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Study of single nucleotide polymorphism in the encoding exons of the AGT gene of an arterial hypertension patient from Azerbaijan

Zumrud Abaszade[1], Karim Gasımov[2], Ilham Shahmuradov[2,3*]

[1]Azerbaijan Medical University, 23 A.A.Bakikhanov Str., AZ1022, Baku, Azerbaijan
[2]Institute of Biophysics, Ministry of Science and Education of the Republic of Azerbaijan, 117 Z.Khalilov Str., AZ1141, Baku, Azerbaijan
[3]Institute of Molecular Biology & Biotechnologies, Ministry of Science and Education of the Republic of Azerbaijan, 11 Izzat Nabiyev Str., AZ1073, Baku, Azerbaijan

***For correspondense:** ilhambaku@gmail.com*

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Arterial hypertension, which affects 20-30% of the world's population, is one of the main causes of cardiovascular disease. According to existing ideas, arterial hypertension is caused by genetic and non-genetic reasons, however, in most cases (more than 90%), the specific cause (s) of this complication in essential hypertension is unknown. It is believed that blood pressure is under the control of numerous genes. One such gene is the AGT gene, which encodes the angiotensinogen/angiotensin protein hormone. To date, a total of 4129 SNPs for the AGT gene have been annotated in the dbSNP database https://www.ncbi.nlm.nih.gov/snp/?term=AGT). In this study, the nucleotide sequences of the 3rd, 4th, 5th and 6th coding exons of the AGT gene in a sample of Azerbaijani patients living in Azerbaijan diagnosed with high arterial hypertension were read and compared with the corresponding reference sequences. It was found that there are 8 mutations in these exons, and all of them have been annotated in dbSNP resources. The results of studying the possible functional role of these mutations are interpreted below.

Keywords: *Human, arterial hypertension (AH), angiotensin (AGT) gene, exon analysis, single nucleotide polymorphism (SNP)*

INTRODUCTION

Arterial high (140/90 mm Hg and above) blood pressure (hypertension), which affects 20-30 percent of the world's population, is one of the main causes of cardiovascular disease. Studies conducted to date indicate that the cause of arterial hypertension (AH) is genetic and non-genetic factors (environment, nutrition and stress). There are two types of hypertension: (1) essential hypertension (primary hypertension, idiopathic hypertension), the specific causes of which are unknown and affect more than 90 percent of people whose specific causes are unknown and who suffer from this pathology, and (2) secondary hypertension caused by known diseases and causes such as pathologies kidneys or lungs, stress, diet, excess weight, smoking, etc. (O'Rourke, 2003; Nguen and Jaisser, 2012; Kunutsor and Powles, 2010; Gruesser et al., 2007; Artman et al., 2007; for summary: Messerley, Williams and Ritz, 2007; Jordan, Kurschat and Reuter, 2018). Existing knowledge confirms that it would be wrong to consider all cases of arterial hypertension as essential (primary) hypertension.

Arterial pressure is estimated to be controlled by numerous genes, and in most known cases, hypertension manifests itself as a feature of non-Mendelian syndrome (Delles et al., 2010; Austin and Loyd, 2014; Garcia-Rivas et al., 2017; Morrell et al., 2019). To date, more than 40 genes have been detected related to hypertension (see https://www.omim.org/entry/145500).

For example, AGT, which encodes the renin-

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angiotensin-aldosterone-related hormone hypertensogen / angiotensin protein Jeunemaitre et al., 1992; Caulfield et al., 1994; Lifton, 1996), AGTR1 (Bonnardeaux et al., 1994), which encodes the first type of receptor of the angiotensin II protein, ACE, which encodes an angiotensin-converting enzyme (Zhang et al., 2004), the APA gene, which encodes aminopeptidase (Ferreira and Raizada, 2008), REN, which encodes the enzyme renin (Persson, 2003; Wu et al., 2018), and MTHFR encoding the enzyme methylenetetrahydrofolate reductase (McNulty et al., 2016; https://www.omim.org/entry/607093).

Angiotensin I secreted by the liver undergoes successive transformations by renin and angiotensin-converting enzyme, resulting in the active hormone angiotensin II, which raises blood pressure (Lifton, 1996). There are significant differences in the concentration of angiotensinogen in the blood plasma of arterial hypertension patients with different AGT genotypes (Jeunemaitre et al., 1992; Caulfield et al., 1994). The AGT gene is mainly expressed in the liver hepatocytes. An angiotensinogen from α globulin family is a 485 amino acid (at) long protein that is the precursor of the angiotensin II protein and the sole substrate of the renin protein. After cleavage of the signal peptide, 452 a.a. mature angiotensinogen protein is formed. The mature form of the angiotensinogen protein is the sole substrate of the renin protein, which consists of 340 a.a.: after the peptide bond between Leu and Val amino acids is cut by renin, the 452 a.a. protein is converted to the 10 a.a. peptide hormone - angiotensin I (Asp-Arg-Val-Tyr - Ile-His-Pro-Phe-His-Leu). The ACE enzyme then converts the angiotensin I peptide to the 8 amino acid (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) angiotensin II peptide hormone. Besides, the enzyme aminopeptidase A cleaves the angiotensin II peptide to 7 a.a. peptide hormone angiotensin III (Arg-Val-Tyr-Ile-His-Pro-Phe), and then the peptide angiotensin III to 6 a.a. peptide hormone angiotensin IV (Val-Tyr-Ile-His-Pro-Phe). The separate biological function of angiotensin I peptide is unknown. Angiotensin III peptide has approximately 40% of the antihypertensive activity of angiotensin II peptide. Angiotensin IV hormone is characterized by the least antihypertensive properties but has a wide range of activities in the central nervous system. Circulating levels of angiotensinogen in the body are regulated by various stimuli, including glucocorticoids, estrogens, thyroid hormone, insulin, and some cytokines (Lifton, 1996; Ferreira and Raizada, 2008; Lu et al., 2016; Wu et al., 2018; Chappell, 2019).

AGT gene with a total length of 43061 bp, including introns, is located on the opposite (negative) strand of DNA [complement (230702523..230745583)] on human chromosome 1, consists of 6 exons. Since the transcription of this gene is initiated from alternative TSSs, this results in partially 2 different mRNAs but single DNA coding sequence (CDS) (NC_000001.11; https://www.genecards.org/cgi-bin/carddisp.pl?gene=AGT&keywords=AGT; Fig. 1). As a result of the translation of this CDS, the initial (precursor) form of angiotensin polypeptide - angiotensinogen - is formed.

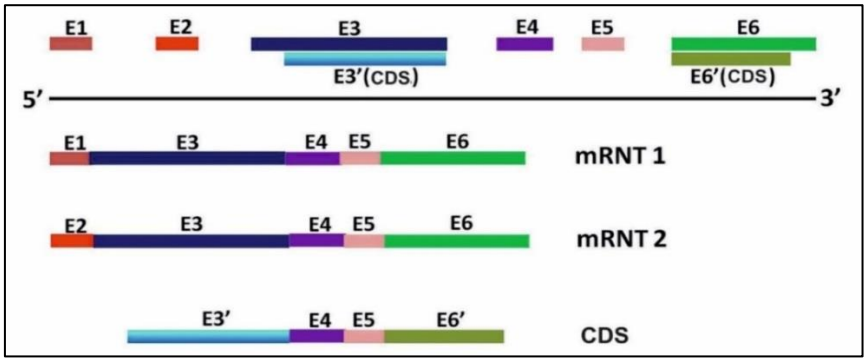

Fig. 1. Exon-intron structure of AGT gene. Localization of exons: E1: c(230745515.. 230745583), E2: c(230714086..230714122), E3: c(230709995..230710853), E4: c(230705933.. 230706200), E5: c(2307.04193). 230704337), E6: c(230702523..230703329). The parts of E3 and E6 exons included in CDS were marked as "E3'" and "E6'"respectively. E3': c(230709995.. 230710823), E6': c(230703141.. 230703329).

In the DNA sequence of the human AGT gene (CDS), the T>C mutation (in the Met268Thr or M268T protein; SNP M235T or rs699 in the scientific literature in dbSNP resources) at position 803 (codon 268) is associated with cardiovascular disease (Mohammadi et al., 2018). For example, in people with arterial hypertension, the frequency of 235T/T genotype and 235T allele is relatively high compared to the control group, and these people are included in the risk group (Cheng, Wang and Wan, 2012; Borai et al., 2018; Azova et al., 2021). On the other hand, it has been noted that people with 235M/M genotype and 235M allele of the AGT gene are in the risk group for high blood pressure (Kim et al., 2015). The 28A>T mutation in exon 2 may play a role in damage to the AGT protein. 90T>C mutation in the 3'-untranslated region (TOR) severely affects mRNA stability and translation (Padma et al., 2015).

In total, 4129 SNPs (3862 substitutions and 267 insertions; https://www.ncbi.nlm.nih.gov/snp/?term=AGT) have been annotated in the dbSNP database so far in the AGT gene.

In this study, the nucleotide sequences of the 3rd, 4th, 5th and 6th coding exons (E3, E4, E5 and E6) of the AGT gene were read in a sample of Azerbaijani patients living in Azerbaijan and diagnosed with high AH, and their SNP spectrum was analyzed by comparison with relevant reference sequences. The results of these studies are interpreted below.

MATERIALS AND METHODS

For the study, one of 127 patients (27 women and 65 men under 35 years old, 20 women and 15 men over 35 years old) were randomly selected for treatment with a diagnosis of hypertension at the Research Institute of Cardiology named after J.M.Abdullayev. The total DNA fraction from the blood sample of the selected patient was isolated using the "QIAamp DNA Mini Kit, Blood Mini (50)" reagent set (catalog no. 51104) manufactured by the QIAGEN company (Germany). The obtained blood sample was first lysed by incubating with proteinase K at 56 ºC for ~2 hours in a buffer containing SDS and EDTA. The lysis process was completed by adding AL buffer and incubating at 70 ºC for ~10 min. The reaction mixture was then vortexed by the addition of 200-400 µl (96%) ethanol. The resulting mixture was transferred to a mini-spin column and centrifuged at 6000 g for 1 minute, the remaining mixture after discarding the supernatant was transferred to a new tube, and washed with 500 µl of washing buffer (Buffer AW2) by centrifugation at 20000 g for 3 minutes. Then, the column was transferred to a new tube to remove the remnants of the washing buffer and centrifuged at 20000 g for 1 minute. To elute the obtained total DNA fraction from the spin column, it was transferred to a new (1.5 ml) tube and incubated for 1-2 min at room temperature with the addition of 200 µl AE buffer (10 mM Tris·Cl; 0.5 mM EDTA; pH 9.0). for DNA solubilization) and centrifuged at 6000 g for 1 min. Finally, the concentration of the resulting total DNA fraction was determined and stored at –20 ºC until use.

The following oligonucleotide primers were used to sequence 4 exons of the AGT gene (E3 and E3', E4, E5, E6) in the total DNA fraction. E3:5′-GGTATGCGGAAGCGAGCAC-3′; E3':5′-CTTGGAAGTGGACGTAGGT-3′; E4:5′-GGAAGATGAAGGGCTTCTC-3′; E5:5′-GACCATCCACCTGACCATG-3′; E6:5′-TGCTTTGCAGGTGCTGAAC-3′.

Exon nucleotide sequences were read using the Sanger method at the Afgen Genetic Diagnostic Center operating at the Baku Clinic of Biological Medicine using a Genetic Analyzer 3130xl manufactured by Applied Biosystems.

The localization of exons of the AGT gene and reference nucleotide sequences, as well as data on the reference amino acid (at) sequence of the corresponding protein, were taken from the GRCh38.p13 (NC_000001.11) version of the GenBank annotation (Refseq) of the human genome, and information on the localization and functional role of known SNPs in these exons was taken from the dbSNP resources (https://www.ncbi.nlm.nih.gov/snp/?term=AGT).

The comparison of nucleotide and amino acid sequences was carried out using the **BLAST** (Altschul S.F. et al. (1997) software package, the study of the distribution spectrum in terms of the number and functional significance of known SNPs in exons was carried out using the computer

program **getsnpp2** (I.Shahmuradov, unpublished). All bioinformatic analyses were implemented in the LINUX operating system.

RESULTS AND DISCUSSION

Nucleotide sequences of the newly sequenced coding exons of the AGT gene and their comparison with the corresponding reference sequences

The nucleotide sequence of 4 exons (E3-E6) included in the CDS of the AGT gene was sequenced and compared with the relevant reference sequences (figures 2-5). There are 2 point mutations (SNP - substitution) between each of these exons and the relevant reference exon: C>A (G>T in the complementary coding strand, dbSNP ID: rs61762539) at position 415 (chromosome 230710439) on exon 3 and G>A (C>T; rs545870660) transversions at position 607 (230710284), G>A (C>T; rs61762530) transition at position 33 (230706168) of exon 4, and at position 260 T>A (A>T; rs7080) transversion (230705941), C>T (G>A) transitions at position 98 (230704240) and at position 137 (230704201) of exon 5; rs886046081 and rs61751067), A>G (T>C; rs5043) transition at position 366 (230702974; rs5043) and G>T (C>A; rs7079) transversion at position 755 (230702585) of exon 6. These SNPs are found within the CDS in exons 2, 3, and 4 and in the 3'-UTR region in exon 6

```
AGT_E3    1  GGTATGCGGAAGCGAGCACCCCAGTCTGAGATGGCTCCTGCCGGTGTGAGCCTGAGGGCC
AGT_E3r   1  ............................................................

AGT_E3   61  ACCATCCTCTGCCTCCTGGCCTGGGCTGGCCTGGCTGCAGGTGACCGGGTGTACATACAC
AGT_E3r  61  ............................................................

AGT_E3  121  CCCTTCCACCTCGTCATCCACAATGAGAGTACCTGTGAGCAGCTGGCAAAGGCCAATGCC
AGT_E3r 121  ............................................................

AGT_E3  181  GGGAAGCCCAAAGACCCCACCTTCATACCTGCTCCAATTCAGGCCAAGACATCCCCTGTG
AGT_E3r 181  ............................................................

AGT_E3  241  GATGAAAAGGCCCTACAGGACCAGCTGGTGCTAGTCGCTGCAAAACTTGACACCGAAGAC
AGT_E3r 241  ............................................................

AGT_E3  301  AAGTTGAGGGCCGCAATGGTCGGGATGCTGGCCAACTTCTTGGGCTTCCGTATATATGGC
AGT_E3r 301  ............................................................

AGT_E3  361  ATGCACAGTGAGCTATGGGGCGTGGTCCATGGGGCCACCGTCCTCTCCCCAACGtCTGTC
AGT_E3r 361  ............................................................g.....

AGT_E3  421  TTTGGCACCCTGGCCTCTCTCTATCTGGGAGCCTTGGACCACACAGCTGACAGGCTACAG
AGT_E3r 421  ............................................................

AGT_E3  481  GCAATCCTGGGTGTTCCTTGGAAGGACAAGAACTGCACCTCCCGGCTGGATGCGCACAAG
AGT_E3r 481  ............................................................

AGT_E3  541  GTCCTGTCTGCCCTGCAGGCTGTACAGGGtCTGCTAGTGGCCCAGGGCAGGGCTGATAGC
AGT_E3r 541  ............................c...............................

AGT_E3  601  CAGGCCCAGCTGCTGCTGTCCACGGTGGTGGGCGTGTTCACAGCCCCAGGCCTGCACCTG
AGT_E3r 601  ............................................................

AGT_E3  661  AAGCAGCCGTTTGTGCAGGGCCTGGCTCTCTATACCCCTGTGGTCCTCCCACGCTCTCTG
AGT_E3r 661  ............................................................
```

Figure 2 continued

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AGT_E3  721  GACTTCACAGAACTGGATGTTGCTGCTGAGAAGATTGACAGGTTCATGCAGGCTGTGACA
AGT_E3r 721  ............................................................

AGT_E3  781  GGATGGAAGACTGGCTGCTCCCTGATGGGAGCCAGTGTGGACAGCACCCTGGCTTTCAAC
AGT_E3r 781  ............................................................

AGT_E3  841  ACCTACGTCCACTTCCAAG
AGT_E3r 841  ...................
```

Fig. 2. Comparison of the nucleotide sequence of the 3rd exon (E3) of the AGT gene in a hypertensive patient belonging to the Azerbaijani population with the reference sequence of this exon. Here and in the following figures, rreference sequences, dots denote the same nucleotides. Here, as in Figures 3, 4 and 5, SNPs are marked with small and red letters on a gray background. The translation initiation codon is marked in pink.

```
AGT_E4    1  GGAAGATGAAGGGCTTCTCCCTGCTGGCCGAGtCCCAGGAGTTCTGGGTGGACAACAGCA
AGT_E4r   1  ..................................c...........................

AGT_E4   61  CCTCAGTGTCTGTTCCCATGCTCTCTGGCATGGGCACCTTCCAGCACTGGAGTGACATCC
AGT_E4r  61  ............................................................

AGT_E4  121  AGGACAACTTCTCGGTGACTCAAGTGCCCTTCACTGAGAGCGCCTGCCTGCTGCTGATCC
AGT_E4r 121  ............................................................

AGT_E4  181  AGCCTCACTATGCCTCTGACCTGGACAAGGTGGAGGGTCTCACTTTCCAGCAAAACTCCC
AGT_E4r 181  ............................................................

AGT_E4  241  TCAACTGGATGAAGAAACTtTCTCCCCG
AGT_E4r 241  ..................a.........
```

Fig. 3. Nucleotide sequence of the 4th exon of the AGT gene of a hypertensive patient belonging to Azerbaijani population and comparison with the reference sequence of that exon.

```
AGT_E5   1  GACCATCCACCTGACCATGCCCCAACTGGTGCTGCAAGGATCTTATGACCTGCAGGACCT
AGT_E5r  1  ............................................................

AGT_E5  61  GCTCGCCCAGGCTGAGCTGCCCGCCATTCTGCACACCaAGCTGAACCTGCAAAAATTGAG
AGT_E5r 61  ........................................g...................

AGT_E5  121  CAATGACCGCATCAGGaTGGGGGAG
AGT_E5r 121  ................g........
```

Fig. 4. Nucleotide sequence of the 5th exon of the AGT gene of a hypertensive patient belonging to the Azerbaijani population and comparison with the reference sequence of that exon.

```
AGT_E6    1  TGCTTTGCAGGTGCTGAACAGCATTTTTTTTGAGCTTGAAGCGGATGAGAGAGAGCCCAC
AGT_E6r   1  ............................................................

AGT_E6   61  AGAGTCTACCCAACAGCTTAACAAGCCTGAGGTCTTGGAGGTGACCCTGAACCGCCCATT
AGT_E6r  61  ............................................................

AGT_E6  121  CCTGTTTGCTGTGTATGATCAAAGCGCCACTGCCCTGCACTTCCTGGGCCGCGTGGCCAA
AGT_E6r 121  ............................................................
```

Figure 5 continued

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AGT_E6    181  CCCGCTGAGCACAGCATGAGGCCAGGGCCCCAGAACACAGTGCCTGGCAAGGCCTCTGCC
AGT_E6r   181  ............................................................

AGT_E6    241  CCTGGCCTTTGAGGCAAAGGCCAGCAGCAGATAACAACCCCGGACAAATCAGCGATGTGT
AGT_E6r   241  ............................................................

AGT_E6    301  CACCCCCAGTCTCCCACCTTTTCTTCTAATGAGTCGACTTTGAGCTGGAAAGCAGCCGTT
AGT_E6r   301  ............................................................

AGT_E6    361  TCTCCcTGGTCTAAGTGTGCTGCATGGAGTGAGCAGTAGAAGCCTGCAGCGGCACAAATG
AGT_E6r   361  .....t......................................................

AGT_E6    421  CACCTCCCAGTTTGCTGGGTTTATTTTAGAGAATGGGGGTGGGGAGGCAAGAACCAGTGT
AGT_E6r   421  ............................................................

AGT_E6    481  TTAGCGCGGGACTACTGTTCCAAAAAGAATTCCAACCGACCAGCTTGTTTGTGAAACAAA
AGT_E6r   481  ............................................................

AGT_E6    541  AAAGTGTTCCCTTTTCAAGTTGAGAACAAAAATTGGGTTTTAAAATTAAAGTATACATTT
AGT_E6r   541  ............................................................

AGT_E6    601  TTGCATTGCCTTCGGTTTGTATTTAGTGTCTTGAATGTAAGAACATGACCTCCGTGTAGT
AGT_E6r   601  ............................................................

AGT_E6    661  GTCTGTAATACCTTAGTTTTTTCCACAGATGCTTGTGATTTTTGAACAATACGTGAAAGA
AGT_E6r   661  ............................................................

AGT_E6    721  TGCAAGCACCTGAATTTCTGTTTGAATGCGGAACaATAGCTGGTTATTTCTCCCTTGTGT
AGT_E6r   721  ..................................c.........................

AGT_E6    781  TAGTAATAAACGTCTTGCCACAATAAGCCTCCAAAAA
AGT_E6r   781  .....................................
```

Fig. 5. Nucleotide sequence of the 6th exon of the AGT gene of a hypertensive patient belonging to Azerbaijani population and comparison with the reference sequence of that exon. The translation termination codon is marked in pink.

Known SNPs in Recently Sequenced Coding Exons of the AGT Gene and Their Possible Functional Significance

Integrative data on the distribution of SNPs collected for AGT gene on the 4 mRNA/CDS exons and their influence on the phenotype in dbSNP resources are indicated in Table 1. In total, 851 and 586 TNPs are known in these 4 exons only for mRNA and KDA, respectively. However, so far only 27 (26) SNPs have been found to have functional consequences: only 4 SNPs have a pathogenic effect, 1 SNP belongs to the risk group, and 22 SNPs had no deleterious effects. In total, 1290 of the 1343 known SNPs in the AGT gene have no known functional consequences.

To assess the possible functional role of SNPs found in the AGT gene in a patient with arterial hypertension belonging to the Azerbaijani population, the nucleotide sequences of newly read and reference variants of the 3rd, 4th, 5th and 6th exons of CDSs and the amino acid sequences of the corresponding proteins were compared (Figure 6).

rs61762539 (C>A, G>T in the complementary DNA strand) variation in exon 3 of the AGT gene results in the replacement of the amino acid arginine (R) with the amino acid serine (S) in the protein sequence. According to dbSNP data, this mutation has some clinical role,

but the specific mechanism of that effect is unknown. Another SNP in exon 3 (rs545870660; G>A, C>T) is a synonymous mutation and does not play any clinical role.

The rs61762530 (G>A, C>T) mutation in exon 4 of the AGT gene results in the substitution of the amino acid proline (P) for the amino acid serine and has a certain clinical role with an unknown specific mechanism of action. Another SNP in this exon (rs7080; T>A, A>T) is synonymous in nature and does not play any clinical role.

Mutation rs886046081 (C>T, G>A) in exon 5 of the AGT gene leads to the replacement of glutamic acid (E) by lysine (K), and mutation rs61751067 (C>T, G>A) leads to the replacement of the amino acid valine (V) to methionine (M). These mutations have a specific clinical role, the mechanism of action of which is unknown.

Table 1. Distribution and functional role of known SNPs on the coding exons E3, E4, E5 and E6 of the AGT gene

Exons (E)	**SNPs**				
	Pathogenic	**Risky**	**Harmless**	**Unknown**	**Total**
mRNT-E3, CDS-E3'	1 (1)	1 (1)	11 (11)	351 (339)	364 (352)
mRNT-E4, CDS-E4	2 (2)	0 (0)	6 (6)	95 (95)	103 (103)
mRNT-E5, CDS-E5	0 (0)	0 (0)	1 (1)	59 (59)	60 (60)
mRNT-E6, CDS-E6'	1 (0)	0 (0)	4 (3)	219 (65)	224 (69)
Total across 4 exons	**4 (3)**	**1 (1)**	**22 (21)**	**724 (558)**	**751 (584)**

```
                  ------> E3
AGT_Protⁿ          M  R  P  R  G  V  S  L  R  R  T  I  L  C  L  L  R  W  R  G
AGT_CDSⁿ       1   ATGGCTCCTGCCGGTGTGAGCCTGAGGGCCACCATCCTCTGCCTCCTGGCCTGGGCTGGC
AGT_CDSʳ       1   ATGGCTCCTGCCGGTGTGaGCCTGAGGGCCACCATCCTCTGCCTCCTGGCCTGGGCTGGC
AGT_Protʳ          M  R  P  R  G  V  S  L  R  R  T  I  L  C  L  L  R  W  R  G

AGT_Protⁿ          L  R  R  G  D  R  V  Y  I  H  P  F  H  L  V  I  H  N  E  S
AGT_CDSⁿ      61   CTGGCTGCAGGTGaCCGGGTGTACATACACCCCTTCCACCTCGTCATCCACAATGAGAGT
AGT_CDSʳ      61   CTGGCTGCaGGTGACCGGGTGTACATACACCCCTTCCACCTCGTCATCCACAATGAGAGT
AGT_Protʳ          L  R  R  G  D  R  V  Y  I  H  P  F  H  L  V  I  H  N  E  S

AGT_Protⁿ          T  C  E  Q  L  R  K  R  N  R  G  K  P  K  D  P  T  F  I  P
AGT_CDSⁿ     121   ACCTGTGAGCAGCTGGCAAAGGCCAATGCCGGGAAGCCCAAAGACCCCACCTTCATACCT
AGT_CDSʳ     121   ACCTGTGAGCAGCTGGCAAAGGCCAATGCCGGGAAGCCCAAAGACCCCACCTTCATACCT
AGT_Protʳ          T  C  E  Q  L  R  K  R  N  R  G  K  P  K  D  P  T  F  I  P

AGT_Protⁿ          R  P  I  Q  R  K  T  S  P  V  D  E  K  R  L  Q  D  Q  L  V
AGT_CDSⁿ     181   GCTCCAATTCAGGCCAAGACATCCCCTGTGGATGAAAAGGCCCTACAGGACCAGCTGGTG
AGT_CDSʳ     181   GCTCCAATTCAGGCCAAGACATCCCCTGTGGATGAAAAGGCCCTACAGGACCAGCTGGTG
AGT_Protʳ          R  P  I  Q  R  K  T  S  P  V  D  E  K  R  L  Q  D  Q  L  V

AGT_Protⁿ          L  V  R  R  K  L  D  T  E  D  K  L  R  R  R  M  V  G  M  L
AGT_CDSⁿ     241   CTAGTCGCTGCAAAACTTGACACCGAAGACAAGTTGAGGGCCGCAATGGTCGGGATGCTG
AGT_CDSʳ     241   CTAGTCGCTGCAAAACTTGACACCGAAGACAAGTTGAGGGCCGCAATGGTCGGGATGCTG
AGT_Protʳ          L  V  R  R  K  L  D  T  E  D  K  L  R  R  R  M  V  G  M  L

AGT_Protⁿ          R  N  F  L  G  F  R  I  Y  G  M  H  S  E  L  W  G  V  V  H
AGT_CDSⁿ     301   GCCAACTTCTTGGGCTTCCGTATATATGGCATGCACAGTGAGCTATGGGGCGTGGTCCAT
AGT_CDSʳ     301   GCCAACTTCTTGGGCTTCCGTATATATGGCATGCACAGTGAGCTATGGGGCGTGGTCCAT
AGT_Protʳ          R  N  F  L  G  F  R  I  Y  G  M  H  S  E  L  W  G  V  V  H

AGT_Protⁿ          G  R  T  V  L  S  P  T  S  V  F  G  T  L  R  S  L  Y  L  G
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Figure 6 continued

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AGT_CDSn    361  GGGGCCACCGTCCTCTCCCCAACGtCTGTCTTTGGCACCCTGGCCTCTCTCTATCTGGGA
AGT_CDSr    361  GGGGCCACCGTCCTCTCCCCAACGgCTGTCTTTGGCACCCTGGCCTCTCTCTATCTGGGA
AGT_Protr         G  R  T  V  L  S  P  T  R  V  F  G  T  L  R  S  L  Y  L  G

AGT_Protn         R  L  D  H  T  R  D  R  L  Q  R  I  L  G  V  P  W  K  D  K
AGT_CDSn    421  GCCTTGGACCACACAGCTGACAGGCTACAGGCAATCCTGGGTGTTCCTTGGAAGGACAAG
AGT_CDSr    421  GCCTTGGACCACACAGCTGACAGGCTACAGGCAATCCTGGGTGTTCCTTGGAAGGACAAG
AGT_Protr         R  L  D  H  T  R  D  R  L  Q  R  I  L  G  V  P  W  K  D  K

AGT_Protn         N  C  T  S  R  L  D  R  H  K  V  L  S  R  L  Q  R  V  Q  G
AGT_CDSn    481  AACTGCACCTCCCGGCTGGATGCGCACAAGGTCCTGTCTGCCCTGCAGGCTGTACAGGGt
AGT_CDSr    481  AACTGCACCTCCCGGCTGGATGCGCACAAGGTCCTGTCTGCCCTGCAGGCTGTACAGGGc
AGT_Protr         N  C  T  S  R  L  D  R  H  K  V  L  S  R  L  Q  R  V  Q  G
AGT_Protn         L  L  V  R  Q  G  R  R  D  S  Q  R  Q  L  L  L  S  T  V  V
AGT_CDSn    541  CTGCTAGTGGCCCAGGGCAGGGCTGATAGCCAGGCCCAGCTGCTGCTGTCCACGGTGGTG
AGT_CDSr    541  CTGCTAGTGGCCCAGGGCAGGGCTGATAGCCaGGCCCAGCTGCTGCTGTCCaCGGTGGTG
AGT_Protr         L  L  V  R  Q  G  R  R  D  S  Q  R  Q  L  L  L  S  T  V  V

AGT_Protn         G  V  F  T  R  P  G  L  H  L  K  Q  P  F  V  Q  G  L  R  L
AGT_CDSn    601  GGCGTGTTCACAGCCCCAGGCCTGCACCTGAAGCAGCCGTTTGTGCAGGGCCTGGCTCTC
AGT_CDSr    601  GGCGTGTTCACAGCCCCAGGCCTGCACCTGAAGCAGCCGTTTGTGCAGGGCCTGGCTCTC
AGT_Protr         G  V  F  T  R  P  G  L  H  L  K  Q  P  F  V  Q  G  L  R  L

AGT_Protn         Y  T  P  V  V  L  P  R  S  L  D  F  T  E  L  D  V  R  R  E
AGT_CDSn    661  TATACCCCTGTGGTCCTCCCACGCTCTCTGGACTTCACAGAACTGGATGTTGCTGCTGAG
AGT_CDSr    661  TATACCCCTGTGGTCCTCCCaCGCTCTCTGGACTTCACAGAACTGGATGTTGCTGCTGAG
AGT_Protr         Y  T  P  V  V  L  P  R  S  L  D  F  T  E  L  D  V  R  R  E

AGT_Protn         K  I  D  R  F  M  Q  R  V  T  G  W  K  T  G  C  S  L  M  G
AGT_CDSn    721  AAGATTGACAGGTTCATGCAGGCTGTGACAGGATGGAAGACTGGCTGCTCCCTGATGGGA
AGT_CDSr    721  AAGATTGACAGGTTCATGCAGGCTGTGACAGGATGGAAGACTGGCTGCTCCCTGATGGGA
AGT_Protr         K  I  D  R  F  M  Q  R  V  T  G  W  K  T  G  C  S  L  M  G
                                                  E3 <------ ------> E4
AGT_Protn         R  S  V  D  S  T  L  R  F  N  T  Y  V  H  F  Q  G  K  M  K
AGT_CDSn    781  GCCAGTGTGGACAGCACCCTGGCTTTCAACACCTACGTCCACTTCCAAGGGAAGATGAAG
AGT_CDSr    781  GCCAGTGTGGACAGCACCCTGGCTTTCAACACCTACGTCCACTTCCAAGGGAAGATGAAG
AGT_Protr         R  S  V  D  S  T  L  R  F  N  T  Y  V  H  F  Q  G  K  M  K

AGT_Protn         G  F  S  L  L  R  E  S  Q  E  F  W  V  D  N  S  T  S  V  S
AGT_CDSn    841  GGCTTCTCCCTGCTGGCCGAGtCCCAGGAGTTCTGGGTGGACAACAGCACCTCAGTGTCT
AGT_CDSr    841  GGCTTCTCCCTGCTGGCCGAGcCCCAGGAGTTCTGGGTGGACAACAGCACCTCAGTGTCT
AGT_Protr         G  F  S  L  L  R  E  P  Q  E  F  W  V  D  N  S  T  S  V  S

AGT_Protn         V  P  M  L  S  G  M  G  T  F  Q  H  W  S  D  I  Q  D  N  F
AGT_CDSn    901  GTTCCCATGCTCTCTGGCATGGGCACCTTCCAGCACTGGAGTGACATCCAGGACAACTTC
AGT_CDSr    901  GTTCCCATGCTCTCTGGCATGGGCACCTTCCAGCACTGGAGTGACATCCAGGACAACTTC
AGT_Protr         V  P  M  L  S  G  M  G  T  F  Q  H  W  S  D  I  Q  D  N  F

AGT_Protn         S  V  T  Q  V  P  F  T  E  S  R  C  L  L  L  I  Q  P  H  Y
AGT_CDSn    961  TCGGTGACTCAAGTGCCCTTCACTGAGAGCGCCTGCCTGCTGCTGATCCAGCCTCACTAT
AGT_CDSr    961  TCGGTGACTCAAGTGCCCTTCACTGAGAGCGCCTGCCTGCTGCTGATCCAGCCTCACTAT
AGT_Protr         S  V  T  Q  V  P  F  T  E  S  R  C  L  L  L  I  Q  P  H  Y

AGT_Protn         R  S  D  L  D  K  V  E  G  L  T  F  Q  Q  N  S  L  N  W  M
```

Figure 6 continued

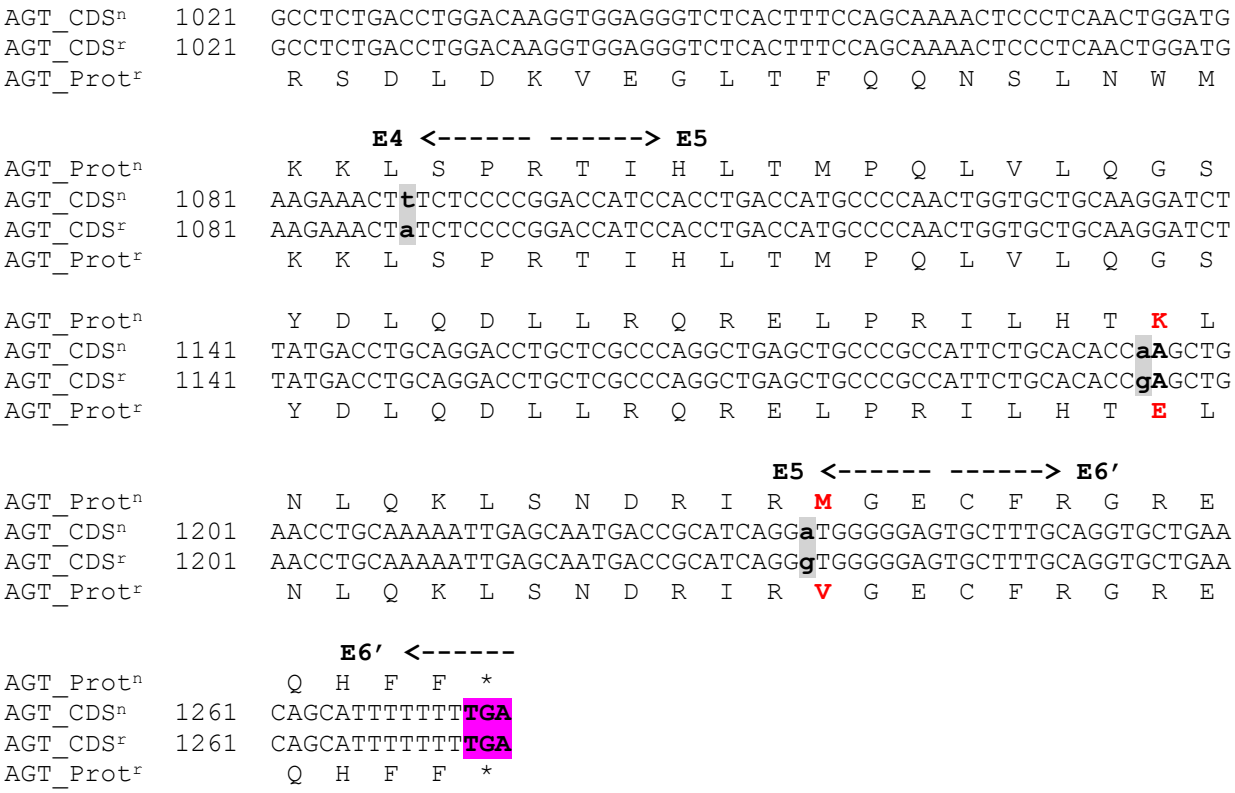

Fig. 6. Comparison of the CDS consisting of the 3rd, 4th, 5th and 6th exons of the AGT gene in a hypertensive patient from Azerbaijan and the nucleotide and amino acid sequences of the corresponding protein with the reference gene and protein sequences. Portions of exons represented in CDS: E3' - 1..830, E4 - 831..1097, E5 - 1098..1242, E6' - 1243..1275. SNPs are shown in gray background, amino acids changed as a result of mutation are shown in red. Translation initiation and termination codons are marked in bold on a pink background.

Despite the fact that mutations rs5043 (A>G, T>C) and rs7079 (G>T, C>A) in the 6th exon of the AGT gene fall into the 3'-UTR region, they have a certain clinical role, which mechanism of action is unknown.

Thus, 6 of the 8 mutations detected in the AGT gene of the hypertensive patient belonging to the Azerbaijani population occurred in the CDS, and 2 occurred in the 3'-UTR region. 4 SNPs causing amino acid substitutions in the protein sequence and 2 SNPs in 3'- UTR have a definite clinical role (according to dbSNP data), while 2 SNPs in CDS are of synonymous character.

It should be noted here that the rs699 SNP (T>C; Met268Thr or M268T; Kim et al., 2015, Mohammed et al., 2018) found in position 803 (codon 268) of the CDS of the AGT gene and associated with cardiovascular disease was not detected in the AGT gene of the hypertensive patient belonging to the Azerbaijani population studied in this research. Furthermore, it is not known whether 558 of the 584 SNPs identified so far in the CDS portion of the human AGT gene play any clinical role (Table 1). All these facts and uncertainties indicate that the role of genetic factors in the formation of AH is unknown in most cases.

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ORCIDS:

Ilham Shahmuradov: https://orcid.org/0000-0001-5533-5906
Karim Gasimov: https://orcid.org/0009-0009-2306-0114
Zumrud Abaszade: https://orcid.org/0009-0000-6790-0466