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The relationship between -C344/T aldosterone synthase (*CYP11B2*) gene polymorphism, enzyme activity level and increased risk of nonvalvular atrial fibrillation

Związek polimorfizmu SNPs -C344/T genu *CYP11B2* syntazy aldosteronu z poziomem aktywności enzymu i ze wzrostem ryzyka niezastawkowego migotania przedsionków

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A – Study Design, **B** – Data Collection, **C** – Statistical Analysis, **D** – Data Interpretation, **E** – Manuscript Preparation, **F** – Literature Search, **G** – Funds Collection

Summary **Background.** Aldosterone plays an important role in the pathogenesis of non-familial AF. The aldosterone synthase gene (-C/344T *CYP11B2*) encodes aldosterone synthase – the enzyme that catalyzes the final reaction to generate aldosterone. Despite several investigation studies of association between -C/344T *CYP11B2* gene polymorphism, enzyme activity and atrial fibrillation (AF) presence, their results still remain controversial.

Objectives. To examine the association between the -C/344T *CYP11B2* gene polymorphism, enzyme activity level and risk of nonvalvular AF development.

Material and methods. The authors observed 45 patients with nonvalvular paroxysmal (group 1, *n* = 28) and persistent AF (group 2, *n* = 17) on the background of ischemic heart disease and/or hypertension, without significant structural myocardial damage. The third – control group – consisted of 39 subjects without cardiovascular diseases and history of arrhythmias. Patients were genotyped for the *CYP11B2* gene polymorphism using the polymerase chain reaction. Aldosterone synthase enzyme activity in plasma was also determined.

Results. In patients with AF the frequency of T/T genotype and T-allele was significantly higher than in the control group. The total number of AF-rhythm disorders was associated with the increased T-allele frequency. Aldosterone synthase enzyme activity level was significantly higher in patients with persistant AF and TT-genotype versus patients of other genotypes in this group and in all patients in the control group.

Conclusions. TT-genotype and T-allele of -C344/T *CYP11B2* gene associates so with nonvalvular AF presence, as with significantly higher aldosterone synthase activity level in patients with persistant AF. Patients with TT-genotype have higher risk of AF development.

Key words: atrial fibrillation, -C344/T aldosteronsynthase (*CYP11B2*) gene polymorphism, aldosterone synthase activity.

Streszczenie **Wstęp.** Aldosteron pełni ważną rolę w rozwoju migotania przedsionków (AF). Ostatnie trzy stadia syntezy aldosteronu katalizuje enzym syntaza aldosteronu, za pierwotną strukturę którego odpowiada gen -C344/T *CYP11B2*. Mimo że istnieją pewne badania związku wzajemnego między polimorfizmem genu *CYP11B2* -C344/T i poziomem aktywności enzymu a AF, ich wyniki pozostają niejednoznaczne.

Cel pracy. Badanie związku SNPs -C344/T genu *CYP11B2* syntazy aldosteronu z poziomem aktywności enzymu i ze wzrostem ryzyka niezastawkowego migotania przedsionków.

Materiał i metody. Zakwalifikowano 45 pacjentów z niezastawkowym migotaniem przedsionków w jego postaci napadowej (grupa 1, *n* = 28) oraz postaci utrwalonej (grupa 2, *n* = 17) przy obecności współistniejącej choroby niedokrwiennej mięśnia sercowego i/lub nadciśnienia tętniczego bez uszkodzenia mięśnia sercowego. Do grupy kontrolnej zakwalifikowano 39 pacjentów bez chorób sercowo-naczyniowych i niemiarowości rytmu serca w wywiadzie. Polimorfizm genu *CYP11B2* -C344/T badano z wykorzystaniem łańcuchowej reakcji polimerazy. Również określano poziom aktywności enzymu w osoczu.

Wyniki. U pacjentów z AF częstość genotypu T/T i T-allelu była istotnie wyższą niż w grupie kontrolnej. Ogólna liczba epizodów nawrotów AF wiązała się ze zwiększeniem częstości allelu -344/T. Poziom aktywności syntazy aldosteronu w osoczu był istotnie wyższy u pacjentów z utrwalonym AF i genotypem TT, niż u pacjentów w tej samej grupie, ale z innymi genotypami oraz u wszystkich pacjentów z grupy kontrolnej.

Wnioski. Obecność genotypu TT oraz allelu T wiążą się z obecnością AF, jak również z istotnie wyższym poziomem syntazy aldosteronu u pacjentów z utrwalonym AF. Pacjenci z obecnością genotypu TT wykazują obecność większego ryzyka rozwoju AF.

Słowa kluczowe: syntaza aldosteronu, migotanie przedsionków, polimorfizm genu -C344/T syntazy aldosteronu (*CYP11B2*).

Background

Atrial fibrillation (AF) is the most common cardiac arrhythmia, the incidence of which increases regularly with age [1].

Etiological and pathophysiological aspects of AF are complex and not fully understood. One of the important mechanisms of the AF pathogenesis is that anatomical properties of the atria associate with subsequent activation of the rennin–angiotensin–aldosterone system (RAAS) [2]. Effect of the elevated aldosterone levels on the AF development is being actively studied. Its proarrhythmic effect is known due to atrial fibrosis and stretch, altered cellular ion channel activities, changes in cellular ion expression, heterogeneity of conduction, increased sympathetic activity as well as apoptosis, necrosis, hypertrophy of atrial cardiomyocytes [3, 4].

In patients with AF there was not only plasma aldosterone concentration increasing but also an increased aldosterone receptor activity [5, 6]. At the same time, aldosterone level decrease after cardioversion [7]. Many factors, along with the RAAS, control aldosterone biosynthesis – potassium ions, brain natriuretic peptide (BNP), adrenocorticotropic hormone and dopamine, but the aldosterone synthase – is a key enzyme catalyzes this reaction, for the primary structure of which corresponds -C344/T *CYP11B2* gene [8]. Recent studies suggest that -C344/T *CYP11B2* nucleotide polymorphism affects the aldosterone-renin ratio: the 344T-allele was associated with an increased aldosterone–renin activity in plasma [9]. As for AF, -C/344T gene polymorphism was shown to be an independent predictor of AF development [10] and its occurrence [11, 12].

Objectives

The aims of this study was to examine the association between the -C/344T aldosterone synthase gene polymorphism, aldosterone synthase activity level and risk of nonvalvular AF development.

Material and methods

The authors observed 45 nonvalvular AF patients on a background of ischemic heart disease and/or hypertension, without significant structural myocardial damage, admitted due to such rhythm disorder to the Arhythmology Department of the Grodno Regional Clinical Cardiology Centre (Belarus). Among them there were patients with paroxysmal AF (n = 28) (82.1% male), mean age 55 (48; 63.5) years, that consisted group 1, and persistent AF (n = 17) (82.3% male), mean age 52 (46; 57) years, that consisted group 2. The third – control group – included 39 subjects without cardiovascular disease and history of arrhythmia (54% male), mean age 50 (39; 64) years. Patients with thyroid dysfunction, acute stroke, myocardial infarction, myocarditis, known chronic heart failure (NYHA ≥ 2), valvular heart disease, left ventricular dysfunction, diabetes, severe chronic diseases (eg, severe renal or liver failure) or inflammation in the last 6 months were excluded from the study. In all patients there was no history of familial arrhythmias. Routine laboratory and physical examinations were used to eliminate cardiovascular diseases.

All patients underwent instrumental and laboratory study after signing an informed consent form that was approved by authors institution's committee on human investigation.

Genotyping

To extract genomic DNA from the peripheral blood leukocytes the authors used "Nukleosorb" ("Praymteh", RB) reagents with the followed -C344/T gene polymorphism genotyping by PCR amplification (Rotor Gene-Q, «Qiagen», Germany) in real time mode TaqMan.

Laboratory methods

Aldosterone synthase (*CYP11B2*), a mitochondrial enzyme activity determination in plasma was performed using a set of Human Cytochrome P450 11B2, mitochondrial (ELISA Kit – catalog Number CSB – EL006391HU) and enzyme immunoassay analyzer TECAN («Sunrise», Austria).

Statistical analysis

Continuous variables were presented as medians with the corresponding range. Comparisons of genotype and allele distributions in different groups were made using χ^2 tests. To compare groups the authors used unpaired Student's t-tests, differences in continuous variables across genotype groups were tested using ANOVA. Odds ratios and associated confidence intervals (CI) were calculated in the standard way. The differences were considered statistically significant when $p < 0.05$ (2-tailed). The analysis was performed using Statistica 6.0 software package (Statsoft, US).

Results

It was revealed that in patients with AF the frequency of T/T genotype and T-allele were significantly higher than in relatively healthy subjects of control group ($p < 0.05$) (Tab. 1, Fig. 1). Binary logistic regression revealed that TT-genotype was a significant risk factor of AF development (OR = 3.44; 95% CI 1.07–11.07; p = 0.038).

Table 1. -C/344T *CYP11B2* allele frequencies in studied patients groups

		AF patients (n = 45)		Controls (n = 39)		p-value
		n	%	n	%	
Allele	T	48	53.3	28	36	0.03
	C	42	46.7	50	64	0.03

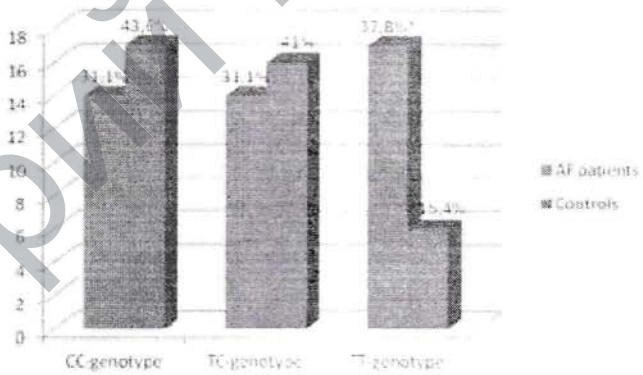

Figure 1. -C/344T *CYP11B2* genotypes frequencies in studied patients group

Note: * – the difference of genotypes frequencies is significant in comparison with control group ($p < 0.05$).

Regression analysis revealed that increased frequency of the T-allele was associated with the total number of AF-rhythm disorders (β = 0.30; p = 0.04).

Association between the -C344/T *CYP11B2* gene polymorphism and aldosterone synthase plasma levels

In order to explore possible ways in which gene polymorphism can influence on the aldosterone synthesis, aldosterone synthase activity levels have been measured. There were no significant differences between studied patients groups (Tab. 2).

But when all three patients groups were divided into subgroups depending on the genotype, it was revealed that in patients with persistent AF and TT-genotype the aldosterone synthase activity level was significantly higher than in patients of other genotypes (TC- and CC-) in this group and in all control patients group without AF (Tab. 3). Despite such significant differences in patients with paroxysmal AF and TT-genotype enzyme activity level was lower and didn't differ from the control patients group with the same genotype.

Table 2. Aldosterone synthase activity level in studied groups

Indicator	Group 1 Paroxysmal AF (n = 28)	Group 2 Persistent AF (n = 17)	Group 3 Controls (n = 39)	p-value
Aldosterone synthase activity, pg/ml	337.6 (82.9; 443.4)	364.9 (95.3; 435.6)	315.7 (117.2; 421.75)	NS

Table 3. Aldosterone synthase activity level depending on genotypes

		Aldosterone synthase activity, pg/ml		
		Group 1 Paroxysmal AF (n = 28)	Group 2 Persistent AF (n = 17)	Group 3 Controls (n = 39)
Genotype	CC	252 (44.7; 454.2)*	174.8 (38.6; 364.9)*	315.7 (57.9; 479.3)*
	TC	337.6 (277.5; 469.3)	252.5 (79; 381.3)*	328 (169; 400.4)*
	TT	266 (79.2; 423.8)	**518.5 (440.1; 576.1)**	264.8 (181.5; 421.8)*

Note: * – significant differences compared with patients with TT-genotype of group 2 (by U Mann-Whitney test).

Discussion

So it is still unclear how the -C/344T *CYP11B2* gene polymorphism affects the steroids biosynthesis at the molecular level. On chromosome 8q24 genes, encoding aldosterone synthase *CYP11B2* and 11B-hydroxylase *CYP11B1*, are located in a close proximity. It gives a possible explanation why the -C344/T *CYP11B2* gene polymorphism can be associated with changes that may be the result of processes occurring in the *CYP11B1* gene [13, 14]. It may also explain the lack of differences in enzyme activity levels despite the particular association between -C344/T *CYP11B2* gene polymorphism and AF history.

In the present study the authors investigated the relationship between aldosterone synthase activity, encoding it -C/344T *CYP11B2* gene polymorphism and risk of nonvalvular AF development in patients with paroxysmal and persistent AF, and first established the associations between enzyme activity level, encoding it gene polymorphism and increased risk of nonvalvular AF. However, studied patients sample was rather small, so despite the significant association of the -C/344T *CYP11B2* gene polymorphisms and the AF development according to other authors [15], the authors of the present paper indicate the need for further study of their association.

Conclusions

TT-genotype and T-allele of the -C344/T *CYP11B2* gene associates so with increased risk of nonvalvular AF, as with significantly higher aldosterone synthase activity level in patients with persistent AF. Patients with TT-genotype have higher risk of AF development. Increased frequency of T-allele was associated with the total number of AF-rhythm disorders. This finding may be important for the development of diagnosis and treatment strategies in patients with AF.

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