of the β 1-adrenoceptor Arg389 homozygous, β 2-adrenoceptor Glu27 homozygous and β 2-adrenoceptor 16Gly homozygous was observed only between TTS patients and normal controls.

Citro and coworkers evaluated [27] a population of 29 TTS patients and more than 1000 healthy controls for the presence of variants in the protein Bcl2- associated athanogene 3 (*BAG3*). Concerning SNP rs35434411 (R71Q), two TTS patients were found heterozygous, while all analysed controls (n = 1043) were homozygous for the major allele (p = 0.0007). In addition, two TTS patients (7%) were homozygous for SNP rs3858340 (P407L) of *BAG3*, versus 1% of analysed controls (p = 0.045). Yet, no statistical differences in the frequency of SNP rs2234962 (C151R) between patients and controls was also detected. This study was extended by sequencing exons 2–4 of the coding sequence and the entire 3'-UTR of *BAG3* in a total of 70 women TTS patients [28]. Among the genomic variants identified, a particularly frequent variant in the 3'UTR (rs8946) was identified: 62.8% of TTS patients carried this nucleotide variant (12.8% were homozygous), while the variant was present in only 45.6% of the samples of the control group (7.4% were homozygous). Since miR-371-5p binds to *BAG3* 3'UTR region, a novel posttranscriptional epi-induced mechanism may be hypothesized for which *BAG3* 3'UTR variants can impair miR-371-5p activity.

In 2018, by genotyping a large TTS cohort, *Mattson* and co-workers [29], demonstrated a lack of association of candidate SNPs with TTS in the *ADRB1*, *GRK5* and *BAG3* genes, previously suggested to contribute to this disease. Precisely, 1438 samples (461 TTS patients, 403 controls with CAD and 574 healthy controls) were genotyped for the following polymorphisms: rs1801253,

rs2230345 and rs8946 in *ADRB1*, *GRK5* and *BAG3* genes, respectively. The genotype distribution did not differ among the three study groups.

In 2016, Cecchi and co-workers [30], analysed 75 TTS women compared to acute coronary syndromes (ACS) patients and control subjects for the detection of the G1691A polymorphism in *the factor V* gene (factor V Leiden) and the G20210A polymorphism in *the factor II* gene. For both variants no significant difference in their distribution was detected.

Successively, a genotyping study was conducted evaluating the SNPs in genes encoding oestrogen receptors, since data literature demonstrate a correlation between myocardial infarction and genetic variants in genes of *ESR1* and *ESR2* oestrogen receptors. Two variants (rs2234693 and rs9340799) in the *ESR1* gene and two (rs1271572 and rs1256049) in the *ESR2* gene were examined in 81 consecutive white women: 22 with TTS, 22 with acute myocardial infarction and 37 asymptomatic healthy controls [31]. Women carrying the T allele at the rs2234693 locus of the *ESR1* gene and carrying a T allele at the rs1271572 locus of the *ESR2* gene had an even higher risk of occurrence of TTS.

Since the studies on polymorphisms did not reveal particular data, some groups focused on genome-wide association study (GWAS) or Whole exome sequencing (WES). GWAS is an approach used in genetics research to associate specific genetic variations with particular diseases. The method involves scanning the genomes from many different people and looking for genetic markers that can be used to predict the presence of a disease. Precisely, GWAS analysis was conducted in 96 TTS patients (91 females, 5 males) and 475 healthy controls [32] showing 68 promising candidate loci. In 18 (rs12612435, rs7070797, rs9392780, rs13273616, rs1154275, rs72970558, rs62253104, rs4676168, rs4961212, rs6944978, rs4812257, rs162487, rs4605019, rs113154180, rs13179382, rs12444925, rs17146144 and rs56403110) of them, the top SNPs were supported by SNPs in high Linkage Disequilibrium ($r > 0.8$) and $p < 10^{-3}$.

The exome sequencing may be used for identifying causal variants of rare disorders by using the Next Generation Sequencing (NGS) technology. Exome analysis, genotyping array analysis, and array comparative genomic hybridization were carried out on 28 Christchurch EqSCM cases [33]. Using WES and Cardio-MetaboChip genotyping analyses, no obvious role for exonic mutations in a monogenic model, or SNPs in a polygenic model were assessed. The most striking finding was the observation of a markedly elevated rate of rare, heterogeneous copy number variants (CNV) of uncertain clinical significance (in 12/28 subjects). Several of these CNVs impacted on genes of cardiac relevance including *RBFOX1*, *GPC5*, *KCNRG*, *CHODL*, and *GPBP1L1*.

Table 2: Summary of polymorphisms found in patients affected by Takotsubo cardiomyopathy

Reference	Year	Population	Analysed variant	Evidence
Sharkey	2009	41 female TTS patients and 43 females control	<i>ADRB1</i> (amino acid positions 389 and 49) and <i>ADRA2C</i> (deletion 322_325)	Genotype polymorphism frequencies are not significantly different in TTS patients compared to controls
Spinelli	2010	22 TTS patients and 740 control	rs35230616 and rs1801253 for <i>ADRB1</i> ; rs1042713, rs1042714, and rs1800888 for <i>ADRB2</i> ; rs11554276 for <i>GNAS</i> ; rs17098707 and rs34679178 for <i>GRK5</i>	The prevalence of polymorphisms of <i>ADRB1</i> , <i>ADRB2</i> , and <i>GNAS</i> were similar between patients and controls. Conversely, the percentage of patients who presented rs17098707 polymorphism of the <i>GRK5</i> gene was significantly higher.
Vriz	2011	61 TTS patients and 109 controls	rs1801253 for <i>ADRB1</i> , rs1042713 and rs1042714 for <i>ADRB2</i>	The rs1801253 for <i>ADRB1</i> genotype frequencies were significantly different in the two groups.
Citro	2013	29 TTS patients and 1043 controls	<i>BAG3</i> polymorphisms: R71Q (rs35434411); C151R (rs2234962); P407L (rs3858340)	Two TTC patients were heterozygous for SNP rs35434411, whereas all analyzed controls were homozygous for the major allele
Figtree	2013	92 TTS patients	rs17098707 for <i>GRK5</i> , rs1801253 for <i>ADRB1</i> ; rs1800888 for <i>ADRB2</i> ; rs4680 <i>COMT</i> ; rs6915267 and rs71017521 for <i>ESR1</i>	They have found no association of genetic variants in the <i>ESR1</i> , <i>ADRB1</i> , <i>ADRB2</i> , or <i>COMT</i> and <i>GRK5</i> genes
D'Avenia	2015	70 TTS women patients and 81 healthy donors	They sequenced exons 2–4 of the coding sequence and the entire 3'UTR of <i>BAG3</i> .	Mutations and polymorphisms detected in the <i>BAG3</i> gene included a frequent nucleotide in the 3'-UTR region of TTS patients, resulting in loss of binding of microRNA-371a-5p
Novo	2015	20 TTS patients	Analysis of the L41Q polymorphism of <i>GRK5</i>	They found a significant difference in the frequency of <i>GRK5</i> polymorphism between TTS patients and controls
Vriz	2015	97 patients with TTS, 81 patients with anterior STEMI and 101 controls	rs1801253 for <i>ADRB1</i> , rs1042713 and rs1042714 for <i>ADRB2</i>	In a TTS cohort compared with anterior STEMI patients, β -adrenoceptor polymorphisms were similar. β -Adrenoceptor polymorphisms in TTS patients differed from normal subjects
Cecchi	2016	75 age- and sex-matched acute coronary syndrome (ACS) patients, both enrolled during the acute phase, and in 75 control subjects	factor II (G20210A) and V (G1691A) polymorphisms	No significant difference between the three groups was observed
Eitel	2017	96 TTS patients and 475 healthy controls	A genome-wide association study (GWAS)	The results of GWAS analysis showed several promising candidate 68 loci
Lacey	2017	28 women who suffered TTS	exome analysis, genotyping array analysis, and array comparative genomic hybridization	Heterogeneous copy number variants (CNV) of uncertain clinical significance were observed in 12 patients. Several of these CNVs impacted on genes of cardiac relevance including <i>RBFOX1</i> , <i>GPC5</i> , <i>KCNRG</i> , <i>CHODL</i> , and <i>GPBP1L1</i>
Pizzino	2017	81 TTS women	rs2234693 and rs9340799 in <i>ESR1</i> gene; rs1271572 and rs1256049 in <i>ESR2</i>	The study reports preliminary findings suggesting a possible link between <i>ESR</i> polymorphisms and the occurrence of TTS.
Mattson	2018	461 TTS patients, 403 controls with CAD and 574 controls without CAD.	rs1801253 for <i>ADRB1</i> , rs2230345 for <i>GRK5</i> and rs8946 for <i>BAG3</i>	By genotyping a TTS cohort, they demonstrate a lack of association between candidate SNPs in the <i>ADRB1</i> , <i>GRK5</i> and <i>BAG3</i> genes, earlier suggested to contribute to TTS.

3. Sequencing of Takotsubo cohort

In 2007, *Nef* group [34] studied 3 female patients with TTS. Complementary RNA was isolated from left ventricular biopsies taken in the acute phase (group A) and after functional recovery (group B). It was profiled for gene expression by using cDNA microarrays and the validation of selected genes was performed by means of real-time PCR and immunohistochemistry. This study demonstrated a significant contribution of oxidative stress to the pathophysiology of TTS, probably triggered by an excess of catecholamine. Increased protein biosynthesis and an activated cell survival cascade might be interpreted as potential compensatory mechanisms.

In 2009, the same group of research detected expression levels of Ca²⁺-regulatory proteins by means of real-time PCR, western blot, and immunohistochemistry in 10 consecutive patients [35]. They demonstrated that TTS is associated with specific alteration of Ca²⁺-handling proteins, which might be crucial for contractile dysfunction.

In 2009, *Kleinfeldt* and coworkers reported for the first-time data on a female individual with TTS, who happened to be carrier of an *FMR1* gene mutation, alleles of an intermediate size between 40–55 triplet pre-mutations [36].

In 2014, *Goodloe* and colleagues [37] used exome sequencing for comprehensively genotyping a TTS cohort (28 TTS subjects, including a mother–daughter pair), enabling to investigate 486 genes of a pathways network related to adrenergic signaling. Two-thirds of TTS cases carried more than one filtered adrenergic pathway variant, and 11 genes harboured a variant in ≥ 2 cases. The mother–daughter pair shared missense variants in highly conserved functional domains of *ADH5*, *CACNG1*, *EPHA4*, and *PRKCA* genes. An adrenergic pathway-independent analysis of the cohort exposed no common gene for TTS. Therefore, the data obtained consent to support the genetic heterogeneity in TTS susceptibility and a likely polygenic basis, conferring a cumulative effect on adrenergic pathway dysregulation in a subset of individual subjects.

In 2016, *Kalani* group performed exome sequencing of 7 female sporadic patients with TTS [38]. Exome-sequencing analysis revealed that each patient carried predicted deleterious variants affecting known cardiomyopathy genes. In each case, the identified variant was either not previously found in public human genome data or previously annotated in a database of clinical variants associated with cardiac dysfunction.

Keller and co-workers, in 2018, presented a patient with TTS having the initial manifestation of a hitherto unrecognized genetic cardiomyopathy [39]. Upon genetic work-up by means of a gene panel, the heterozygous mutation c.1489G > T (p. E497X) in exon 9 of the *TTN* gene was detected and made responsible for the phenotype.

Lacey and coworkers performed WES on 24 of the 28 Christchurch earthquake-associated TTS cases [32]. The most striking finding was the observation of a markedly elevated rate of rare, heterogeneous copy number variants (CNV) of uncertain clinical significance (in 12/28 subjects). Several of these CNVs impacted on genes of cardiac relevance including *RBFOX1*, *GPC5*, *KCNRG*, *CHODL*, and *GPBP1L1*.

In 2020, *Pan* group analysed microarray datasets GSE95368 derived from the Gene Expression Omnibus (GEO) database [40], and in a first time they identified differentially expressed genes (DEGs) between TTS and controls. Then, the DEGs were used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Lastly, the protein-protein interaction (PPI) network was constructed and Cytoscape was used to find the key genes. In total, 25 DEGs were identified, including 10 upregulated genes and 15 downregulated genes. Moreover, seven genes including *APOE*, *MFGE8*, *ALB*, *APOB*, *SAA1*, *A2M*, and *C3* were identified as *hub* genes of TTS, which might be used as diagnostic biomarkers or molecular targets for the treatment of TTS.

In 2021, *Khurana* and coworkers [41] tested the hypothesis that the cardioprotective benefit of Suberanilohydroxamic acid (SAHA) in a pre-clinical model of TTS is conferred by an epigenetic

acetylation/deacetylation (Ac/Dc) axis. RNA-sequencing showed SAHA treatment influenced the transcriptional expression index (TEI) of genes with a shift towards cardiac benefit. These results suggested that the core pathways identified might be regulated by SAHA treatment. The recent SCAAR screen found no genetic link with Takotsubo for polymorphisms in the *ADRB1*, *BAG3* and *GRK5* genes. To determine whether the cardiac benefit conferred by SAHA treatment was regulated by an Ac/Dc axis, they performed chromatin immunoprecipitation assays from LV tissues followed by sequencing (ChIP-seq). SAHA treatment influenced acetylation and deacetylation of genes implicated with TTS. Surprisingly, this included prominent lysine deacetylation of the SCAAR-associated genes *ADRB1*, *BAG3* and *GRK5*. While H3K9/14ac selectivity was previously described for EP300 gene targets⁵ in this study they observed that the expression of stress-induced genes were influenced in the REV model (ISO/SAHA vs. Control) as well as the Ac/Dc index in the REV (ISO/SAHA) vs. ISO groups.

4. Epigenetic factors and TTS

Recently, the role of epigenetic factors, including MicroRNAs (miRNAs/miRs) and acetylation/deacetylation (Ac/Dc) axis (lastly as abovementioned and discussed [41]), is emerging in different pathologies. MicroRNAs (miRNAs/miRs) are a class of highly conserved, small (19–25 nucleotides) noncoding post-transcriptional regulators of proliferation, angiogenesis, differentiation, and apoptosis, but also recently suggested as biomarkers of several cardiovascular diseases, such as heart failure, stable coronary artery disease, and acute MI [42–47]. Such evidence also led us to search from the international web databases PubMed, Medline and Web of Science articles published before June 2021 on circulating miRNAs associated with TTS. The following data have been reported. Precisely, in 2014, Templin group demonstrated that circulating miR-16 and miR-26 resulted increased in TTS *versus* STEMI or healthy controls. While, *Braun* group, in 2013, evidenced that miR-1 [48] and miR-133a resulted to be largely increased in STEMI vs. TTS, even if

elevated in both vs. controls. miR-1 and miR-133a are, indeed, typical of cardiac tissue, consequently they likely reproduce the grade of myocardial injury than other pathological conditions. Accordingly, they were more discreetly elevated in TTS respect to STEMI associated with levels of cardiac troponin and creatine kinase-MB in circulation [49].

No other data have been reported on miRs in TTS in literature until now. However, some researchers have evaluated whereas miR-16 and miR-26a, specifically increased in TTS as above described, have a close relation with TTS or simply are the result of the activation of catecholamines or their damage. In addition, it has been also investigated the organ or tissue of origin of miR-16 and miR-26a in TTS, because assessed only in blood samples from TTS patients. However, it is well known that miR-16 and miR-26a have been detected in circulation in stress, depression and anxiety conditions, as documented by the results of three recent studies [50-52]. In addition, *Templin* group has elegantly reported that TTS has a significant relationship with pre-existing psychiatric disorders, i.e., anxiety and depression [53]. Consequently, miR-16 and miR-26a could have the role of effectors in the brain–heart interaction in TTS cases. Some responses to these questions recently arrive from a study conducted in 2021. Precisely, the researchers demonstrated that miR-16 and miR-26a reduce the TTS-like variations in contractile system from TTS rats (*in vivo* and *in vitro*) and human cardiomyocytes, and permitted to detect the involved fundamental mechanisms [54].

Further studies on TTS-associated miRs are imperative, because they can contribute to clear their role, as well as their potential capacity to be active players for TTS onset and complications. Consequently, the evaluation of their blood levels in TTS patients clinically followed or recovered during successive stress periods might predict the probability of reappearance of acute episodes, which represent a significant risk in these cases, by consenting the application of preventive strategies.

On the other hand, the knowledge of the mechanisms involved may also allow the development of other preventive pharmacological therapies, including the pre/anti-miRNA constructs which are now demonstrating to influence the clinic.

5. Conclusions

The clear TTS causes, such as the predisposing genetic factors, are uncertain, given the restricted literature evidence on this intriguing syndrome, until now. However, the increasing frequency of TTS cases in the last years, and particularly correlated to SARS-CoV-2 pandemic [55,56] is leading to imperative necessity of a complete knowledge of TTS pathophysiology, and to urgently develop specific and effective treatments.

To support of these necessities, this systematic review (**Figure 1**) focused its attention on evidencing the potential genetic and epigenetic factors closely associated with TTS, by performing a systematic search of published studies before June 2021 from international web databases PubMed, Medline and Web of Science. The data obtained have led us to suggest that genetic variants of *ADRB1*, *GRK5* and *BAG3* genes, or the variants of *APOE*, *MFGE8*, *ALB*, *APOB*, *SAA1*, *A2M*, and *C3* genes significantly associated with TTS, can probably interact with the environment, and predispose certain individuals than others to develop TTS more easily and with more severe complications, by justifying the heterogeneity of having acute episodes and the different clinical pictures observed in TTS cases.

Certainly, the data evidenced in this review could show the major limitation, typical of a systematic literature revision, that is to originate from retrospective studies. This enhances the probability of selection's and information's bias.

In conclusion, we can affirm that the current available literature has not comprehensively addressed the possibility of identifying clear genetic traits facilitating the TTS management, as well as the increased risk of recurrence of acute episodes in TTS cases. Consequently, future research

with omics investigations and involving larger populations is warranted.

In addition, the low power for studies of this size in detecting an effect of a common polymorphism, leads to encourage further studies with high-quality phenotyping, and sharing high-number/high-quality data are necessary to estimate the potential role of the genetics and epigenetics as predisposing factor in TTS.

Finally, we also suggest that the better strategy might be the integration of multi-omics approaches as a promising tool for the identification of appropriate biomarkers, such as genetic and epigenetic risk factors, and therapeutic targets for TTS, as previously indicated for other cardiovascular diseases [57]. The analysis of genetic, epigenetic, metabolomics, microbiomic, and nutrigenomic profiles could be encouraging, and lead to the identification of promising biomarkers, and personalized medicine in near future. Such analyses could also consent to understand the impact of gender differences in TTS pathophysiology, which has been widely demonstrated, where gender is referred to the complex interrelation and integration of sex (as a biological and functional marker of the human body) and psychological and cultural behavior (due to ethnical, social, and religious background).

Author Contributions: Conceptualization, V.F., F.S. and C.R.B.; methodology, V.F., F.S. and C.R.B.; investigation: V.F., D.V., B.B., writing—original draft preparation V.F., F.S. and C.R.B, writing—review and editing, V.F., F.S., C.R.B and G.N. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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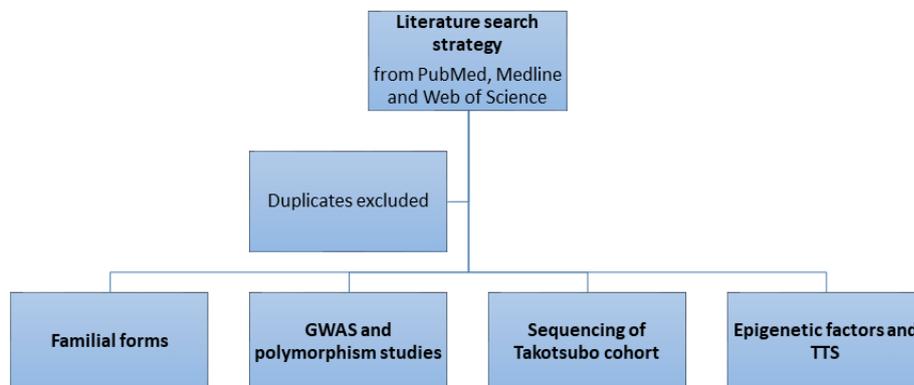


Figure 1: Workflow for this project