

Single-nucleotide Polymorphisms of AGT, SOD2, CAT in Risk Estimates for Arterial Hypertension among Young People

A. V. Kovaleva, I. V. Astratenkova, N. E. Golovanova,
O. G. Orlova, O. V. Rybalchenko

St. Petersburg State University,
7–9, Universitetskaya nab., St. Petersburg, 199034, Russian Federation

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Arterial hypertension is one of the most common cardiovascular diseases and one of the main causes of mortality and disability of population. Opportunity to predict emergence of arterial hypertension and to take preventive measures in pre-nosological diagnostics of disease arouse research interest in this issue. Possibility to predict progression of arterial hypertension based on polymorphic variants of *AGT* gene (C521T, rs4762) identification, which encodes angiotensinogen protein in combination with polymorphic variants of *CAT* (–262C/T, rs1001179), encoding catalase, and polymorphic variants of *SOD2* (C47T, rs4880), encoding mitochondrial superoxide dismutase, are discussed in this article. Decrease of antioxidant enzymes (catalase and superoxide dismutase) activity leads to accumulation of reactive oxygen species in cells. They may activate NF- κ B transcription factor, which increases expression of individual genes involved in sodium homeostasis and in blood pressure control, including angiotensinogen. Increased angiotensinogen concentration in blood constitutes a risk factor in arterial hypertension development. In our opinion, combination of polymorphic variants of *AGT*, *CAT* and *SOD* genes expands the possibilities of personalized medicine. Performed investigation involved 65 medical students of St Petersburg State University. DNA was extracted from epithelial cells of the oral cavity. Genotyping of DNA samples was determined by real-time PCR. Obtained results of our investigation enabled to reveal *AGT* genotypes associated with hypertension in 12 students. The article discusses combinations of TT and CT genotypes of *AGT* with unfavorable alleles of antioxidant enzymes. Carriers of heterozygous *SOD2* genotype and homozygous CC genotype *CAT* are in more unfavorable conditions compared to carriers of the CC genotype *SOD2* and CT genotype *CAT*, as *SOD2* is a key antioxidant enzyme protecting from ROS.

Keywords: arterial hypertension, genetic polymorphism, angiotensinogen, superoxide dismutase, catalase.

Introduction

Modern personalized medicine assumes development of basic methods of diseases prevention and control based on the knowledge of the individual characteristics. Such individual characteristics include patient genome and epigenome information. Identification of genetic predisposition is important for the multifactorial diseases prevention since

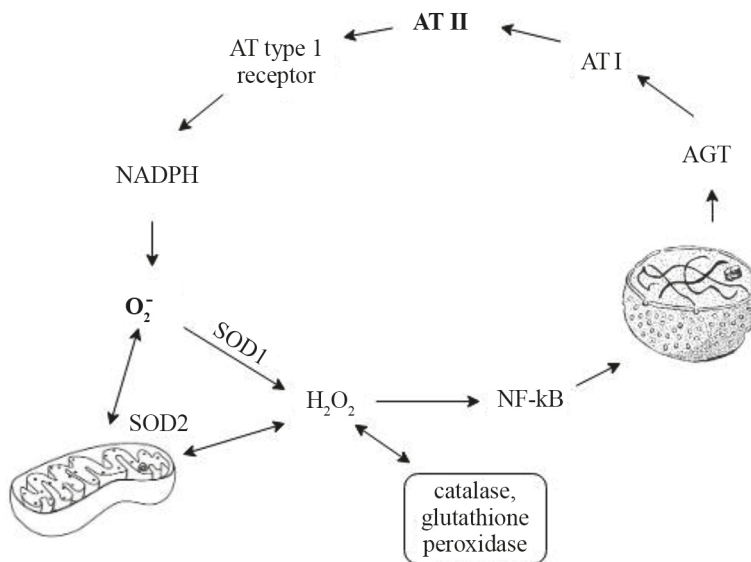


Fig. 1. RAS and antioxidant system enzymes in blood pressure regulation

disease manifestation depends on person's lifestyle, diet, physical activities, rest regime, sleep duration, etc.

Cardiovascular diseases (CVD) are multifactorial ones for which genetic determination was proven. Numerous studies of the cardiovascular pathology, demonstrate that 20–60 % of blood pressure changes are genetically determined. Thus arterial hypertension (AH) is associated with gene polymorphisms of angiotensinogen, β 1-adrenergic receptor, cytochrome P450 [1; 2; 3].

Arterial hypertension as increased stressful conditions risk, lack or excessive physical activity risk, consumption of junk food, smoking, and alcohol abuse risks are widely spread in highly developed countries. It is well known that multifactor diseases are associated with the development and activation of oxidative stress, which can be caused by decrease in antioxidant defense effectiveness. Arterial hypertension and ischemic heart disease can trigger acute myocardial infarction and further development of heart failure. One of the mechanisms developing such pathologies is mitochondrial dysfunction associated with disruption of antioxidant system enzymes. Antioxidant enzymes are widely represented in the body cells. So, superoxide dismutase (SOD) catalyses superoxide radical breakdown in the mitochondria and cytoplasm, catalase (CAT) decomposes H₂O₂ at high concentrations in the cytoplasm.

It is well known that angiotensin II (Ang II) activates NADPH oxidase in blood vessels, kidneys, brain, increasing superoxide radical and H₂O₂ formation [4; 5]. Modern studies demonstrate that H₂O₂ plays a role of paracrine mediator of cardiovascular and renal dysfunctions. Therefore, H₂O₂ activates the nuclear factor NF-kB which increases genes expression involved in sodium homeostasis and blood pressure control, including angiotensinogen gene (Fig. 1). T.Sousa and co-authors demonstrated that increase in the H₂O₂ level is directly involved in the angiotensin-II-induced hypertension development and intrarenal activation of renin-angiotensin system (RAS) [5]. Accordingly, concen-

trations of H₂O₂ and angiotensinogen (AGT) are interdependent. Increased blood AGT concentration and decreased activity of SOD and CAT lead to accumulation of reactive oxygen species (ROS) in cells and further development of mitochondrial dysfunction.

This investigation aims to assess young people's predisposition to hypertension based on determination of the polymorphism C521T of the *AGT* gene, polymorphisms -262C/T of the *CAT* gene and C47T gene of the *SOD2*.

Methods

The study involved 65 medical students of St Petersburg State University (18–22 years old) who have no cardiovascular pathology. DNA was isolated from oral epithelial cells using commercial DNA-sorb-A kits (InterLabService, Moscow). Genotyping of DNA samples was carried out using Sintol's real-time PCR reagent (Moscow). Gene polymorphisms were determined using "DTlite — DNA technology".

AGT gene polymorphism (C521T, rs4762), encoding an angiotensinogen protein, was determined in the group of students examined. Then, genetic polymorphisms of *CAT* gene (-262C/T, rs1001179), encoding catalase, and *SOD2* gene (C47T, rs4880), encoding mitochondrial superoxide dismutase, were identified in carriers of the T *AGT* allele.

Results and discussion

The study results of the C521T polymorphism frequency of the *AGT* gene in students group are presented in Fig. 2. Obtained results distributed as follows: CC — 53 persons, CT — 8 persons, TT — 4 persons. Rates of alleles occurrence: C — 87.7 %, T — 12.3 %.

Distribution of *CAT* (-262C/T) genotypes and *SOD2* (C47T) genes among polymorphic variants of *AGT* (C521T) gene is presented in Fig. 3. In the group of CT *AGT* genotype carriers, two students had CC genotype of *CAT* and six — CT; two students had CC genotype of *SOD2* and six — CT. There was one CC and three CT genotype carriers of *CAT*, one CC and three CT genotype carriers of the *SOD2* in TT genotype carriers of *AGT*.

Polymorphic variant (C521T) of *AGT* is characterized by replacement of cytosine (C) by thymine (T) at position 521 of gens. It leads to replacement of tryptophan by methionine at position 174 of amino acid sequence (Thr174Met). Presence of T allele is associated with increased risk for hypertension [6]. Therefore, TT genotype of the *AGT* gene seems to be the most clinically significant for risk assessment for cardiovascular pathology. This genotype is associated with blood angiotensinogen elevation, which is responsible for increased production of angiotensin II and activation of ROS. At the same time, the role of *SOD2* and catalase increases.

Structural variability of the *SOD2* gene affects amount and activity of the Mn-dependent superoxide dismutase, synthesized in cells. C47T polymorphism leads to replacement of alanine (Ala) by valine (Val) amino acid, modifies secondary protein structure, alters mitochondrial targeting sequence and can affect the enzyme transfer from the cytoplasm to the mitochondrial matrix. It was found out that the enzyme activity in mitochondria is decreased by 30–40 % and the risk of developing cardiovascular pathology is elevated in T allele carriers [7].

CAT -262C/T polymorphism is a single nucleotide substitution of cytosine (C) to thymine (T) in promoter region at position 262. It is known that CC genotype carriers

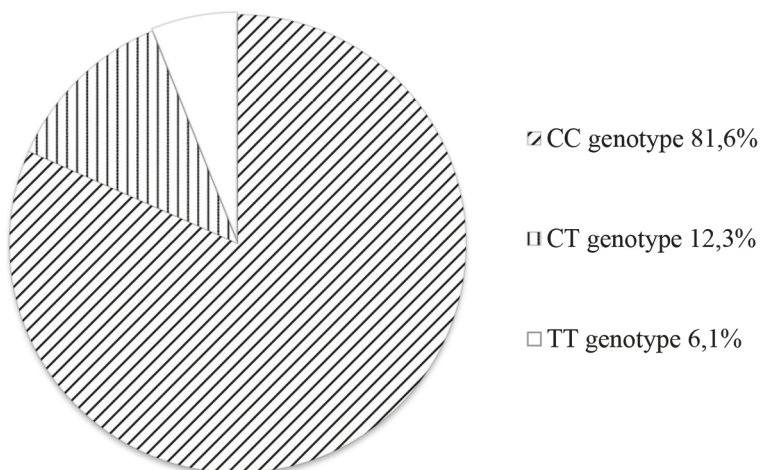


Fig. 2. The genotype distribution of the *AGT* C521T

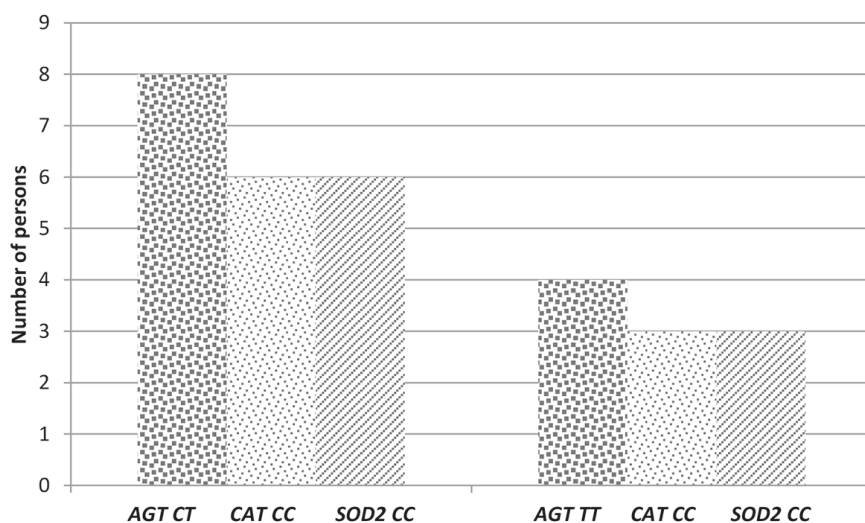


Fig. 3. The distribution of genotypes of *CAT* (-262C/T) and *SOD2* (C47T) genes among the polymorphic variants of the *AGT* (C521T) gene

have a higher catalase enzyme activity than CT and TT genotypes carriers. Thereby C allele increases the antioxidant status of cells and consequently reduces the risk of cardiovascular diseases. However, nowadays there is no proof that the T allele correlates with cardiovascular pathology and with risk of arterial hypertension [8].

According to our study results, *AGT* genotypes associated with hypertension were identified in 12 students (Table). However, student No. 58 had *SOD2* and *CAT* genotypes that indicate a normal level of antioxidant system enzymes. This might indicate that cells are well protected when oxidative processes are activated by Ang II. Students No. 2, 9, 11, 28, 38 are heterozygous carriers of all studied genotypes and, therefore, have a moderate risk of developing hypertension due to increased concentration of Ang II and de-creas-

ing SOD2 and CAT activity. There are carriers of the heterozygous genotype *SOD2* and homozygous CC genotype *CAT* in more adverse conditions compared to carriers of the CC genotype *SOD2* and CT genotype *CAT*, as *SOD2* is a key antioxidant enzyme in the defense against oxidative stress. We assume that the risk of developing hypertension is decreased among the students No. 42, 61, 12, 67. In our opinion, students No. 49 and 66 that carried 4 unfavorable alleles of the studied genes have the greatest risk of developing hypertension.

Table. The SOD2 and CAT genotypes distribution among the students with CT and TT genotypes of the AGT

AGT CT	SOD2	CAT	AGT TT	SOD2	CAT
No. 2	CT	CT	No. 42	CT	CC
No. 9	CT	CT	No. 49	CT	CT
No. 11	CT	CT	No. 61	CC	CT
No. 12	CT	CC	No. 66	CT	CT
No. 28	CT	CT			
No. 38	CT	CT			
No. 58	CC	CC			
No. 67	CC	CT			

It is necessary to consider that lifestyle of medical student is rather stressful and further doctor's work is accompanied by high level of stressful situations, therefore, a significant number of risk factors can lead to early development of arterial hypertension. Methods of assessing genetic predisposition to multifactorial diseases are the basis of personalized medicine and should be used for an early individual disease prevention.

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Authors' information:

Alexandra V. Kovaleva — student; 9xaasaax9@gmail.com

Irina V. Astratenkova — PhD, Associate Professor; astratenkova@mail.ru

Natalya E. Golovanova — PhD, Associate Professor; nesh1764@mail.ru

Olga G. Orlova — PhD, Associate Professor; st003724@spbu.ru

Oksana V. Rybalchenko — Dr. Sci., Professor; ovr@inbox.ru