

# Investigating Changes in $\beta$ -Adrenergic Gene Expression (ADRB1 and ADRB2) in Takotsubo (Stress) Cardiomyopathy Syndrome

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## Research Article

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# Abstract

**Background:** Takotsubo Cardiomyopathy (TC) is a rare disorder that is mostly caused by stress and is often misdiagnosed. We aimed to analyze Takotsubo Syndrome at the molecular level by using the Oxford Nanopore Minion Device and its protocol.

**Methods and Results:** Ten patients who were previously diagnosed with Takotsubo Syndrome (increased after decrease in ejection fraction and without critical stenosis in coronary arteries) and 10 healthy individuals in the control group were included in our project. The mean age was  $53 \pm 12.2$  for the patient group and  $52.4 \pm 9.9$  for the control group, and the left ventricular ejection fraction was  $50.3 \pm 11.5$  for the patient group and  $64.2 \pm 2.8$  for the control group ( $p < 0.05$ ). Peripheral blood of patients and healthy individuals was taken and their DNA was obtained. By making long reads throughout the genome, the most studied regions responsible for  $\beta$ -adrenergic signaling pathways; The gene expression level of cardiac  $\beta$ -1 ADRB1(rs1801253-ENST00000369295.4), G>C, (Gly389Arg) and cardiac  $\beta$ -2 ADRB2 (rs1800888-ENSG00000169252), C>T, (Thr165Ile) adrenoceptors was investigated. As a result; no structural variation was detected leading to Takotsubo Cardiomyopathy. The results obtained from the bioinformatics analysis were also checked from the VarSome Tools and similar results were found.

**Conclusions:** Many publications in TC susceptibility have that may lead to adrenergic pathway dysregulation, most studied adrenergic receptor genes in the similar literatures too. We searched for genetic variants in  $\beta$ 1AR and  $\beta$ 2AR genes in our study and however we could not find any variants in this study, we think larger numbers of cohort studies are needed.

## Introduction

Takotsubo cardiomyopathy (TC) is characterized by the apical balloon appearance known for about 25 years, of the left ventricle; it is a reversible cardiomyopathy characterized by its similarity to the earring used by the Japanese to catch octopuses. Catecholamine increase plays an important role in the pathophysiology of TC; For this reason, it is also called "Stress Cardiomyopathy"[1]. It causes TC, which is a disease in which the effects of physical and psychological stress are also seen, to gain different characteristics from known cardiomyopathies [2, 3].

It is usually seen in the postmenopausal period of women, after emotional or physical stress [4]. They reported that the reason why it is frequently seen in the postmenopausal period is that it makes the heart sensitive to catecholamines in connection with the decrease in estrogen, and therefore systolic dysfunction can develop more easily in the left ventricle of the heart under stress [5]. Although its physiopathology has not been clarified yet, increased catecholamine levels are thought to play an important role (Fig. 1). In addition, enlarged myocardium, hypertension, chronic obstructive pulmonary disease, decreased estrogen level, small vessel disease, myocarditis, insufficiency of myocardial fatty acid metabolism have also been blamed. Symptoms and electrocardiogram (ECG) findings mimic acute coronary syndrome [5, 6].

Acute changes in myocardial function are predominantly controlled by the beta-adrenergic receptor ( $\beta$ -AR) and occasionally result in changes in an intracellular signaling pathway [7]. Specifically, transmissions in signal transduction pathways triggered by the agonist response of  $\beta$ -ARs act as essential regulators of heart rate, systolic and diastolic function, and myocardial metabolism [7, 8].

Until now, all major genome sequencing technologies have been used as modifications of polymerase-mediated DNA synthesis. While these methods have contributed immensely to our genome recognition so far, nanopore-based sequencing is a new technology that represents entirely different genome-wide read-along approaches in which nucleic

acid sequences are inferred from changes in ionic current across a membrane as a single DNA molecule passes through a protein nanopore [9].

Genetic variation in both ADRB1 and ADRB2 has been associated with disease phenotypes, both in vitro and clinically. Recent studies are; they are studies investigating the potential interaction between variations in ADRBs and drugs for these important receptors [10, 11]. In our study, we aimed to investigate whether there is any variation in ADRB1 and ADRB2 genes in the patient and control group diagnosed with Takotsubu Cardiomyopathy using Nanopore technology. Beta-adrenergic receptors (ADRBs) are cell surface receptors that play a central role in the sympathetic nervous system. The pharmacological selection of two of these receptors, ADRB1 and ADRB2, is due to the fact that they can be evaluated for a widely used therapeutic approach for common and important diseases such as asthma, hypertension and heart failure.

## Materials And Methods

### Patients

Within the framework of the project; 10 patients who were admitted to Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Cardiology and hospitalized with the diagnosis of acute coronary syndrome or acute heart failure and diagnosed with Takotsubu Cardiomyopathy according to the diagnostic criteria of Mayo Clinic TK, included in the study. As the control group, 10 healthy individuals included in the study. Medical history, physical examination, laboratory findings, electrocardiography, echocardiography, coronary angiography and ventriculography of the patients were evaluated. 10 ml of venous blood samples taken from the forearm with the vacutainer system from the patients were transferred to the Medical Genetics Department on the same day. One of the 20 subjects (10 patients and 10 controls) diagnosed with Takotsubu Cardiomyopathy included in the study did not want to donate blood. Genomic DNA (gDNA) was obtained from a total of 18 cases (9 patients and 9 controls) using the QIAamp DNA Blood Mini Kit (Qiagen) and quantified with the NanoDrop ND-1000 Spectrophotometer. Therefore, the number of control groups was started to be 9 to equalize the number of study and control groups. In our study group, menopausal women are more affected by Takotsubu cardiomyopathy, so the number of male cases is less. The characteristics of the demographic and clinical variables of the study and control groups are given in Table-1, and the echocardiographic results of our study and control groups are given in Table-2.

### Library Prep with Native Barcoding Amplicons

Library preparation was completed using the Native Barcoding Kit 1D (catalog number EXP-NBD104) and Ligation Sequencing Kit (catalog number SQK-LSK109; Oxford Nanopore Technologies, Oxford, UK) (Fig. 2). ONT libraries were prepared as follows: 48 µl of genomic DNA per library was 3.5 µl of NEBNext FFPE DNA Repair Buffer, 2 µl of NEBNext FFPE DNA Repair Mix (New England Biolab, M6630), and 3.5 µl of nuclease-free water (NFW) and incubated at 20°C for 15 min for DNA repair, re-pooled and cleaned up using a 0.8×volume of AMPure XP beads (Beckman Coulter) according to the manufacturer's instructions, with final elution in 30 µl of EB (10mM Tris pH 8.0).

The DNA recovered from the elution well (~ 40 µl) was brought to 50 µl and end-repaired by the addition of 3.5µl NEBNext Ultra II End Prep Reaction Buffer and 3 µl NEBNext Ultra II End Prep Enzyme Mix (New England Biolab, E7546) with incubation for 5 min at 20°C followed by 5 min at 65°C. The sample was cleaned up with 1×volume AMPure XP beads and eluted in 30 µl of EB.

PCR adapters (20 µl) from Oxford Nanopore Technology (SQK-LSK109 Ligation Sequencing Kit 1D) were ligated to the end-repaired DNA with 50 µl of NEB Blunt/TA Ligase Master Mix (M0367) at room temperature for 10 min, followed by

clean-up with 1×volume AMPure XP beads and elution in 48 µl of EB.

Combined 22.5 µL of purified DNA, 2.5 µL of Native Barcode and 25 µL of blunt/TA ligase master mix in a new 1.5 mL DNA-low binding reaction tube, mix was by tapped and spined briefly. It was incubated for 10 minutes at room temperature. Then 65 µL pooled barcoded DNA, 5 µL Adapter MixII (AMII), 20 µL NEBNext Quick Ligation Buffer (5x) and 10 µL Quick T4 DNA Ligase were prepared for Adaptor ligation and clean-up steps, and mix was gently by flicked the tube and spin down. Finally, 61.5 ng/ml of the control group and 58.8 ng/ml of the patient group's pooled DNA were washed twice with 250 µL of Short Fragment Buffer (SFB) Buffer and loaded into the MinION device by adding 15 µL of Elution Buffer. In a new tube, prepared the library for loading as follows; 37.5 µl Sequencing Buffer,(SQB), 25.5µl Loading Beads, ~ 14µl DNA library. Then gently loaded SpotOn Flowcell. As the first operation; Flow-Check was performed to check pore numbers, then samples were loaded separately as study and control groups using the MinION Mk1b sequencer with two FLO-MINSP6 flow cells. Assuming the sample count as 10, respectively, 1M reads with an average length of 100,000 bases were obtained. Raw data (FAST5 files) were obtained using MinKNOW software version 21.06.0 (Oxford Nanopore Technologies), which were converted to FASTQ files and sorted into each sample depending on their barcode sequences by using Guppy software version 4.4.0 (Oxford Nanopore Technologies).

## Data Analysis

The data obtained as a result of MinION (Oxford Nanopore Technologies) sequencing were processed in fast5 format. In the first stage, basecalling and demultiplexing operations of fast5 files were performed with Guppy basecaller 4.4.0 software, and the obtained sequences were obtained in fastq format and separated into barcodes. Sequences in Fastq format were loaded into the Geneious Prime 2021.0.3 ([www.geneious.com](http://www.geneious.com)) program in separate files according to each barcode, and the sequences consisting of different fragments with the same barcode were combined. The sequences were made ready for the mapping process by performing filtering processes according to the quality scores of the obtained sequences. The reference sequence required for the mapping process was obtained after the analysis of the presented primers. Given for the ADRB1 gene; forward 5'-AGACGTGCTATGTGTGACGG-3' and reverse 5'-AGCACTTGGGGGTCGTTGTAG-3' primer pair, given for the ADRB2 gene; forward 5'-TGGATTGTGTCGGGCCTTA-3' and reverse 5'-TGGCACGGTACCAGTGAT-3' PCR was performed.

## Results

Demographic data of all participants are seen in Table 1. There was no difference in the all parameters between two groups. Echocardiographic results of our study and control groups are given in Table 2. Ten patients who were previously diagnosed with Takotsubo Syndrome (increased after decrease in ejection fraction and without critical stenosis in coronary arteries) and 10 healthy individuals in the control group were included in our project. The mean age was  $53 \pm 12.2$  for the patient group and  $52.4 \pm 9.9$  for the control group, and the left ventricular ejection fraction was  $50.3 \pm 11.5$  for the patient group and  $64.2 \pm 2.8$  for the control group ( $p < 0.05$ ). Peripheral blood of patients and healthy individuals was taken and their DNA was obtained. By making long reads throughout the genome, the most studied regions responsible for  $\beta$  adrenergic signaling pathways; The gene expression level of cardiac  $\beta$ -1 ADRB1(rs1801253-ENST00000369295.4), G > C, (Gly389Arg) and cardiac  $\beta$ -2 ADRB2 (rs1800888-ENSG00000169252), C > T, (Thr165Ile) adrenoceptors was investigated. As a result; No structural variation was detected leading to Takotsubo Cardiomyopathy. The results obtained from the bioinformatics analysis were also checked from the VarSome Tools and similar results were found. The data of the Alignment results of ADRB1 and ADRB2 gene regions according to the analysis are shown in Table 3. The results obtained show very high coverage and accuracy results for the ADRB1 region, but not significant results for the ADRB2 region. Therefore, the results and statistical information shown in the report apply only to the ADRB1 region.

Table 1  
Demographic and clinical characteristics of the study group.

<b>Variables</b>	<b>Takotsubo (n:9)</b>	<b>Control (n:9)</b>	<b>P value</b>
<b>Male</b>	2 (%22.2)	3 (%33.3)	0.599
<b>Women</b>	7 (%77.8)	6 (%66.7)	
<b>Age (years)</b>	53 ± 12.2	52.4 ± 9.9	0.917
<b>BMI (kg/m<sup>2</sup>)</b>	28.6 ± 4.06	27.6 ± 4.09	0.616
<b>HT</b>	2 (%22.2)	2 (%22.2)	1
<b>DM</b>	3 (%33.3)	1 (%11.1)	0.257
<b>Throid Dys.</b>	0	1 (%11.1)	0.303
<b>Total cholesterol, mg/dl</b>	161.5 ± 45.8	204.5 ± 65	0.125
<b>Trigliserid (mg/dl)</b>	182.2 ± 80.5	141.3 ± 60.5	0.255
<b>HDL cholesterol (mg/dl)</b>	43.3 ± 17	51.5 ± 12.5	0.261
<b>LDL cholesterol (mg/dl)</b>	101.5 ± 44.3	155.7 ± 60.3	0.045
<b>White blood cell count, µl</b>	10.09 ± 1.6	7.3 ± 2.1	0.007 <sup>#</sup>
<b>Haemoglobin, g/dl</b>	13.9 ± 1.7	13.8 ± 1.6	0.891
<b>Platelet, 10<sup>3</sup>/mm<sup>3</sup></b>	292.4 ± 75.4	276 ± 91.3	0.683
<b>Lymphocyte count, µl</b>	2.7 ± 0.8	2.5 ± 0.5	0.560
<b>Neutrophil count, µl</b>	6.3 ± 1.7	3.8 ± 1.7	0.009 <sup>#</sup>
<b>Monocyte count, µl</b>	0.67 ± 0.098	0.59 ± 0.17	0.261
<b>Alt</b>	22.1 ± 12.4	24.4 ± 16.4	0.739
<b>Ast</b>	19.2 ± 4.6	24.1 ± 9.1	0.173
<b>Kreatinine</b>	0.8 ± 0.2	0.7 ± 0.16	0.559
<b>F.glukoz</b>	117.3 ± 27.7	100.7 ± 7.1	0.102
<b>TSH</b>	1.5 ± 1.1	1.6 ± 0.8	0.720
<b>T4</b>	1.3 ± 0.18	1.2 ± 0.21	0.411
<b>C-reactive protein, mg/dl</b>	0.4 ± 0.27	0.3 ± 0.19	0.425

Table 2  
 Echocardiographic results of the Study and Control groups.

Variables	Takotsubo (n:9)	Control (n:9)	P value
LVEDD (mm)	47.5 ± 4.9	44.2 ± 1.7	0.072
LVESD (mm)	33.2 ± 7.8	27.7 ± 1.5	0.059
LVEF Simpson (%)	50.3 ± 11.5	64.2 ± 2.8	0.003 <sup>#</sup>
IVS (mm)	11.5 ± 1.58	11.1 ± 1.53	0.555
PW (mm)	10.8 ± 1.53	10.6 ± 1.1	0.730
LA (mm)	34.1 ± 3.8	32.2 ± 4.7	0.372
Ao (mm)	25 ± 2.1	26 ± 2.1	0.339

LVEDD: left ventricular end diastolic diameter, LVESD: left ventricular end systolic diameter, LVEF: left ventricular ejection fraction, IVS: interventricular septum, PW: posterior Wall, LA: left atrium, Ao: Aort root diameter, # p < 0.05 statistical significance, n: number

Table 3

Data of the Alignment results of ADRB1 and ADRB2 gene regions according to the analysis performed.

Analysis type	Primer F-R*	Barcode kiti	Barcode no	Sequencing Kit	Flowcell type	Received data size (MBfastq/barcode)	ADRB1 Aligned Read number	ADRB2 Aligned Read number
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD01	SQK-LSK109	FLO-MIN106	137	137701	7
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD02	SQK-LSK109	FLO-MIN106	183	165975	8
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD03	SQK-LSK109	FLO-MIN106	95	87236	8
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD04	SQK-LSK109	FLO-MIN106	168	156156	24
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD05	SQK-LSK109	FLO-MIN106	88	96652	3
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD06	SQK-LSK109	FLO-MIN106	35	37285	3
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD07	SQK-LSK109	FLO-MIN106	123	109483	19
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD08	SQK-LSK109	FLO-MIN106	66	72119	0
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD09	SQK-LSK109	FLO-MIN106	151	154890	11
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD01	SQK-LSK109	FLO-MIN106	114	121707	2
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD02	SQK-LSK109	FLO-MIN106	110	117779	6
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD03	SQK-LSK109	FLO-MIN106	131	143815	1
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD04	SQK-LSK109	FLO-MIN106	111	123712	0

\*F Primer: ADRB1 5'-AGACGTGCTATGTGTGACGG-3' ADRB2 5'-TGGATTGTGTCGGGCCTTA-3'

R Primer: ADRB1 5'-AGCACTTGGGGTCGTTGTAG-3' ADRB2 5'-TGGCACGGTACCAGTGCAT-3'

Analysis type	Primer F-R*	Barcode kiti	Barcode no	Sequencing Kit	Flowcell type	Received data size (MBfastq/barcode)	ADRB1 Aligned Read number	ADRB2 Aligned Read number
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD05	SQK-LSK109	FLO-MIN106	118	124389	2
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD06	SQK-LSK109	FLO-MIN106	81	88196	1
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD07	SQK-LSK109	FLO-MIN106	118	127283	2
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD08	SQK-LSK109	FLO-MIN106	107	117082	2
ADRB1-2 target region	ADRB1-ADRB2	EXP-NBD104	NBD09	SQK-LSK109	FLO-MIN106	157	169639	4
*F Primer: ADRB1 5'-AGACGTGCTATGTGTGACGG-3' ADRB2 5'-TGGATTGTGTCGGGCCTTA-3'								
R Primer: ADRB1 5'-AGCACTTGGGGTCGTTGTAG-3' ADRB2 5'-TGGCACGGTACCAGTGCAT-3'								

Considering these matches, the gene region "Homo sapiens - NG\_012187.1 Homo sapiens adrenoceptor beta 1 and 2 (ADRB1 and ADRB2), RefSeqGene on chromosome 10" was downloaded in full genbank format and pulled into Geneious Prime 2021.0.3 ([www.geneious.com](http://www.geneious.com)) (Fig. 3 and Fig. 4).

Using these references, mapping for the sequences of each barcode was performed with the minimap2.17 software "Command Line: minimap2.exe-xmap-ont-frag = yes-k21-secondary = yes-N5-p0.7-arefSeq.fastainput.fastq-output.sam" commands.

While the results obtained showed very high coverage and accuracy results for the ADRB1 region, they did not yield any significant results for the ADRB2 region. Therefore, the results and statistical information shown in the report are inclusive only for the ADRB1 region.

The fasta file for each sample is additionally provided. The threshold value taken while creating fasta files was determined as coverage over 1000. Images of each alignment are given below in order. Statistical information is given as an appendix in the "stats.xlsx" file.

No significant changes were observed in the alignment analysis performed with the obtained consensus sequences. In the picture given (Fig. 5 and Fig. 6), the multiple alignment graph made with the consensus sequences and the images taken by zooming the regions with the change in this graph are given.

## Discussion

Although the recovery rate is high in TC, hemodynamic and respiratory support may be required and sometimes, unfortunately, death may result. A better understanding of the mechanisms underlying the disease can aid in targeted management and better survival. Identifying the role of genetic variants will help both to understand the abnormalities

in signaling pathways in these patients and to inform new therapeutic targets. In another study thought to be effective in Takotsubo syndrome; although there is no evidence for specific genetic variants in GRK5 or  $\beta$ AR that play a role in an individual's susceptibility to TC, this has been proposed as an interesting hypothesis. Therefore, further exploration of alternative candidates in the proposed signaling pathways or non-candidate approaches such as genome-wide screening is recommended [12, 13].

It is usually seen in the postmenopausal period of women, after emotional or physical stress. They reported that the reason why it is frequently seen in the postmenopausal period is that it makes the heart sensitive to catecholamines in connection with the decrease in estrogen, and therefore systolic dysfunction may develop more easily in the left ventricle of the heart under stress. Although its physiopathology has not been clarified yet, increased catecholamine levels are thought to play an important role. In addition, enlarged myocardium, hypertension, chronic obstructive pulmonary disease, decreased estrogen level, small vessel disease, myocarditis, insufficiency of myocardial fatty acid metabolism have also been blamed. Symptoms and electrocardiogram (ECG) findings mimic acute coronary syndrome [14].

Sharkey et al. (2009) published their study in which they genotyped three adrenergic receptor polymorphisms in a cohort of 41 patients with stress cardiomyopathy (SK). Also known as takotsubo cardiomyopathy and apical balloon syndrome, this idiopathic but reversible disorder was typically seen in postmenopausal women. It was also manifested by ischemia-like chest pain, transient ECG changes, and minor cardiac biomarker elevation after acute emotional or physical stress. Coronary arteries were not occluded in angiography of Takotsubo patients. Sharkey et al. investigated functional polymorphisms of B1 and  $\alpha$ 2c adrenergic receptors. Despite the increased activation of the sympathetic nervous system, in which catecholamines increase (epinephrine, norepinephrine, dopamine) and this elevation stimulates adrenergic receptors, it has been reported that there is no significant difference between Takotsubo patients and female controls when the polymorphism frequencies are compared [15]. This result was similar our research results.

Figtree et al. (2013) examined the potential association of genetic variants in the adrenergic and estrogen signaling pathways with TC in a large cohort of 92 Takotsubo patients recruited from four major Australian centers. While genotypic variation is an attractive way to explain disease susceptibility, this study demonstrates that major candidate polymorphisms in adrenergic, estrogen, and GRK5 signaling pathways genes are not associated with TC [12].

Studies have led to the hypothesis that epinephrine is the main circulating catecholamine under stress, leading to the hypothesis that regional differences in epinephrine-sensitive B2 receptors may explain the myocardial response to the catecholamine surge seen in Takotsubo Cardiomyopathy [16]. Therefore, it has been hypothesized that its mutation in adrenergic receptors may increase the sensitivity of the heart to adrenergic stress. In polymorphism studies with primers designed for the ADRB1 and ADRB2 genes, comprehensive DNA sequencing of these adrenergic receptor genes did not reveal any mutations in the familial Takotsubo Cardiomyopathy case. While a molecular defect in adrenergic signaling remains a plausible pathogenic mechanism, our data, along with the findings of Sharkey et al., suggest that Takotsubo Cardiomyopathy is probably not based on genetic variation in adrenergic receptors [17, 18].

Eitel et al. (2017), in a large GWAS study; It has been concluded that the genes in the  $\pm$  500kb upstream and downstream regions of the SNPs in the gene regions that are thought to cause TC may be associated with different cancer types, obesity and heart rate variability, but cannot be directly responsible for TC. According to these results; although there is a familial predisposition for TC in several families, or studies have been conducted in postmenopausal women; it has been reported that larger-scale TC cohorts should be established [19].

In a study in rats; Expression of  $\beta$ -1AR and  $\beta$ -2AR mRNA in Dilated Cardiomyopathies treated with doxorubicin was evaluated by RT-qPCR and reported no change in  $\beta$ -1AR and  $\beta$ -2AR gene expressions [20].

Although a few familial cases of Takotsubo have been reported, no responsible gene mutation, variation or polymorphism has been clearly identified so far, so the genetic causes underlying TC have not yet been clearly elucidated.

## Conclusion

Beta1-adrenergic receptors (beta1ARs) are interesting candidates for pharmacogenetic studies, particularly in heart disease, as they mediate the action of catecholamines in the sympathetic nervous system. These receptors are important both in the progression of the disease and in its treatment (beta blocker therapy). For this reason, it has been a matter of curiosity what kind of changes occur in gene expression levels [12]. From the results of studies to date, it has been considered very likely that beta1AR variants have a genetic component that defines the response to beta-blockers in heart failure and hypertension. However, due to the inconsistency in the results of the polymorphism study, the polymorphism results cannot be used in practice yet, and we did not find any variation in the ADRB1 and ADRB2 genes in our study. Therefore, multicenter studies with different races and a large number of patients are needed.

## Declarations

**Conflicts of interest/Competing interests** The authors declare that they have no conflict of interest.

**Ethics approval** The studies were approved Ethic Committee of Afyonkarahisar Health Science University.

**Consent for publication** All authors approved the final manuscript and agreed to publication in Basic Research in Cardiology.

**Author Contributions** ZY and STO designed and performed the experiments, analysed data, and wrote the manuscript. EO, ZY and IED performed polyclinic examinations, Takotsubo Cardiomyopathies diagnosis and other cardiological data.

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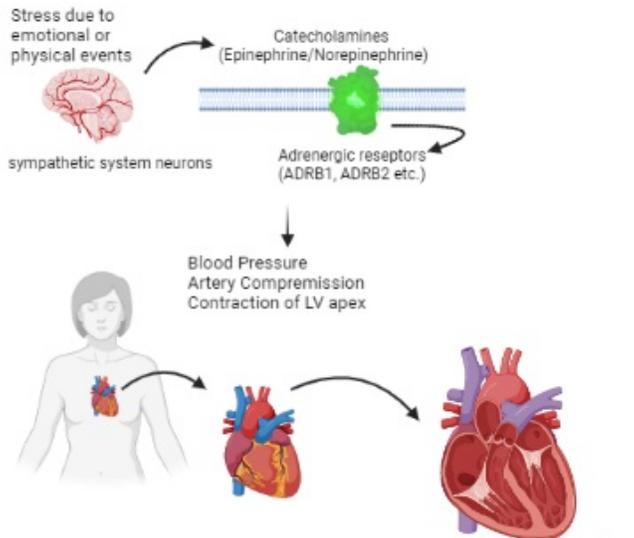
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## Figures



**Figure 1**

Increased activation of the sympathetic nervous system due to stress-related emotional or physical events, increases catecholamines (epinephrine, norepinephrine, dopamine), and this increase stimulates adrenergic receptors. Specifically, transmissions in signal transduction pathways triggered by the agonist response of  $\beta$ -ARs act as essential regulators of heart rate, systolic and diastolic function, and myocardial metabolism and thus systolic dysfunction may more readily develop in the left ventricle of the heart under stress.(Created in BioRender.com)



ADRB1 Reference-Homo sapiens-NG\_012187.1 Homo sapiens adrenoceptor beta 1 (ADRB1), RefSeqGene on chromosome10–Geneious Prime 2021.0.3 (www.geneious.com) view.

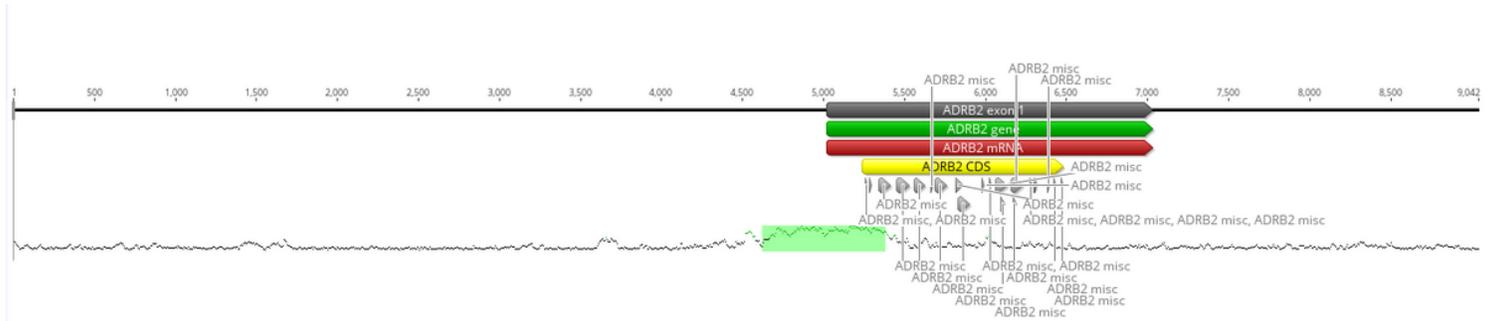


Figure 4

ADRB2 Reference - Homo sapiens - NG\_016421.2 Homo sapiens adrenoceptor beta 2 (ADRB2), RefSeqGene on chromosome 5 – Geneious Prime 2021.0.3 (www.geneious.com) view



Figure 5

Alignment file created with consensus sequences

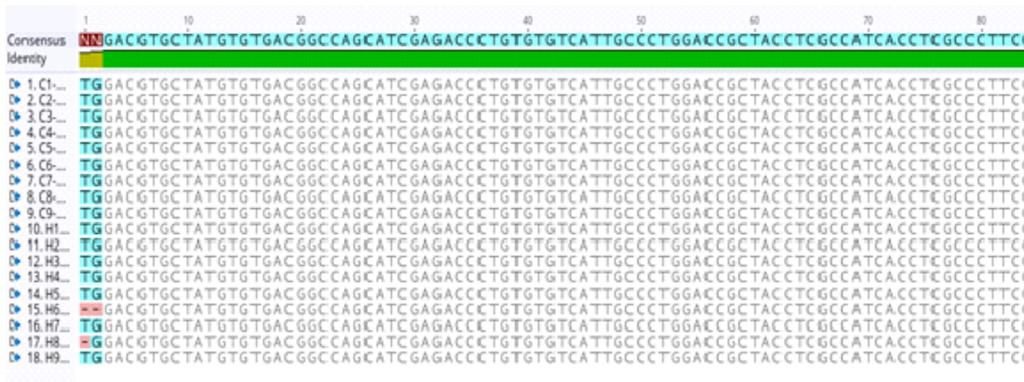


Figure 6

The starting region where the change is seen in the alignment file created with the consensus sequences