DISTRIBUTION OF RS6795970 SNP VARIANTS IN SCN10A GENE IN YOUNG POPULATION OF THE REPUBLIC OF MOLDOVA

Cristina BUTOVSCAIA - research assistant,
Anastasia BUZA - research assistant,
Daniela GALEA-ABDUȘA - PhD, research assistant,
Ghenadie CUROCICHIN - MD, PhD, professor.

Laboratory of genetics, *Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

tel.: +373 69 654 423; cristina.butovscaia@usmf.md

Summary

Background. PR interval reflects atrial and atrioventricular nodal conduction time and is an important determinant of arrhythmia risk. Genome-wide association studies (GWAS) have identified association of nonsynonymous SNP, rs6795970, in the SCN10A gene with PR interval in individuals of European ancestry. **Purpose.** To estimate the distribution of rs6795970 variants, associated with PR interval in young population of Republic of Moldova. **Methods**. 1390 young participants from Republic of Moldova with age range: 19-25 years, were genotyped for rs6795970 in the SCN10A gene, using TaqMan technology. **Results.** The genotype A/A, A/G, G/G distributions of rs6795970 among the young participants were 15%, 48%, 37% respectively ($\chi 2 = 0.161$, $p = 0.688$). The allele frequencies for A and G in young participants were 39% and 61% respectively. **Conclusion.** The minor allele frequency (MAF) in young Moldavian population was 0.39 for rs679570 and was consistent with 1000 Genomes Project data in the European population – 0.41. 15% of all participants (the AA genotype), may have an increased risk of conduction abnormalities.

Key-words: SNP, PR interval, genotype, GWAS.

Rezumat. Distribuția variantelor polimorfismului de un singur nucleotid rs6795970 al genei SCN10A la populația tânără din Republica Moldova

Introducere. Intervalul PR reflectă timpul de conducere nodală atrială și atrioventriculară și este un predictor important al riscului de aritmie. Prin studiile de tip *GWAS* a fost identificată asocierea polimorfismului de un singur nucleotid (SNP) rs6795970, nonsinonim, al genei SCN10A, cu intervalul PR la indivizii din populația europeană. **Scopul**. Determinarea distribuției variantei genetice ale polimorfismului rs6795970 asociat cu intervalul PR în populația tânără din Republica Moldova. **Metode.** Genotiparea polimorfismului rs6795970 al genei SCN10A la 1390 de participanți tineri din Republica Moldova, cu vârstele cuprinse între 19-25 de ani, s-a efectuat prin tehnica *TaqMan*. **Rezultate**. S-a stabilit că distribuțiile genotipurilor A/A, A/G, G/G de rs6795970 între participanți tineri au fost 15%, 48% și, respectiv, 37% ($\chi 2=0,161$, $p=0,688$). Frecvența alelei A a fost de 39%, iar a alelei majore G de 61%. **Concluzie**. Frecvența de 0,39 a alelei minore (*MAF*) în populația tânără din Republica Moldova, pentru rs679570 este în concordanță cu datele Proiectului *1000 Genomes* pentru populația europeană – 0,41. 15% din toți participanții (genotipul AA) pot avea un risc crescut de anomalii de conducere.

Cuvinte-cheie: *SNP*, interval PR, genotip, *GWAS*.

Резюме. Распределение вариантов однонуклеотидного полиморфизма rs6795970 гена SCN10A среди молодого населения Республики Молдова

Введение. PR интервал отражает время прохождения возбуждения по предсердиям и атриовентрикулярному соединению до миокарда желудочков и является важной детерминантой риска аритмий. Полногеномные исследования ассоциаций (*GWAS*) выявили связь несинонимичного однонуклеотидного полиморфизма (ОНП) rs6795970 в гене SCN10A с PR интервалом у лиц европейского происхождения. **Цель.** Определить распределение генетических вариантов rs6795970, связанного с PR интервалом у молодого населения Республики Молдова. **Методы.** Генотипирование полиморфизма rs6795970 гена SCN10A среди 1390 молодых участников из Республики Молдова в возрасте от 19 до 25 лет было выполнено с использованием методики *TaqMan*. **Результаты.** Распределение генотипов A/A, A/G, G/G rs6795970 среди участников составило 15%, 48%, 37% соответственно ($\chi 2 = 0,161$, $p = 0,688$). Частоты аллелей A и G составили 39% и 61% соответственно. **Вывод.** Частота минорного аллеля (*MAF*) rs679570 в популяции молодого населения Республики Молдова составила 0,39 и согласуется с данными проекта *1000 Genomes* в европейской популяции - 0,41. 15% от всех участников (генотип AA) могут иметь повышенный риск нарушений проводимости.

Ключевые слова: ОНП, PR интервал, генотип, GWAS.

Introduction

The electrocardiogram (ECG) is a valuable clinical tool for assessing the function of the cardiac conduction system. The electrocardiographic PR interval represents conduction through the atria and atrioventricular (AV) node to the Purkinje fibers. This

interval represents the time between the excitation of the atria and ventricles, which normally ranges from 120 to 200 milliseconds in duration on the standard 12-lead electrocardiogram [1].

Delayed conduction in the above parts of the cardiac conduction system, results in prolongation of this ECG parameter. Prolongation of the PR interval leads to increased risk of atrial fibrillation, heart block, and mortality. The duration of the PR interval has a important heritable component, with heritability estimates ranging up to 50% in populations of European and Asian ancestry [2, 3]. Genome-Wide Association studies (GWAS) have identified a common loci associated with PR interval duration. The strongest association was observed between nonsynonymous single nucleotide polymorphism, rs6795970 (G > A), in the *SCN10A* gene and the PR interval [1-4].

The SCN10A gene is mapped to chromosome 3p22.2 and encodes the alpha subunit, type X, of a voltage-gated sodium channel. The SCN10A gene is expressed in the dorsal root ganglion (DRG), nociceptive nerve fibers, retina, in the myocardium and preferentially in the Purkinje fibers of the cardiac conduction system. The allele A of the *SCN10A* gene polymorphism (rs6795970) was associated with increased risk of first-degree heart block, bundle-branch block, bifascicular heart block, idiopathic sick sinus syndrome [1-4].

Early determination of the genetic characteristics of the functioning of the cardiac conduction system in young people is important to form groups for early preventive interventions with the potential to reduce, in the future, the incidence of cardiovascular diseases.

The purpose of the study was to determine distribution of the genetic variants of rs6795970, associated with PR interval in young population of Republic of Moldova.

Material and methods. 1390 students from *Nicolae Testemitsanu* State University of Medicine and Pharmacy, aged between 19-25 years, enrolled in our cross-sectional study. Written informed consent was obtained from all the participants. Personal identifiers associated with medical information and blood samples were encrypted with a special codification and then analyzed. The study was approved by the *Nicolae Testemitsanu* SUMPh Research Ethics Committee.

DNA isolation. Genomic DNA was isolated from buffy coat using silica-based membrane technology in the form of a spin column Gene JET Genomic DNA Purification Kit (Thermo Scientific, USA) according to the manufacturer's protocol. Quality evaluation and quantification of isolated DNA samples was performed by the spectrophotometry using NanoDrop 2000c.

TaqMan SNP Genotyping. Genotype analysis of all 1390 participants to detect rs6795970 (G>A) in the *SCN10A* gene was performed with commercially available TaqMan assay kit (Assay ID: C__29261054_10) on a QuantStudio 6 Flex instrument (Thermo Fisher Scientific). The data analysis has been performed using TaqMan Genotyper Software (v.1.3.1., Applied Biosystems, ThermoFisher Scientific). The differences of genotype frequencies of the rs6795970 have been analyzed by the chi-square Test ($\chi 2$), also used to test deviations of genotype distribution from the Hardy-Weinberg equilibrium.

Results. Out of 1390 samples, the genotyping successful call rate was 99.7%. The validity of the genotyping results is in concordance with the allele frequency distribution predicted by Hardy-Weinberg equilibrium for rs6795970 ($\chi 2 = 0.161$, $p = 0.688$). The distribution of genotypes and allele frequencies of the rs6795970 in the sample tested is shown in *table 1*. The genotype A/A, A/G, G/G distribution of rs6795970 was 15%, 48% and 37% respectively. The allele frequencies for A and G were 39% and 61% respectively.

Table 1

The genotypes and alleles distribution of the rs6795970 *SCN10A* polymorphism

	Young population of Republic of Moldova	
	(n=1390)	%
Genotype frequency		
G/G	515	37%
G/A	668	48%
A/A	207	15%
Allele frequency		
G allele	848	0.61
A allele	542	0.39

Discussion. Initially, SCN10A was found expressed in the dorsal root ganglion and the encoded Nav1.8 channels were thought to play a role in pain pathways [5]. Recently genome-wide association studies (GWAS) have identified strong association of the *SCN10A* with cardiac conduction. The Nav1.8 blocker, A-803467, prolonged the PR and QRS intervals in mice [6] and reduced the late sodium current (INa, L) in mouse and rabbit cardiomyocytes, and slows action potential firing in sympathetic intracardiac neurons, confirming a direct role for SCN10A in cardiac conduction [7]. Chambers et al.

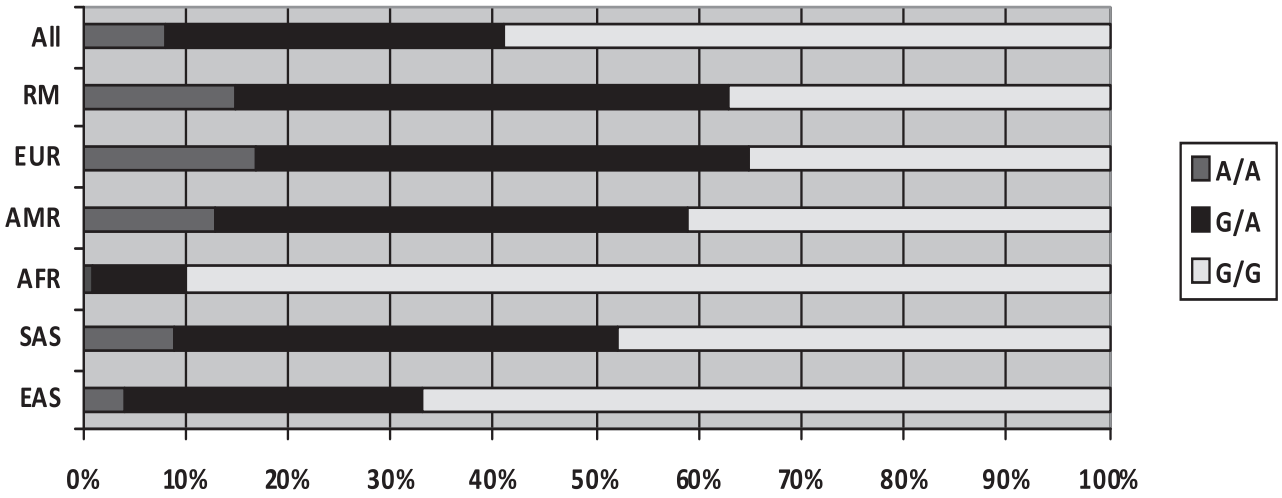

Fig. 1. Genotype frequency of the *SCN10A* polymorphism rs6795970

Notes:

ALL – All (phase 3 individuals, 1000 Genomes Project); RM - Population in Republic of Moldova; EUR – European; AMR- American; AFR – African; SAS – South Asian; EAS – East Asian (1000 Genomes Project).

In addition, it important to determine the association of rs6795970 genotypes with electrocardiographic parameters (FCC, PR, QRS, QT) to form risk groups for early general preventive efforts targeting with the potential to reduce, in the future, the incidence of cardiovascular diseases.

conducted a study involving 6,243 Indian Asians and 5,370 Europeans and identified association of rs6795970 with PR interval duration. It was reported that rs6795970 (G > A) leads to longer P-wave duration, PR interval and QRS duration, higher risk of heart block, and lower risk of ventricular fibrillation [4]. At the same time, Pfeufer et al. meta-analyzed the results of genome-wide association studies for PR interval in European-ancestry individuals and demonstrated a specific association of rs6795970 with atrial fibrillation and flutter [3]. A multi-centre study conducted in London revealed that A allele, encoding the Valine, was strongly associated with Brugada Syndrome (66.9%) compared with controls (40.1%), and demonstrated loss of Nav1.8 function [8]. Chiharuko Iio et al. proved the association between the rs6795970 polymorphism of the SCN10A gene and cardiac conduction disorders in Japanese patients with hypertrophic cardiomyopathy [9]. The AA genotype of the rs6795970 was associated with predisposition to idiopathic sick sinus syndrome in Siberian population sample [10]. Thus, several studies have established the association of minor allele (A allele) with higher risk of conduction abnormalities. In our study, the minor allele frequency (MAF) was 0.39 for rs679570, consistent with 1000 Genomes data in the European population – 0.41. Furthermore, we performed the comparative analysis of the obtained frequencies with those established in other studies on populations *(Figure1)*.

Conclusions.

In this study, we determined that distribution of the genetic variants of rs6795970, associated with PR interval in young population of Republic of Moldova was consistent with 1000 Genomes data in the European population.

Thus, our data demonstrate that at least 15% of all participants (the AA genotype), may have an increased risk of conduction abnormalities.

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