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Single Nucleotide Polymorphism rs950880 in IL1RL1 Gene and sST2 Plasma Concentration in Men with Essential Hypertension

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Abstract— Searching for heart remodelling legitimate biomarkers in patients with hypertension is an important branch of modern cardiology. ST2, a soluble representative of interleukin-1 receptor familyis a promising yet underinvestigated myocardial remodelling marker. The genetic basis of ST2 production, i. e. SNP rs950880 polymorphism of IL1RL1 gene, also demands detailed studying. The purpose of the study was to investigate the prevalence and special characteristics of the phenotypic implementation of SNP rs950880 in IL1RL1 gene, namely sST2 plasma level in men (residents of the Podolsk Oblast of Ukraine) with essential hypertension (EH) of varying severity. The study included 170 men residing in Vinnytsia Oblast, Ukraine: 70 men without any cardiovascular disease and 100 men with EH of varying severity aged 40 to 60 years. Among the residents of Vinnytsia Oblast, Ukraine, both among men without any cardiovascular disease and among patients with EH of varying severity, the most common variant of SNP rs950880 in IL1RL1 which includes C allele. The lowest plasma concentration of sST2 was observed in all groups of AA variant carriers; however, sST2 plasma level among these patients did not increase significantly even in case of EH development.

Key words: essential hypertension, soluble ST2, single nucleotide polymorphism rs950880 in IL1RL1 gene.

Introduction

In the fast-paced world of modern medicine, the arterial hypertension (AH) incidence is steadily increasing while its consequences present a heavy medical and social burden for many countries and, in particular, for Ukraine. A number of complications caused by a persistent blood pressure (BP) increase are stemmed from myocardial remodelling. The development of left ventricular hypertrophy (LVH) immediately puts AH patients at a high cardiovascular risk and, thus, increases several times their chances of getting a disabling condition or fatal outcome. The early detection of changes in the heart on the background of hypertension is the prerequisite to successful secondary prevention. An important branch of such diagnostics is the use of biomarkers which can be used for screening programmes independently of expensive equipment or complex technologies. Soluble ST2, growth stimulating factor which belongs to interleukin-1 receptors family, is a relatively new biomarker in the cardiac biomarkers cohort. At present, the processes involving IL-33/ST2 system, its anti-inflammatory effects and cardioprotection have been rather thoroughly studied [15]. The peptide is known to be expressed on cardiomyocytes, fibroblasts, and endothelial cells while its increased blood level has been associated with the myocardial fibrosis factors activation [8]. The given data allow to range soluble ST2 in the category of perspective biomarkers of heart remodelling. Indeed, a number of studies have confirmed that high plasma peptide concentrations are observed in acute coronary syndrome, acute and chronic heart failure (HF), and other cardiovascular diseases [1, 4, 8, 10, 11, 12, 13]. The 2017 ACC/AHA guidelines for the management of patients with acute and chronic HF suggest that sST2 plasma levels be determined to predict hospitalisation and fatal outcome rates in such patients [1]. However, the data on sST2 production in hypertension and due to LVH development are limited. According to a number of researchers, LVH development and sST2 production are interrelated [2, 3, 5, 6, 14]. Nevertheless, there are studies which have not confirmed

such correlation [16]. It is reasonable to assume that this difference in the clinical trials results may be due to unaccounted genetic component of peptide production. In general, there are very few studies of sST2 production genetic basis, although it has been established that genetic factors can determine up to 40% of inter-individual variability of sST2 levels [7]. The mechanism of IL1RL1 gene upon presence of SNP rs950880 polymorphism is promising in terms of studying the said aspect. It is now known that the C/A polymorphism rs950880 (replacement of cytosine by alanine) affects IL1RL1 gene expression in various tissues, as well as sST2 blood concentration [7]. However, there is no research information on the phenotypic implementation of this polymorphism in the form of peptide production in various cardiovascular diseases, in particular, in hypertension, taking into account possible subpopulation differences.

Thus, **the purpose of the study was to investigate the prevalence and special characteristics of the phenotypic implementation of SNP rs950880 in IL1RL1 gene and sST2 plasma level in men (residents of Podolsk Oblast of Ukraine) with essential hypertension (EH) of varying severity.**

Materials and Methods

In the furtherance of this goal, 170 men (residents of Vinnytsia Oblast, Ukraine) were examined, of which 70 men without cardiovascular diseases (mean age 48.82 ± 0.78 years) were randomised in the control group, while 100 men with confirmed EH (mean age 50.17 ± 0.48) were randomised in the study group. The inclusion criteria were as follows: age from 40 to 60 years, male, diagnosis of EH verified according to the current recommendations, residence in Vinnytsia Oblast in the third generation, at a distance over 5 km from each other's place of residence, as well as lack of family relations with each other. All study participants signed the Informed Consent on participation and their personal data processing prior to any study procedures. The following exclusion criteria were applied: confirmed secondary HT, endocrine diseases, blood system disorders, chronic obstructive pulmonary disease, renal (glomerular filtration rate less than 60 ml/min by MDRD formula) or hepatic dysfunction, history of and documents confirming EH complications (myocardial infarction, acute cerebrovascular event), symptoms of clinically significant (III-IV functional class stable angina) or unstable forms of coronary heart disease (CHD), anamnestic data on coronary heart disease which preceded EH. In the study group, 50 men had asymptomatic EH, 50 patients had EH complicated by HF, II-III functional classes by NYHA. EH stages were verified according to the recommendations of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH), 2018.

All study participants were subjected to SNP rs950880 polymorphism determination in IL1RL1 gene using polymerase chain reaction (PCR).

sST2 plasma level was determined by enzyme-linked immunosorbent assay using a kit of reagents manufactured by RayBiotech, Inc. (USA) and Humareader Single ELISA analyser (Germany).

Results and Discussion

At the first stage of the study, the prevalence of SNP rs950880 polymorphism in IL1RL1 gene among the residents of Vinnytsia Oblast was studied. For Vinnytsia Oblast, it was found that the proportion of C allele carriers was 65.00% while A allele was detected only in 35.00% of the study population. In general, CC genotype and CA genotype occurred with almost the same frequency (72 men [42.35%] and 77 men [45.30%], respectively) while AA genotype occurred 3 times less often (21 men [12.35%]) ($p<0.05$).

Further, the prevalence of different variants of SNP rs950880 in IL1RL1 gene were analysed separately in the control group and study group. It was found that in the control group, C allele carriers (67.14%) and CC genotype and CA genotypes (45.72% and 42.86%, respectively) also prevailed, while A allele carriers (11.86%) and AA homozygous genotype were significantly less common (11.42%) (see Fig. 1). The similar data were obtained for the study group. Thus, the

detection frequency was 63.50% for C allele and 36.50% for A allele. For different SNP rs950880 genotype among patients with EH, the frequency distribution was as follows: CC genotype was registered in 40 men (40.00%), AC genotype – in 47 men (47.00%), and AA genotype – in 13 men (13.00%). Thus, even among the residents of Vinnytsia Oblast with EH, C allele carriers predominate while A allele and AA homozygous genotype were significantly less common ($p < 0.05$).

The prevalence of the studied gene polymorphism was further compared between the control group and men having EH (see Fig. 1).

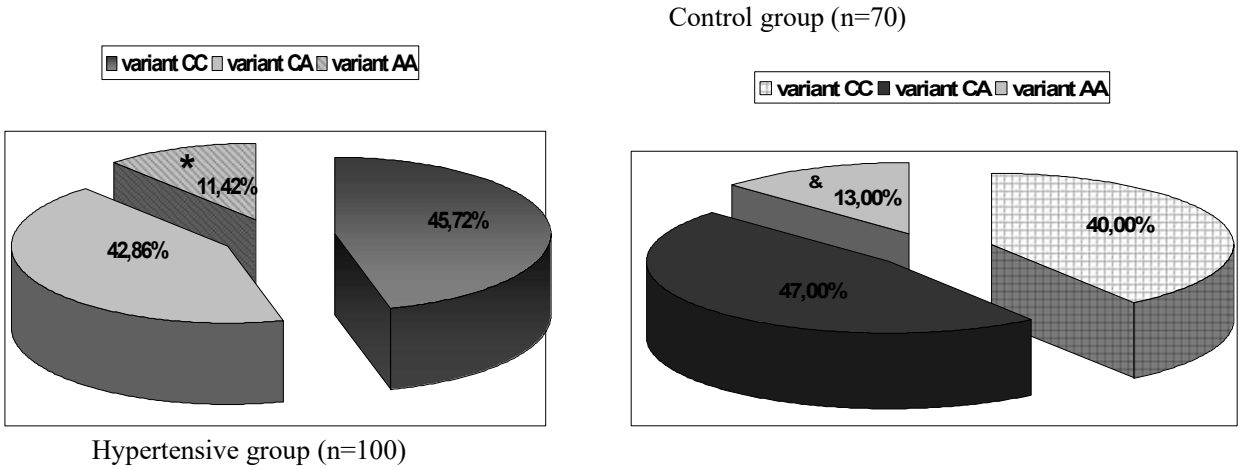


Fig. 1. The prevalence of SNR rs950880 in IL1RL1 gene among men without cardiovascular diseases residing in Vinnytsia Oblast (%)

Note: * differences are reliable in comparison with CC genotype and CA genotype carriers, $p < 0,01$, control group;

&– differences are reliable in comparison with CC genotype and CA genotype carriers, $p < 0,01$, study group with EH.

It was found that the frequency of different genotype variants and SNR rs950880 alleles in IL1RL1 gene in the control group and study group with EH (regardless of the severity of the disease) did not differ significantly.

However, further analysis of the frequency distribution of SNP rs950880 in IL1RL1 gene among men with EH of varying severity showed that C allele carriers with asymptomatic EH and EH complicated by HF were found among the patients significantly more often (65.00% and 62.00%, respectively) than A allele carriers (35.00% and 38.00%, respectively) ($p < 0.05$). These findings generally correspond to the data obtained for the general population of men residing in Vinnytsia Oblast. In the group of asymptomatic EH, the portion of men with CC homozygous genotype was 42.00%, with CA heterozygous genotype – 46.00%, and AA homozygous genotype – 12.00%; in the HF group complicated by HF, the findings were the same: CC genotype occurred with a frequency of 38.00%, CA genotype – 48.00%, AA genotype – 14.00%. Therefore, the frequency distribution of SNP rs950880 in IL1RL1 gene was almost the same and did not differ significantly in patients with EH of varying severity.

Thus, the C allele carriers and CC and CA genotypes of SNR rs950880 in IL1RL1 gene prevail among the residents of Vinnytsia Oblast, Ukraine, both in men without cardiovascular diseases and in patients with EH of various severity. According to the frequency distribution of the studied

gene polymorphic variants, portion of men with EH did not differ significantly from the general population of Vinnytsia Oblast.

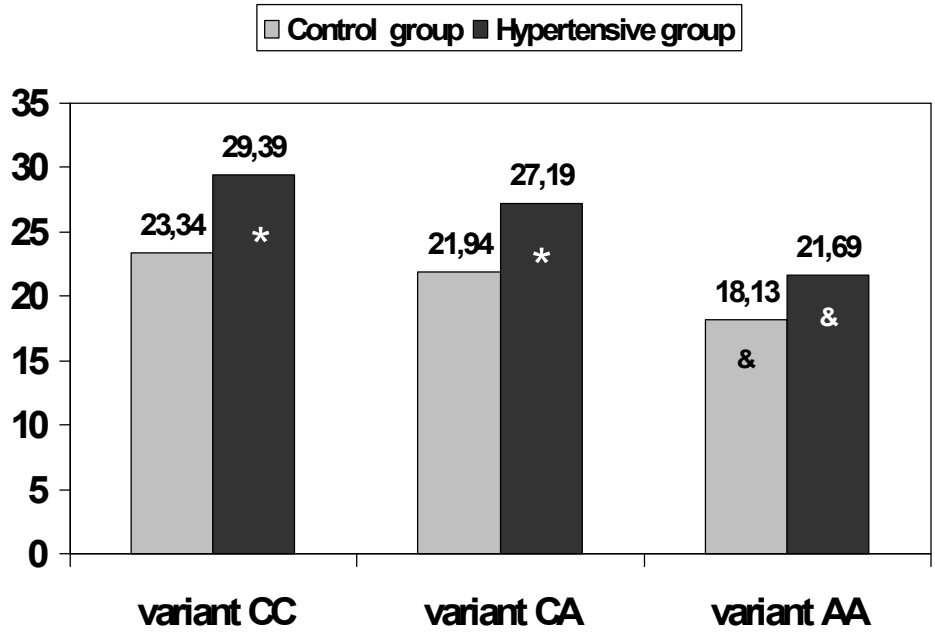
In general, there is a lack of literature data on IL1RL1 gene SNP rs950880 polymorphism. In relation to our study, it is worth mentioning the extensive work of Thai scientists [9] who studied SNP rs950880 in patients with clinical coronary and peripheral arteries atherosclerosis testing the hypothesis of a possible correlation between polymorphism and long-term effects of the disease. The researchers found that Thai carriers of C allele also predominated among C allele carriers in all study groups. However, it was found that the presence of AA variant of genotype is an independent predictor of overall mortality, however it is not correlated with the risk of myocardial infarction, stroke, or a higher probability of cardiovascular death.

The next step of the study involved investigation of such an aspect of the phenotypic implementation of SNP rs950880 polymorphism in IL1RL1 gene as sST2 plasma level.

It was found that sST2 plasma concentration in men residing in Vinnytsia Oblast, Ukraine (general population) was the lowest in the carriers of AA homozygous genotype of SNP rs950880 and was 20.34 ± 1.04 ng/ml ($p < 0.05$). In C allele carriers, the blood level of peptide was significantly higher (26.70± 1.57 ng/ml for CC genotype carriers and 25.14 ± 1.15 ng/ml for AC genotype carriers).

Peptide plasma levels were further analysed separately in the control group and among men with EH of varying severity. For the control group, the findings were similar to those for the general population, namely, in the carriers of AA homozygous genotype, the plasma concentration of peptide was significantly lower (18.13 ± 0.85 ng/ml) compared with the carriers of CC homozygous genotype (23.34 ± 1.22 ng/ml) ($p < 0.05$) (see Fig. 2). In the study group with EH regardless of its severity, sST2 plasma concentration was also the lowest in case of AA homozygous genotype of SNP rs950880 in IL1RL1 gene (21.69 ± 2.44 ng/ml vs. 29.39 ± 1.48 ng/ml in carriers of CC genotype and 27.19 ± 1.57 ng/ml in carriers of CA genotype) (see Fig. 2).

Fig.2. Blood plasma sST2 level in men carrying different genotypes of SNP rs950880 in IL1RL1 gene.



Note: * – differences are reliable when compared with the control group, $p < 0,05$;

& – differences are reliable in comparison with the carriers of CC and CA genotypes, $p < 0.05$.

The plasma level of circulating sST2 in carriers of different genotypes of SNP rs950880 in IL1RL1 gene among patients with EH of different severity was also analysed. The results are presented in Table 1.

Table 1

Blood plasma sST2 in men with EH of varying severity carrying different genotypes of SNP rs950880 in IL1RL1 gene, (M ± m).

Groups	sST2 level, ng/ml (M ± m)
Asymptomatic EH group (n=70)	
1. CC homozygous genotype (n=30)	23.34 ± 1.22
2. AC heterozygous genotype (n=32)	21.94 ± 1.47
3. AA homozygous genotype (n=8)	18.13 ± 0.85
p<0.05 or p<0.01	P_{3-1}
4. CC homozygous genotype (n=21)	28.96 ± 1.78
5. AC heterozygous genotype (n=23)	25.44 ± 1.87
6. AA homozygous genotype (n=6)	21.96 ± 2.54
p<0.05 or $^{*}p < 0.01$	p_{6-4} p_{5-2} p_{4-1}
Complicated EH group (n=50)	
7. CC homozygous genotype (n=19)	29.85 ± 2.47
8. AC heterozygous genotype (n=24)	28.87 ± 2.49
9. AA homozygous genotype (n=7)	21.46 ± 2.74
p<0.05 or $^{*}p < 0.01$	$^{*}p_{9-7}$ p_{9-8} p_{8-2} p_{7-1}

In the groups of patients with asymptomatic EH and EH complicated by stage II HF, the lowest level of peptide was also registered in the plasma of patients with AA homozygous genotype (see Table 1).

The analysis of the peptide blood level in the control group and patients with EH who carried one variant of the genotype revealed interesting results (see Fig. 2). It was found that sST2 plasma concentration in carriers of AA homozygous genotype from the control group and in patients with EH (general population) and separately from groups with asymptomatic EH and EH complicated by HF did not differ significantly (see Table 1). Within the groups with CC variant and AC genotypes, the peptide plasma level was significantly higher in patients with EH both in general population and separately in patients with asymptomatic EH and EH complicated by HF (see Table 1). This result indicates that in the carriers of AA homozygous genotype, sST2 plasma level did not increase significantly even in case of developed EH. From a physiological point of view, free-circulating sST2 somewhat inhibits the cardioprotective effects of IL-33. The fact that the carriers of AA genotype of SNP rs950880 of the gene, which was closely related to the peptide production, had lower plasma level of this peptide can be considered as a certain cardiac protective effect of this genotype variant.

It is obvious that rs950880 allelic polymorphism in IL1RL1 gene plays some role in the regulation of sST2 production both in men without cardiovascular diseases and in patients with EH. Similar conclusions were made by other researchers. Thus, the study involving residents of Northern and Western Europe revealed that the average plasma concentration of peptide in the carriers of CC homozygous genotype was 43% higher than in the carriers of AA homozygous genotype [7]. In the above-mentioned Thai population of AA homozygous genotype carriers, the lowest sST2 plasma levels were also detected in both the control group and patients with coronary and peripheral atherosclerosis [9].

Conclusions

1. Carriers of the C allele and CC and CA genotypes of SNR rs950880 in IL1RL1 gene are significantly more common among the residents of Vinnytsia Oblast, Ukraine, both in men without cardiovascular diseases and in patients with EH of various severity.
2. The lowest sST2 plasma concentration in men without cardiovascular disease and in patients with EH of varying severity was recorded in AA homozygous genotype.
3. In AA homozygous genotype, sST2 plasma concentration was not significantly increased even in case of EH development; in C allele carriers, the peptide level in EH patients was significantly higher than in men without EH in general population and regardless of the severity of the disease.
4. It is conceivable that missense mutation from C to A in rs950880 locus of IL1RL1 gene appears to be protective due to decreased peptide production in AA homozygous genotype, however additional studies are required to clarify the essence of the phenomenon.

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