The association of polymorphisms of β-adrenergic receptors genes with the low triiodothyronine syndrome in patients with a heart failure

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The course of heart failure (HF) and its progression is associated with comorbidities, genetic factors and a dynamics of a number of biomarkers. The low triiodothyronine syndrome (LT_3S) is observed in some patients with HF. Extremely little data are available in the literature regarding the effect of β-adrenoreceptors (β-AR) genes polymorphisms on the development of LT_3S and many contradictory results about their association with HF course. This encourages new research in this area.

***The aim** of study was to evaluate the relationship of β-adrenergic receptors gene polymorphisms with low triiodothyronine syndrome in patients with a heart failure.*

***Materials and methods.** 354 patients with HF on a background of postinfarction cardiosclerosis were included to the study. At 89 (25.1%) patients LT_3S was diagnosed. The course of HF was studied for 2 years. Mean levels of thyroid stimulating hormone (TSH), free T_{3f} and T_{4f} were evaluated. Genotyping of 4 single nucleotide polymorphisms (Gly389Arg of β_1-AR gene, Ser49Gly of β_1-AR gene, Gln27Glu of β_2-AR gene and Ser275 of $GN\beta_3$ gene) was performed by polymerase chain reaction. Genetic and epidemiological analysis was performed using the SNPStats program.*

***Results.** The risk of LT_3S in patients with HF increases with homozygous G/G variant of Gln27Glu polymorphism of the β_2-AR gene (OR=2.21, p=0.037), described as a recessive model of inheritance. There was a tendency to increase the risk of LT_3S development in the presence of the genotype C/T of the Ser275 polymorphism of the GNb_3 gene (OR=1.75, p=0.054), described as an over-dominant model. The genotype C/G of the Gln27Glu polymorphism of the β_2-AR gene was associated with a decreased risk of LT_3S development (OR=0.54, p=0.037), described as over-dominant model. Patients with HF carriers the A allele (A/GA/A) of the Ser49Gly polymorphism of the β_1-AR gene have a lower risk of repeated hospitalization due to HF decompensation (OR=0.50, p=0.032), described as a dominant model. There was a tendency to increase the risk of re-hospitalization in the G-allele (C/G-G/G) variant of the Gln27Glu polymorphism of the β_2-AR gene (OR=1.68, p=0.057), described as a dominant heredity model. At patients with HF in combination with LT_3S the risk of re-hospitalization increases at C/G variant of the Gln27Glu polymorphism of β_2-AR gene (OR=1.25, p=0.025), described as an over-dominant model.*

***Conclusions.** The results suggest that congenital genetic alterations in β-adrenergic pathways may be associated with the development of LT_3S in patients with HF and the features of the HF course.*

Key words: *low triiodothyronine syndrome, β_1-adrenoreceptors, β_2-adrenoreceptors, G-protein, heart failure, single-nucleotide polymorphisms, risk*

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Związek polimorfizmów genów receptorów β-adrenergicznych z zespołem niskiego stężenia trójjodotyroniny u chorych z niewydolnością serca

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Przebieg niewydolności serca (HF – heart failure) i jej rozwój wiąże się ze współistniejącymi chorobami, czynnikami genetycznymi i dynamiką wielu biomarkerów. Zespół niskiego stężenia trijodotyroniny (LT3S) obserwuje się u niektórych chorych z HF. Niezwykle mało danych jest dostępnych w piśmiennictwie, dotyczących wpływu polimorfizmów genów β-adrenoreceptorów (β-AR) na rozwój LT3S i wiele sprzecznych danych wskazujących na ich związek z przebiegiem HF. To zachęca do nowych badań w tej dziedzinie.

***Celem pracy** była ocena związku polimorfizmów genów receptorów β-adrenergicznych z zespołem niskiego stężenia trijodotyroniny u chorych z HF.*

***Materiały i metody.** Do badania włączono 354 chorych z HF po zawale serca. U 89 (25,1%) chorych rozpoznano LT3S. Przebieg HF obserwowano przez 2 lata. Oceniano średnie stężenie hormonu stymulującego tarczycę (TSH), jego wolne frakcje T3f i T4f. Genotypowanie 4 polimorfizmów pojedynczego nukleotydu (Gly389Arg genu β1-AR, Ser49Gly z genu β1-AR, Gln27Glu z genu β2-AR i Ser275 z genu GN β3) przeprowadzono za pomocą reakcji łańcuchowej polimerazy. Analizę genetyczną i epidemiologiczną przeprowadzono za pomocą programu SNPStats.*

***Wyniki.** Ryzyko wystąpienia LT3S u chorych z HF ulega zwiększeniu wraz z homozygotycznym wariantem G/G polimorfizmu genu Gln27Glu genu β2-AR (iloraz szans OR = 2,21, p=0,037), który wykazuje recesywny model dziedziczenia. Obserwowano występowanie tendencji do zwiększania ryzyka rozwoju LT3S w obecności genotypu C/T polimorfizmu Ser275 genu GN β3 (OR=1,75, p=0,054), który ujawniał model nadmiernie dominujący. Genotyp C/G polimorfizmu Gln27Glu genu β2-AR wiązał się ze zmniejszonym ryzykiem rozwoju LT3S (OR=0,54, p=0,037, model nadmiernie dominujący). Chorzy nosiciele HF allel A (A/GA/A) polimorfizmu Ser49Gly genu β1-AR mają mniejsze ryzyko ponownej hospitalizacji z powodu dekompensacji HF (OR=0,50, p=0,032) i rozwijają model dominujący. Występowała ponadto tendencja do zwiększania ryzyka ponownej hospitalizacji w wariancie allelu G (C/GG/G) polimorfizmu Gln27Glu genu β2-AR (OR=1,68, p=0,057), określana jako model he-redity. U chorych z HF w połączeniu z LT3S ryzyko ponownej hospitalizacji zwiększało się w wariancie C/G polimorfizmu Gln27Glu genu β2-AR (OR=1,25, p=0,025), co uznano za model szczególnie dominujący.*

***Wnioski.** Wyniki sugerują, że wrodzone zmiany genetyczne w szlakach β-adrenergicznych mogą być związane z rozwojem LT3S u chorych z HF i wpływać na przebieg HF.*

Słowa kluczowe: *zespół niskiego stężenia trijodotyroniny, β1-adrenoreceptory, β2-adrenoreceptory, białko G, niewydolność serca, polimorfizmy pojedynczego nukleotydu*

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Heart failure (HF) is one of the most common causes of hospitalization and death of patients [24]. It is known that concomitant diseases and the dynamics of a variety of biomarkers and genetic factors are associated with the HF course and progression [15,33]. The low triiodothyronine syndrome (LT_3S) (Low T_3 syndrome, peripheral dysthyroidism syndrome) is observed in some patients with HF. Disruption of the activity of thyroxin (T_4) conversion to triiodothyronine (T_3) by peripheral deiodinases (D) is associated with the clinical course of the HF [11]. The activity of D, among other factors, is also affected by hypercatecholemia, which is inherent in HF. The hypercatecholemia in HF, on the one hand, is an adaptive mechanism, but subsequently it leads to disorder in many systems. These effects are realized through the b-adrenergic receptors' system (β-ARs). There is extremely little data in literature regarding the effect of β-ARs gene polymorphisms on the development of LT_3S and many contradictory results about their association with HF course. This encourages new research in this area.

The aim of the study was to evaluate the relationship of β-ARs genes polymorphisms with LT_3S in patients with HF.

MATERIALS AND METHODS

From January 2015 to June 2019, we included patients in the study and conducted a prospective follow-up for 2 years. 354 patients with HF (110 women and 244 men) of the Caucasian race were included to the study at the time of hospitalization at the department of cardiology due to decompensation of the HF. Their average age was 58.00 (54.00-67.00) years. Inclusion criteria were: signed patient informed consent, history of myocardial infarction (MI), verified diagnosis of HF of II – IV functional class by NYHA. Exclusion criteria were: no informed consent, hemodynamically significant valvular heart diseases, HF other than postinfarction cardiosclerosis etiology, hormone replacement therapy with L-thyroxine, thyroid suppression treatment, clinical or subclinical hypothyroidism, thyrotoxicosis, inflammatory diseases, other serious pathologies (tumor, tuberculosis), that could complicate treatment or reduce life expectancy.

Table 1. Characteristics of the studied population
Tabela 1. Chrakterystyka badanej populacji

Parameters	Control group	Patients with HF	p
Number of subjects	55	354	NA
Median age with interquartile range	57,00 (52,00-65,00)	58,00 (54,00-67,00)	0.121
Males, n (%)	37 (67.3)	244 (68.9)	0.806
Females, n (%)	18 (32.7)	110 (31.1)	χ^2
LT_3S, n (%)	10 (18.2)	85 (25.1)	0.262

Table 2. Characteristics of patients with HF at presence of LT_3S (criterion $T_{3f} \leq 2,07$ pmol/l)
Tabela 2. Charakterystyka chorych z niewydoolnością serca (HF) w odniesieniu do obecności LT_3S (kryterium $T_{3f} \leq 2,07$ pmol/l)

Parameters	Groups of patients with HF (n = 354)		P
	Without LT_3S (n = 265)	With LT_3S (n = 89)	
1	2	3	4
Age, years	58,00 [54,00-68,00]	58,00 [54,00-66,00]	0,657
Males, n (%)	179 (67,5)	65 (73)	0,333

The control group consisted of 55 healthy individuals (without coronary heart disease, HF, thyroid pathology): 18 women (32.7%) and 37 men (67.3%). Their average age was 57.00 (52.00-65.00) years. The statistical analysis did not reveal any significant difference in the frequency distribution by gender, LT_3S and age between the control group and patients with HF (tab.1).

Diagnosis of HF and treatment of patients were performed according to the recommendations of the European Society of Cardiology [20]. To determine serum concentrations of thyroid hormones: thyroid stimulating hormone (TSH) (normal range 0.3-4.0 mIU/l), free T_3 (T_{3f}) (normal range 2.5-5.8 pmol/l) and free T_4 (T_{4f}) (normal range 10-25 pmol/l) reagent kits ("TSH-ELISA", "T_{4f}-ELISA" and "T_{3f}-ELISA" from Hema, Ukraine) were used. The enzyme-linked immunosorbent assay was performed on an Immunochem-2100 semi-automatic immunoassay analyzer (High technology, USA). The follow-up period was 2 years, during which the course of HF was evaluated; the frequency of hospitalizations for decompensation of the disease, and mortality were estimated.

Patients with HF were divided into 2 groups: the first group included 265 (74.9%) patients who had normal levels of T_{3f}, T_{4f}, and TSH; the second – 89 (25.1%) patients with T_{3f} level ≤2.07 pmol/l at normal T_{4f} and TSH levels [22]. These patients were diagnosed with LT_3S. Groups of patients did not differ by gender and age (tab.2).

Selection of polymorphisms and genotyping. In the selection of gene polymorphisms, three main criteria were used: 1) the localization in the genes of the b-AR system; 2) the frequency of minor allele in European population ≥ 5% according to HapMap; 3) little or no research on the role of this polymorphism in LT_3S. DbSNP, SNPinfo, and SNPnexus databases were used to select polymorphisms [7,32]. Totally 4 single nucleotide polymorphisms (SNP) of 3 genes were selected. The table 3 shows the specific primers for genotyping. The laboratory staff did not know to which group the patients belonged; 10% of all DNA samples were genotyped repeatedly for quality control. Blood for molecular genetic studies was sampled in the VACUTEST tubes with K_3EDTA. DNA was isolated from whole blood using a set of reagents "DNA-sorbent-B" (Amplisens, Russian Federation). The isolated DNA was stored before amplification at a minus 20°C for no more than 3 months.

Genotyping of polymorphic sites of genes of β_1-adrenoreceptor (β_1-AR) (rs1801253; c.1165G> C; p.Gly389Arg), β_2-adrenoreceptor (β_2-AR) (rs1042714; c.79C> G; p .Gln27Glu) and the β_3-subunit of G-protein ($GN\beta_3$) (rs5443; c.825C> T; p.Ser275 =) were run by real-time polymerase chain reaction (PCR) using reagent kits "Syntola" according to the manufacturer's instructions. For genotyping of the Ser49Gly polymorphism of the β_1-AR gene (rs1801252; c.145A> G; p.Ser49Gly) TaqMan

Table 3. Studied single nucleotide polymorphisms
Tabela 3. Badane polimorfizmy pojedynczego nukleotytu

Gene	Single nucleotide polymorphism	Nucleotide substitution	Aminoacid substitution	Forward 5'-3' and reverse 3'-5' polymerase chain reaction primers
β_1-AR	rs1801253	c.1165G>C	Gly389Arg	F: ccccgacttccgcaaggccttccag R: gactgctctgctgcgcgcgcagggc
β_1-AR	rs1801252	c.145A>G	p.Ser49Gly	F: ctcgttgctgcctcccgccagcgaa R: gccccgagccgctgtctcagcagtg
β_2-AR	rs1042714	c.79C>G	p.Gln27Glu	F: tgcgccggaccacgacgtcacgcag R: aaagggacgaggtgtgggtggtggg
$GN\beta_3$	rs5443	c.825C>T	p.Ser275=	F: agagcatcatctgcggcatcacgtc R: gtggccttctccctcagtggccgcc

Table 4. *Hardy-Weinberg* equilibrium (p values)
Tabela 4. Równowaga *Hardy-Weinberg'a* (wartość p)

Single nucleotide polymorphism	Control group	Patients with HF	Total
Gly389Arg gene β_1-AP	0.55	0.22	0.14
Ser49Gly gene β_1-AP	0.55	0.44	0.17
Gln27Glu gene β_2-AP	0.43	0.16	0.091
Ser275 gene GNβ_3	0.65	1	1

SNP Genotyping Assay (ID C_8898508_10) and Universal PCR Master Mix (Ref. 4304437) (Thermo Fisher Scientific, USA) were used according to TaqMan® Universal PCR Master Mix USER GUIDE (Applied Biosystems by Life Technologies). The amplification was performed using the "CFX96 Touch Real-Time PCR Detection Systems" (BioRad). CFX Manager Software was used for allelic discrimination.

All polymorphisms we studied were in *Hardy-Weinberg* equilibrium (tab.4). This eliminated the possibility of a genotyping error.

Table 5. Relationship of β-ARs gene polymorphisms and LT_3S at patients with HF
Tabela 5. Zależność między polimorfizmem genu β-AR a obecnością LT_3S u chorych z HF

Model of inheritance	Genotype	Without LT_3S (n = 265)	With 3 LT_3S (n = 89)	OR (95% CI)	p	AIC	HWE
SNP Gly389A of β_1-AR gene (n=281)							
Codominant	G/G	116 (53.7%)	38 (62.3%)	1.00	0.47	296.5	
	G/C	80 (37%)	19 (31.1%)	0.72 (0.39-1.35)			
	C/C	20 (9.3%)	4 (6.6%)	0.61 (0.20-1.90)			
Dominant	G/G	116 (53.7%)	38 (62.3%)	1.00	0.23	294.6	
	G/C-C/C	100 (46.3%)	23 (37.7%)	0.70 (0.39-1.26)			0.22
Recessive	G/G-G/C	196 (90.7%)	57 (93.4%)	1.00	0.5	295.6	
	C/C	20 (9.3%)	4 (6.6%)	0.69 (0.23-2.09)			
Overdominant	G/G-C/C	136 (63%)	42 (68.8%)	1.00	0.39	295.3	
	G/C	80 (37%)	19 (31.1%)	0.77 (0.42-1.41)			
Log-additive	–	–	–	0.76 (0.48-1.20)	0.22	294.6	
SNP Ser49Gly of β_1-AR gene (n= 286)							
Codominant	G/G	157 (71.7%)	52 (82.5%)	1.00	0.12	301.4	
	A/G	59 (26.9%)	11 (17.5%)	0.56 (0.28-1.15)			
	A/A	3 (1.4%)	0 (0%)	0.00 (0.00-NA)			
Dominant	G/G	157 (71.7%)	52 (82.5%)	1.00	0.074	300.4	
	A/G-A/A	62 (28.3%)	11 (17.5%)	0.54 (0.26-1.09)			0.44
Recessive	G/G-A/G	216 (98.6%)	63 (100%)	1.00	0.22	302.1	
	A/A	3 (1.4%)	0 (0%)	0.00 (0.00-NA)			
Overdominant	G/G-A/A	160 (73.1%)	52 (82.5%)	1.00	0.11	301.1	
	A/G	59 (26.9%)	11 (17.5%)	0.57 (0.28-1.17)			
Log-additive	–	–	–	0.53 (0.26-1.06)	0.057	300	
SNP Gln27Glu of β_2-AR gene (n= 285)							
Codominant	C/C	89 (40.8%)	28 (44.4%)	1.00	0.045	298.9	
	C/G	101 (46.3%)	20 (31.8%)	0.63 (0.33-1.19)			
	G/G	28 (12.8%)	15 (23.8%)	1.70 (0.80-3.63)			
Dominant	C/C	89 (40.8%)	28 (44.4%)	1.00	0.61	302.8	
	C/G-G/G	129 (59.2%)	35 (55.6%)	0.86 (0.49-1.52)			0.16
Recessive	C/C-C/G	190 (87.2%)	48 (76.2%)	1.00	0.041	298.9	
	G/G	28 (12.8%)	15 (23.8%)	2.12 (1.05-4.28)			
Overdominant	C/C-G/G	117 (53.7%)	43 (68.2%)	1.00	0.037	298.7	
	C/G	101 (46.3%)	20 (31.8%)	0.54 (0.30-0.98)			
Log-additive	–	–	–	1.16 (0.78-1.71)	0.47	302.6	
SNP Ser275 of GNβ_3 gene (n= 285)							
Codominant	C/C	117 (53.7%)	29 (46%)	1.00	0.066	299.6	
	C/T	81 (37.2%)	32 (50.8%)	1.59 (0.90-2.84)			
	T/T	20 (9.2%)	2 (3.2%)	0.40 (0.09-1.82)			
Dominant	C/C	117 (53.7%)	29 (46%)	1.00	0.29	301.9	
	C/T-T/T	101 (46.3%)	34 (54%)	1.36 (0.77-2.38)			1
Recessive	C/C-C/T	198 (90.8%)	61 (96.8%)	1.00	0.088	300.2	
	T/T	20 (9.2%)	2 (3.2%)	0.32 (0.07-1.43)			
Overdominant	C/C-T/T	137 (62.8%)	31 (49.2%)	1.00	0.054	299.4	
	C/T	81 (37.2%)	32 (50.8%)	1.75 (0.99-3.07)			
Log-additive	–	–	–	1.04 (0.67-1.62)	0.86	303	

AIC – Akaike information criterion, HWE – *Hardy-Weinberg* equilibrium
The same at tables 6 and 7

The analysis of the normality of the distribution of values was performed using the *Shapiro-Wilk* test. Data are given as the median (Me) and the interquartile range (when the data is different from the normal distribution). Quantitative indicators were compared using *Mann-Whitney* non-parametric criterion. The difference between the frequencies in groups was estimated by chi-square distribution. The odds ratio (OR) with 95% confidence interval (CI) was calculated. The difference between values was considered statistically significant at $p < 0.05$. Statistics processing was performed using IBM® SPSS® Statistics, 20.0 (free-download full Version) software package. Genetic and epidemiological analysis was carried out using the SNPStats online program [27].

The protocol of this prospective cohort study was approved by the local Ethics and Deontology Committee of the Institute of Therapy. All patient-based study procedures were performed in accordance with the ethical standards of the Declaration of Helsinki.

RESULTS

In the analysis of the data in the control group, no significant or trend association of the β-ARs gene polymorphisms with the risk of LT_3S was found. In the group of patients with HF, the genotype C/G of the Gln27Glu polymorphism of the β_2-AR gene is associated with a decreased risk of developing LT_3S (OR=0.54 [0.30-0.98], p=0.037, an overdominant model of inheritance) (tab.5). With the homozygous G/G genotype of this gene, the risk of LT_3S increases (OR=2.21 [1.05-4.28], p=0.037, recessive model). We found a tendency to increase the risk of LT_3S development in the presence of the genotype C/T of the Ser275 polymorphism of the GNb_3 gene (OR = 1.75 [0.99-3.07], p=0.054, overdominant model). There were no statistically significant differences for the other studied polymorphisms.

In our previous study [21] we have shown that the presence of LT_3S significantly increases the risk of re-hospitalization due to HF decompensation within 2 years. We analyzed the associations of β-ARs gene polymorphisms with HF course. No association with the frequency of re-hospitalization was found between the Gly389A polymorphism of the β_1-AR gene and the Ser275 polymorphism of the $GN\beta_3$ gene. In addition, it was found that the carrier of the A allele (A/GA/A) of the Ser49Gly polymorphism of the β_1-AR gene was associated with a decrease in the frequency of rehospitalization (OR=0.50 [0.26-0.97], p=0.032, dominant model of inheritance) (tab.6). There was also a tendency to increase the risk of re-hospitalization in the G-allele (C / G-G / G) variant of the Gln27Glu polymorphism of the β_2-AR gene (OR=1.68 [0.98-2.87], p = 0.057, dominant model).

Taking into account (during calculations on SNPstats) the presence of LT_3S, it was found that the risk of re-hospitalization increases with C/G polymorphism of the Gln27Glu β_2-AR gene (OR=1.25 [0.85-1.82], p=0.025, overdominant model) (tab.7). No statistically significant differences for the other studied polymorphisms were revealed.

DISCUSSION

HF is a complex process affecting many organs and systems that leads to hormonal and immune alterations, including low T_3 syndrome. LT_3S is characterized by low T_3 levels, increased or normal reversed T_3 (rT_3) levels, normal or slightly altered TSH, and normal T_4 level [1,10,12]. In our study, the incidence of LT_3S among patients with HF was 25.0%. The main mechanism of low serum T_3 concentration in patients with nonthyroid disorders is decreased D activity in liver. Increased concentration of cytokines, such as interleukin-6 and tumor necrosis factor α, is one of the causes of decreased D activity. Another important factor in reducing D activity is hypercatecholemia [13].

The evidence that catecholamines increase D activity is a decrease in serum T_3 concentration when using β-blockers (β-AB) [13]. The inhibitory effect of β-AB on D activity is manifested in liver homogenates, but it is less shown in renal tubular

Table 6. Relationship between gene polymorphisms of the β-ARs with the frequency of re-hospitalization of patients with HF
Tabela 6. Zależność między polimorfizmem genowym układu β-AR a częstością ponownych hospitalizacji u chorych z HF

Model of inheritance	Genotype	Without rehospitalization (n = 272)	With rehospitalization (n = 109)	OR (95% CI)	p	AIC	HWE
SNP Ser49Gly of β_1-AR gene (n= 286)							
Codominant	G/G	145 (70.7%)	67 (82.7%)	1.00	0.053	341	
	A/G	57 (27.8%)	14 (17.3%)	0.53 (0.28-1.02)			
	A/A	3 (1.5 %)	0	0.00 (0.00-NA)			
Dominant	G/G	145 (70.7%)	67 (82.7%)	1.00	0.032	340.3	
	A/G-A/A	60 (29.3%)	14 (17.3%)	0.50 (0.26-0.97)			0.44
Recessive	G/G-A/G	202 (98.5%)	81 (100%)	1.00	0.16	342.9	
	A/A	3 (1.5%)	0	0.00 (0.00-NA)			
Overdominant	G/G-A/A	148 (72.2%)	67 (82.7%)	1.00	0.057	341.3	
	A/G	57 (27.8%)	14 (17.3%)	0.54 (0.28-1.04)			
Log-additive	–	–	–	0.50 (0.27-0.94)	0.023	339.7	
SNP Gln27Glu of β_2-AR gene (n= 285)							
Codominant	C/C	93 (45.6%)	27 (33.3%)	1.00	0.16	342.6	
	C/G	81 (39.7%)	40 (49.4%)	1.70 (0.96-3.01)			
	G/G	30 (14.7%)	14 (17.3%)	1.61 (0.75-3.46)			
Dominant	C/C	93 (45.6%)	27 (33.3%)	1.00	0.057	340.6	
	C/G-G/G	111 (54.4%)	54 (66.7%)	1.68 (0.98-2.87)			0.16
Recessive	C/C-C/G	174 (85.3%)	67 (82.7%)	1.00	0.59	343.9	
	G/G	30 (14.7%)	14 (17.3%)	1.21 (0.61-2.43)			
Overdominant	C/C-G/G	123 (60.3%)	41 (50.6%)	1.00	0.14	342	
	C/G	81 (39.7%)	40 (49.4%)	1.48 (0.88-2.49)			
Log-additive	–	–	–	1.34 (0.93-1.92)	0.11	341.7	

Table 7. Relationship of gene polymorphisms of the β-AR system with the frequency of re-hospitalization of patients with HF taking into account the presence of LT_3S
Tabela 7. Zależność między polimorfizmem genowym układu β-AR a częstością ponownych hospitalizacji u chorych z HF z obecnością LT_3S

Model of inheritance	Genotype	Without rehospitalization	With rehospitalization	OR (95% CI)	đ	AIC	HWE
SNP Ser49Gly of β_1-AR gene (n= 286)							
Codominant	G/G	143 (70.8%)	66 (82.5%)	1.00	0.16	316.4	
	A/G	56 (27.7%)	14 (17.5%)	0.60 (0.31-1.19)			
	A/A	3 (1.5 %)	0	0.00 (0.00-NA)			
Dominant	G/G	143 (70.8%)	66 (82.5%)	1.00	0.1	315.4	
	A/G-A/A	59 (29.2%)	14 (17.5%)	0.58 (0.29-1.14)			0.44
Recessive	G/G-A/G	199 (98.5%)	80 (100%)	1.00	0.23	316.7	
	A/A	3 (1.5%)	0	0.00 (0.00-NA)			
Overdominant	G/G-A/A	146 (72.3%)	66 (82.5%)	1.00	0.15	316	
	A/G	56 (27.7%)	14 (17.5%)	0.61 (0.31-1.21)			
Log-additive	–	–	–	0.57 (0.30-1.10)	0.081	315	
SNP Gln27Glu of β_2-AR gene (n= 281)							
Codominant	C/C	90 (44.8%)	27 (33.8%)	1.00	0.072	314.3	
	C/G	81 (40.3%)	40 (50%)	2.01 (1.09-3.72)			
	G/G	30 (14.9%)	13 (16.2%)	1.23 (0.53-2.83)			
Dominant	C/C	90 (44.8%)	27 (33.8%)	1.00	0.05	313.8	
	C/G-G/G	111 (55.2%)	532(66.7%)	1.76 (0.99-3.11)			0.33
Recessive	C/C-C/G	171 (85.1%)	67 (83.8%)	1.00	0.7	317.5	
	G/G	30 (14.7%)	13 (16.2%)	0.86 (0.40-1.84)			
Overdominant	C/C-G/G	120 (59.7%)	40 (50%)	1.00	0.025	312.6	
	C/G	81 (40.3%)	40 (50%)	1.90 (1.08-3.34)			
Log-additive	–	–	–	1.25 (0.85-1.82)	0.26	316.3	

cells [13]. Hypercatecholemia at HF exercisess its effects through the β-AR system. β-AR – paired transmembrane proteins are found on cells of the whole organism, including cardiomyocytes, vascular smooth myocytes, and extra-vascular parenchymal organs, including the thyroid gland, liver [13]. Catecholamines, through β-AR, regulate thyroid function [13] and activity of peripheral D in liver and adipose tissue [26]. There are three subtypes of adernoreceptors (β_1, β_2, β_3) [18]. β_1 and β_2-AR stimulate catecholamines on intracellular processes through a cytosolic heterotrimeric G-protein consisting of three subunits: α, β and γ. G-protein in turn regulates the classical cAMP / protein kinase pathway [30]. The active form of G-protein (Gs), associating with the allosteric center of adenylyl cyclase, activates the formation of cAMP. The cAMP serves as a regulator of the activity of many enzymes. As a result of these and a number of other related processes, iodide transportation to thyroid, and its incorporation into thyroglobulin are also activated. β_1-AR only activates G-protein (Gs). In addition, β_2-AR can also suppress it (Gi), reducing cAMP production, which may reduce D activity [30,31,34]. There are evidences that D activity may also depend on β-AR polymorphisms [2,6,9].

In this study, it was found out that the risk of LT_3S in patients with HF increases with homozygous G/G type of Gln27Glu polymorphism of the β_2-AR gene (OR=2.21 [1.05-4.28], p=0.037, recessive model). At the same time, the C/G genotype of the Gln27Glu polymorphism of the β_2-AR gene was associated with a decreased risk of LT_3S (OR=0.54 [0.30-0.98], p=0.037, overdominant model).

The β_2-AR gene is localized in chromosome 5q31_32. Significant mutations are Gly16Arg, Gln27Glu, Val34Met and Thr164Ile. Gly16Arg and Gln27Glu are located in the extracellular part of the receptor, while Thr164Ile is on the transmembrane domain and Val34Met is a rare mutation in the first transmembrane portal domain [4]. The proportion of small β_2-AR polymorphisms in the population is as follows: Arg16, Glu27, Ile164 – 39%, 43% and less than 5%, respectively, with a rare Met34 proportion [19]. For some polymorphisms, interethnic variability in allele frequency was shown: Gln27Glu occurs at 35% of Europeans, 21% of African Americans, 7% of Chinese [3,28].

Single nucleotide replacement of cytosine (C) by guanine (G) at position 79 of the β_2-AR gene results in the replacement of glutamine (Gln) with glutamic acid (Glu) in the 27 codon (rs1042714). The C allele is called the „wild-type" allele because it is most commonly found in the population, and the glutamic acid allele is less common, so it is called „mutant." Allelic frequencies of C and G in the general population are 0.55 / 0.45, respectively [4]. Studies have shown that this polymorphism is closely related to the sensitivity of this receptor to downregulation [28]. *In vitro* work has shown that the Glu27 allelic variant has a high degree of desensitization to the Arg16 variant after isoprenaline use [19]. The Glu27 allele is assumed to have a higher degree of resistance to downregulation than the wild-type, since it causes changes in the β_2-AR conformation [19].

In our study, a trend to increase the risk of re-hospitalization in the G-allele (C/GG/G) carriers of the Gln27Glu polymorphism of the β_2-AR gene (OR=1.68 [0.98-2.87], p=0.057, the dominant model) was found. In the presence of LT_3S in patients with HF, the risk of re-hospitalization increases with C/G polymorphism of the Gln27Glu β_2-AR gene (OR=1.25 [0.85-1.82], p=0.025, over-dominant model). According to the literature, the relationshiop of the Gln27Glu polymorphism of β_2-AR with the clinical course of HF is rather ambiguous. Exercise tolerance was studied in 2002 in patients with compensated HF and it was found that patients with Arg16/Glu27 had greater endurance compared with the Gli16/Gln27 polymorphism group [29]. A decrease in exercise tolerance always precedes the decompensation of HF [29]. Researchers studied the polymorphism of β_2-AR and β_2-AR 5 «LC Arg19Cys in patients with idiopathic dilated cardiomyopathy [19]. The analysis revealed that Arg16 and Gln27 β_2-AR polymorphism may be associated with a low risk of HF [19]. At the same time, other scientists studied 256 cases of HF in 2004, focusing on the β_1-AR Arg389Gly, β_2-AR Arg16Gly, and Gln27Glu polymorphisms, but no significant correlation with HF was found [5]. Two studies examined the effect of β_2-AR

gene polymorphisms on the risk of HF development and progression. The Italian study included 236 patients with HF and 230 healthy volunteers. No associations were found between Arg16Gly, Gln27Glu polymorphisms and HF course [5]. Another group of scientists reported the results of a randomized trial involving a large number of patients with ischaemic and idiopathic cardiomyopathy. No effect of polymorphism 16 and 27 of the β_2-AR gene on the risk of development and features of the HF course was detected [16].

We demonstrated that patients with HF who are carriers of the A allele (A/GA/A) of the Ser49Gly polymorphism of the β_1-AR gene have a lower risk of re-hospitalization for HF decompensation (OR=0.50 [0.26-0.97], p=0.032, the dominant model). The β_1-AR gene is localized on chromosome 10q24-26. There are two clinically relevant gene polymorphisms associated with single-nucleotide substitutions: in position 49 (extracellular N-terminal site) associated with the replacement of the amino acid serine (Ser) by glycine (Gly) and in position 389 (intracellular carboxyterminal site) by replacing arginine (Arg) with glycine. *In vitro* studies have shown that homozygotes for Ser (AA genotype) have lower functional adenylyl cyclase activity compared to carriers of the G allele, but they are more sensitive to adrenaline stimulation [14]. In another study, no differences in basal adenylyl cyclase activity were detected, but high sensitivity to long-term agonist activity was confirmed [23].

We were unable find the relationship of the Gly389Arg polymorphism of the β_1-AR gene with either the risk of LT_3S developing or the likelihood of rehospitalization. There was a tendency to increase the risk of LT_3S in the presence of the genotype C/T of the Ser275 polymorphism of the GNβ_3 gene (OR=1.75 [0.99-3.07], p=0.054, an over-dominant model). G-proteins are expressed in all human cells. The most common polymorphism of the β_3-subunit (GNβ_3) gene is C825T [25]. Although T polymorphism is functionally inactive, it leads to truncated (alternative) splicing of exon 9 (GNβ_3) and ultimately to the truncated β_3 subunit of the G protein. Such an altered subunit increases β-adrenergic activation and is associated with increased activity of signaling pathways [25]. This polymorphism primarily influences vascular reactivity and cardiomyocytes growth [25]. The T allele is more prevalent among the black race compared to the Caucasians [17]. Approximately 50% of Africans are homozygous for the GNβ_3 TT genotype, while among Europeans this value is only 10%. In a genetic study of HF at African Americans (AHeFT), homozygotes of the T allele (GNβ_3 TT) had the most unfavorable course of pathology in the placebo group and received the greatest benefit from therapy [17]. Despite the clear racial differences in the frequency of mutations, there are only isolated and rather contradictory results of studies of the effect of the GNβ_3 TT genotype on the course of HF in Europeans. We were unable to demonstrate the effect of this mutation on the incidence of re-hospitalization of patients with HF for decompensation. Also, we could not find the literature data on the value of this polymorphism, regarding the activity of D.

There was no significant or trend relationship between the risk of LT_3S development and β-ARs gene polymorphism in the absence of HF (control group). It is possible to assume that activity of D (development of LT_3S) is probably more influenced by other factors [8] than hypercatechaemia inherent in patients with HF. In this situation, gene mutation of the β-ARs system is not crucial.

CONCLUSIONS

The obtained results suggest that congenital genetic differences in β-adrenoreceptor pathways may be associated with the development of LT_3S in patients with HF (the risk of LT_3S in patients with HF increases with homozygous G/G type of the Gln27Glu polymorphism of β_2-AR gene and in the presence of the genotype C/T of the Ser275 polymorphism of the GNβ_3 gene; the genotype C/G of the Gln27Glu polymorphism of the β_2-AR gene is associated with a decreased risk of LT_3S) and features of the pathology (patients with HF who are carriers of the A allele (A/GA/A) of Ser49Gly polymorphisms of the β_1-AR gene have a lower risk of re-hospitalization due to HF decompensation, the presence of LT_3S in patients with heart failure the risk of re-hospitalization increased with C/G type of Gln27Glu polymorphism of the gene β_2-AR).

Studies in this area are needed to confirm these results and further investigations the genetic basis of LT_3S development and features of HF course.

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