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Analysis in silico of the single nucleotide polymorphism G–152A in the promoter of the angiotensinogen gene of Indonesian patients with essential hypertension

Akhiyan Hadi Susanto[1,*], Widodo[2], Mohammad Saifur Rohman[3], Didik Huswo Utomo[2], Mifetika Lukitasari[4]

Abstract

Background: Single nucleotide polymorphism (SNP) G–152A (rs11568020) in the promoter of the angiotensinogen gene (*AGT*) may modulate its transcription. Translation of mRNA to angiotensinogen induces hypertension during hypoxia. The G allele at position –152 is located within the hypoxia-response element (HRE) transcription factor-binding site for the hypoxia-inducible factor 1 (HIF-1) heterodimer. However, the function of the –152 site in HIF-1 binding is not fully elucidated.

Objectives: To determine the frequency of SNP G–152A in Indonesian patients with hypertension and the function of this SNP.

Methods: We determined the frequency of the SNP in 100 patients by direct sequencing, and the influence of SNP G–152A on predicted binding of HIF-1 to the HRE using a docking approach in silico.

Results: The *AGT* promoter in our patients had genetic variants –152G and –152A (19:1). Predicted binding indicated that HIF-1 directly contacts the major groove of the G allele, but not the A allele. Scoring according to weighted sum High Ambiguity Driven biomolecular DOCKing showed that the score for the A allele–HIF-1 complex (–47.1 ± 6.9 kcal/mol) was higher than that for the G allele–HIF-1 complex (–94.6 ± 14.1 kcal/mol), indicating more favorable binding of HIF-1 to the G allele.

Conclusions: SNP G–152A reduces the favorability of binding of HIF-1 to the HRE. The occurrence of this SNP in the *AGT* promoter of Indonesian patients with essential hypertension suggests that the G allele is a genetic susceptibility factor in hypertension regulated by HIF-1.

Keywords: angiotensinogen, essential hypertension, genetic promoter region, hypoxia-inducible factor 1, molecular docking, simulation, single nucleotide polymorphism

***Correspondence to: Akhiyan Hadi Susanto,** Basic Nursing Department, School of Nursing, Faculty of Medicine, Universitas Brawijaya, East Java 651445, Indonesia, e-mail: susanto.akhiyan@ub.ac.id

[1]Basic Nursing Department, School of Nursing, Faculty of Medicine, Universitas Brawijaya, Malang 65145, Indonesia

[2]Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang 65145, Indonesia

[3]Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Brawijaya – Saiful Anwar General Hospital, Malang 65145, Indonesia

[4]Medical Surgical Nursing Department, School of Nursing, Faculty of Medicine, Universitas Brawijaya, Malang 65145, Indonesia

Single nucleotide polymorphisms (SNPs) are estimated to occur in 1 of every 1,000 nucleotides in the human genome [1, 2]. Depending on where a SNP occurs, it might have various consequences at the phenotypic level. SNPs that alter the function or structure of the encoded proteins are a necessary and sufficient cause of most of the known recessively or dominantly inherited monogenic disorders. These SNPs are found in the coding regions of genes and are routinely analyzed for medical purposes [3]. SNPs in the promoter region of genes affect the transcriptional activity of the gene. The promoter is the center for regulation of gene transcription because it contains numerous transcription factor-binding sites [4].

Several SNPs in the promoter of the angiotensinogen gene (*AGT*) have also been implicated in the pathogenesis of high blood pressure, including those at nucleotides –6, –20, –217, –517, and –792 [5, 6]. Human *AGT*, which is an important component of the renin–angiotensin system (RAS), is located on chromosome 1 (1q42–q43) and comprises 5 exons [7]. Angiotensinogen (*AGT*) is an important substrate for renin in the RAS, and is ultimately converted to angiotensin II (Ang II), which effects vasoconstriction and plays a pivotal role in the regulation of blood pressure [6, 8]. The G-to-A substitution at nucleotide –152 (SNP G–152A: rs11568020; 5′-untranslated region (UTR) variant at 1q42.2) in the *AGT* promoter is thought to affect the transcriptional activity generating *AGT* [6]. However, the functionality and transcription factor association with the –152 site are not fully elucidated, and the studies that discuss this SNP remain limited.

Molecular variants of *AGT* are thought to be a genetic risk factor for hypertension [7]. The G/A allele at position –152 is located within the transcription factor-binding site of the hypoxia-response element (HRE) as a part of a consensus sequence motif for the hypoxia-inducible factor 1 (HIF-1) DNA-binding domain (DBD). HIF-1 is a heterodimeric transcription factor involved in initiating the inflammatory response related to blood vessel damage caused by intermittent hypoxia and is activated during hypoxic conditions [9]. Another gene upregulated by HIF-1 is the gene for erythropoietin (*EPO*) that contains an HRE in its promoter region [10]. Intermittent hypoxia induces a positive interaction between the HIF-1 complex and the RAS. HIF-1 contributes to upregulation of endothelin 1 (ET-1) and Ang II to induce hypertension during hypoxia. Ang II increases blood pressure during hypoxia, which is caused by reactive oxygen species (ROS) activation. ROS are major factors activating HIF-1, which is followed by Ang II upregulation [10, 11]. Promoters of pre–pro ET-1 comprise an HRE and demonstrate that transcription of pre–pro ET-1 is increased by hypoxia through recruitment of HIF-1 [11].

HIF-1 is a heterodimeric transcription factor that is composed of 2 different subunits, HIF-1α and aryl receptor nuclear translocator (ARNT). There are 826 and 789 amino acids in the 2 subunits, respectively. Both of these subunits belong to the basic helix–loop–helix (bHLH) Per–Ahr/Arnt–Sim family, where Per is the period circadian protein, Ahr/Anrt the aryl hydrocarbon receptor nuclear translocator protein, and Sim the single-minded protein domain. The bHLH domain is responsible for dimerization through 2 helices and the DNA binding through the basic domain [12]. The consensus DNA sequence for HIF-1 recognition and binding is the HRE region 5′–[A/G]CGTG–3′ flanked with or without a second consensus site 5′–[A/C]ACAG–3′. However, when there is a mutation of the consensus sequences, HIF-1 binding and the transcriptional response of the genes to hypoxia will be lost [13, 14]. In the bHLH family, the HLH motif is required for both hetero- and homodimerization, while the basic region is essential for binding to the DNA. All bHLH transcription factors recognize a common core, CANNTG [15]. Both dimerization and DNA binding are essential for the function of HIF-1 [16].

A previous study of the promoter for the presence of SNPs and its transcription factor-binding properties indicates that a nucleotide substitution in the promoter region of the 5′-region upstream of *AGT* affected the transcription levels of the gene [6]. However, the mechanism associated with the transcription-level changes between G–152A/HRE and HIF-1 remained unknown. Therefore, we have investigated the SNP G–152A in the promoter of *AGT* in Indonesian patients with hypertension and analyzed the possible role of HIF-1 binding to the SNP G–152A in regulating the expression of *AGT*.

Methods

Detection of polymorphism

After approval by the Ethics Committee of the Faculty of Medicine, Universitas Brawijaya (UB), and Saiful Anwar General Hospital (ethical clearance No. 332/KEPK/VI/2012) in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki and its contemporary amendments) and after obtaining written informed consent to participate in the study from all patient participants, blood samples were taken from September 2012 to December 2014 from 100 Indonesian patients with essential hypertension. Essential hypertension was defined as a systolic blood pressure of ≥140 mmHg, diastolic blood pressure of ≥90 mmHg [17], or the use of at least one class of antihypertensive agent. Patients who had secondary hypertension, massive bleeding, or liver problems or failure; were pregnant; or taking estrogen

or corticosteroid therapy were excluded. Genomic DNA was isolated from the blood samples using a DNA extraction kit (Geneaid). Each DNA sample was amplified using a polymerase chain reaction (PCR) with forward primer 5′–TTC CAG AAG GCA CTT TTC AC–3′ and reverse primer 5′–TAG TAC CCA GAA CAA CGG CA–3′ [18]. The PCR reactions were conducted using a GeneAmp PCR System 2400 (Perkin Elmer) in a total volume of 20 µL, including 25 ng genomic DNA (1 µL), 10 µL Qiagen PCR mix, 20 pmol/0.5 µL each primer, and 8 µL double-distilled H_2O. Cycling parameters were as follows: 95°C for 3 min, 35 cycles of 95°C for 15 s, 60°C for 15 s, 72°C for 1 min, and a final extension step at 72°C for 10 min. PCR products (3 µL) and loading dye (1 µL TrackIt Cyan/Orange Loading Buffer; Thermo Fisher Scientific) were separated by electrophoresis at 8.5 V/cm for 40 min on 1.5% agarose gels in a Hoefer HE 33 Mini Submarine Electrophoresis Unit (Thermo Fisher Scientific). Agarose gel was prepared in a Tris–borate–ethylenediaminetetraacetate buffer (pH 8.0) to which ethidium bromide was added. Separated bands were observed using ultraviolet light (wavelength 200–400 nm). The PCR products were sequenced using an automatic sequencing method (Macrogen), and the genetic variants were analyzed using an ABI Prism 377 DNA Sequencer (Applied Biosystems).

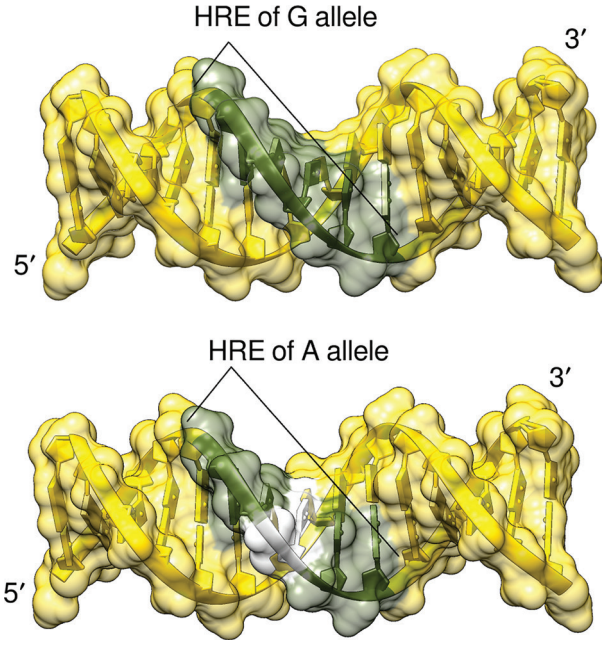

Figure 1. 3D structural models of DNA (3D-DART *.pdb files) from sequences of the *AGT* promoter hypoxia-response element (HRE) region: G allele (top), 5′–GCGTG–3′ (olive green) and A allele (bottom), 5′–GCATG–3′ (olive green) with the mutation region (G→A) (white). Molecular graphics were created using the Chimera package (version 1.11.2), developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from a U.S. National Institutes of Health grant P41-GM103311 [20].

DNA and protein modeling

We generated 3D structural models of DNA (**Figure 1**) from sequences of the *AGT* promoter (HRE region) of the G allele (5′–TCCCA GCGTG AGTGT–3′) and the A allele (5′–TCCCA GCATG AGTGT–3′) using 3D-DART provided by the High Ambiguity Driven protein-protein DOCKing (HADDOCK) webserver (http://haddock.science.uu.nl/services/3DDART/) with B-DNA and the following parameters: 2 blocks per base pair, modeling fashion with global mode, and protein structure database (Protein Data Bank (PDB)) with the following formatting option: convert International Union of Pure and Applied Chemistry to Crystallography and nuclear magnetic resonance (NMR) system notation [19]. Molecular graphics were created and analyzed using the University of California, San Francisco (UCSF) Chimera extensible molecular modeling system package (version 1.11.2; https://www.cgl.ucsf.edu/chimera) [20]. A model of the complex between HIF-1 and its consensus DNA sequence was retrieved from the PDB (http://www.rcsb.org) under the PDB ID code 1D7G (doi: 10.2210/pdb1D7G/pdb) [21]. We visualized the HIF-1 DBD/HRE complex using the Chimera package (**Figure 2**). We extracted and optimized the molecular structure of the pure HIF-1 protein heterodimer (HIF-1α and ARNT/HIF-1β complex)

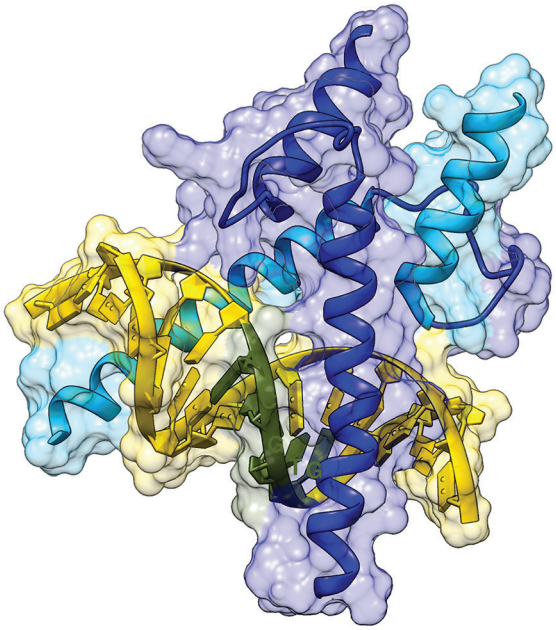

Figure 2. 3D representation of the complex between hypoxia-inducible factor 1 (HIF-1) with its aryl receptor nuclear translocator (ARNT) subunit (dark blue) and HIF-1α subunit (sky blue) and DNA (gold) with hypoxia-response element (HRE) (chain A 5′–ACGTG–3′) (olive green). Molecular graphics were created from Protein Data Base entry 1D7G.pdb [21] with the Chimera package (version 1.11.2) [20].

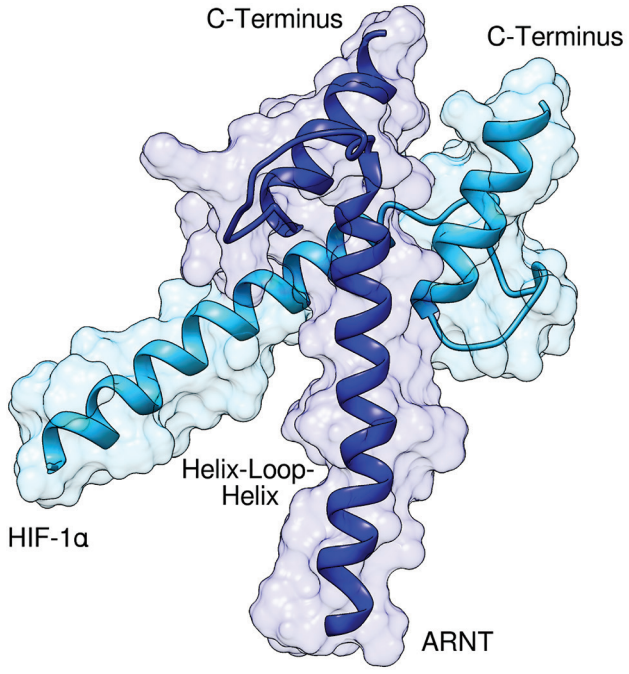

Figure 3. Visualization of isolated hypoxia-inducible factor 1 (HIF-1) protein with its aryl receptor nuclear translocator (ARNT) subunit (dark blue) and HIF-1α subunit (sky blue). We removed the 3D DNA structure and optimized the isolated HIF-1 protein (HIF-1α and ARNT complex) using VEGA ZZ software (release 3.1.1.42) [22] by removing water molecules and adding hydrogen atoms before creating the molecular graphics with the Chimera package (version 1.11.2) [20].

using VEGA ZZ software (release 3.1.1.42) (http://www.ddl.unimi.it) [22] by removing water molecules and adding hydrogen atoms (**Figure 3**).

DNA–protein docking

We modeled the binding interactions between DNA and HIF-1 using HADDOCK2.2 (http://haddock.science.uu.nl/services/HADDOCK) [23]. HADDOCK2.2 docks 1000 structures in a rigid body minimization (it0) mode and refines the top 200 in a semiflexible refinement in the torsion angle space (it1) followed by explicit solvent refinement (water) (the most favorable cluster was listed first) [23, 24]. The HADDOCK approach required near-native complexes for satisfactory results, such as the active site of the DNA and protein that previously had to be determined. To study the interactions between amino acids of HIF-1 and the HRE DNA directly, the DBD of HIF-1 was docked onto its DNA consensus sequence, HRE. The protein was docked onto either the G allele or the A allele. We considered nucleotides: G6, C7, G8/A8, T9, and G10, which are conserved among many related sequences and the amino acids of ARNT (His 8, Glu 12, and Arg 16) and HIF-1α (Ser 67, Ala 71, and Arg 75).

Analysis of hydrogen bonds

Weak intermolecular interactions such as hydrogen bonding are key players in stabilizing energetically favored ligands in the open conformational environment of protein structures [25]. In the present research, we studied the relative contribution of hydrogen bonds in the docking of HIF-1 and DNA of HRE. The parameters used here are a hydrogen atom (H) to acceptor atom (A) (H–A) distance of ≤2.7 Å, a donor atom (D) to acceptor atom (A) (D–A) distance of ≤3.0 Å, and donor proton (H)–acceptor atom (A) angles between 120° and 180°. All the hydrogen bonds were analyzed using NUCPLOT (version 1.0) (which can automatically generate a schematic 2D plot of protein–DNA interactions) [26] and LIGPLOT (which can compute all possible positions for H attached to D that satisfy specified geometrical criteria with A in the vicinity) [27]. Subsequently, we identified all residues that were involved in binding to the DNA, investigated how they interact with the bases and sugar–phosphate backbone of nucleic acids (NUCPLOT), and manually analyzed the D–A distance of hydrogen bonds with LigPlot+ (version 2.1) [28].

Visualization

We visualized the structural files using the UCSF Chimera package (version 1.11.2) [20] and the PyMol Molecular Graphics System (version 2.2.0; Schrödinger (www.pymol.org)). We analyzed the DNA–protein binding pattern, HADDOCK score, and protein–DNA contacts using a descriptive docking approach.

Statistical analysis

HADDOCK scores are presented as mean ± standard deviation.

Results

Analysis of genetic variation

PCR-amplified DNA sample products were separated by agarose gel electrophoresis for *AGT* SNP genotyping (**Figure 4**). Based on alignment results from the BioEdit sequence alignment editor software (version 7.0.5) (http://www.mbio.ncsu.edu/bioedit/bioedit.html) that were confirmed

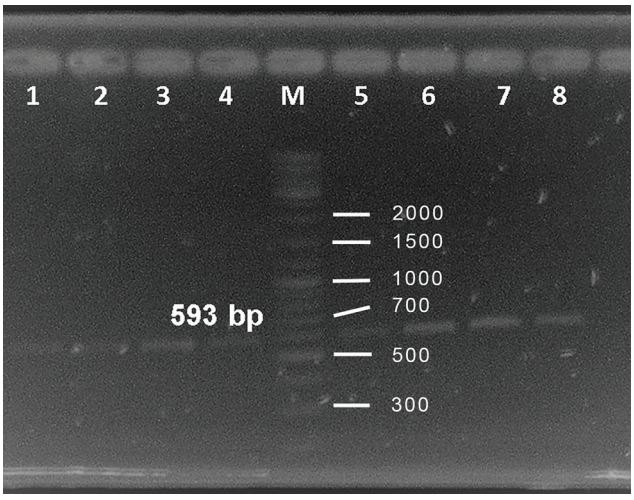

Figure 4. Polymerase chain reaction (PCR) products. Agarose gel (1.5%) electrophoresis showing a 593 bp band for 8 PCR products after amplification of *AGT*; presented is *AGT* in the promoter area; M: Invitrogen TrackIt 100 bp DNA Ladder (Thermo Fisher Scientific).

on GeneBank (https://www.ncbi.nlm.nih.gov/genbank/) [29], we found 2 different alleles at position –152, a G allele and an A allele. The alleles were identified in all patients and showed that the genetic variation at the –152 site with genotypes GG (G allele) (95 patients) and AG (A allele) (5 patients) was in the proportion of 19:1. Sequence analysis of the electropherogram results indicated that there were no patients with an AA genotype (**Figure 5**). Because the HIF-1 bound to DNA at the HRE motif, we searched for the motif in the *AGT* promoter of the DNA from 100 patients that we analyzed. The search revealed that the G/A allele at position –152 constituted the 2 genetic variations of the HIF-1-binding site.

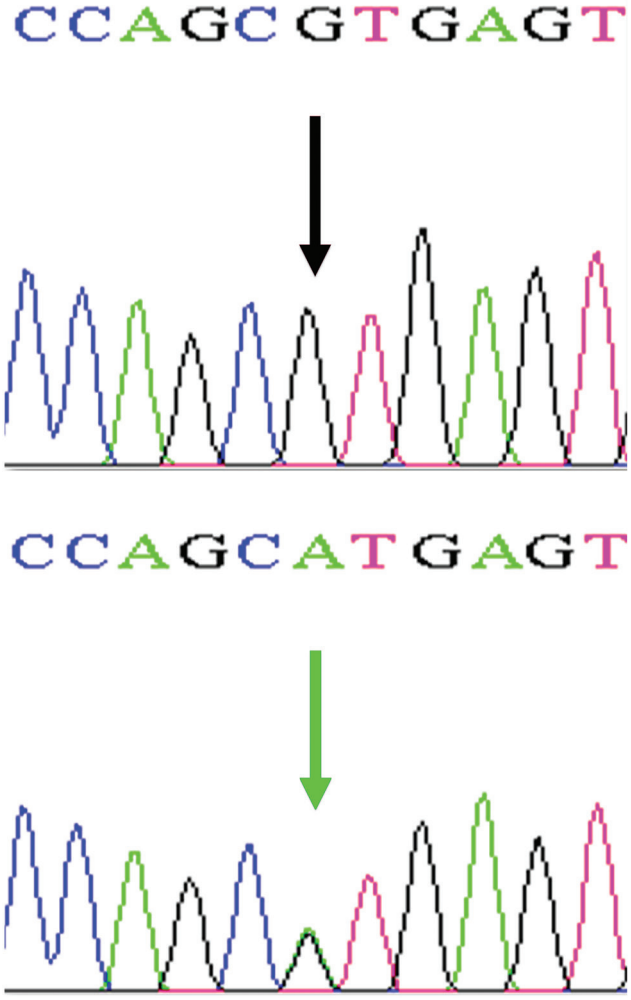

Figure 5. The single nucleotide polymorphism (SNP) G–152A of *AGT* (rs11568020) by direct sequencing. Electropherograms indicate the polymorphic site of GG (G allele) (black arrow, top) and AG (A allele) (green arrow, bottom) genotypes. No patients with AA genotype were found. C, cytosine blue; A, adenine green; T, thymine magenta; G, guanine black.

HIF-1 protein–DNA docking

The docking simulation showed that HIF-1 bound most preferably to DNA in the HRE sequence. The HIF-1 (HIF-1α/ARNT) heterodimer grips directly the *major* groove of the double helix structure of the G allele "like a clothespin on a clothesline" [30]. HIF-1α bound most preferably to the active site of the G allele at nucleotides 6, 7, 8, 9, and 10. HIF-1 makes specific contacts with DNA binding and was bound in the center, HRE 5′–GCGTG–3′. However, HIF-1 did not bind directly with the DNA of the A allele, but instead associated with the *minor* groove. There were changes in the conformation of the binding pattern, and the HIF-1 dimer did not grip the double helix (the angle β << the angle α) (**Figure 6**). This conformational change suggested that HIF-1 lost contact with the center of the HRE 5′–GCATG–3′ sequence of the A allele, and was very likely unable to stimulate transcription of *AGT*.

We analyzed differences between the HADDOCK scores in detail. The HADDOCK score is a weighted sum of the following 4 terms: electrostatic energy (weight 0.2), van der Waals energy (weight 1.0), desolvation energy (weight 1.0), and restraint violation energies (distance, susceptibility anisotropy (SANI)) (weight 0.1) [23, 24]. The HADDOCK score for the A allele–HIF-1 complex (–47.1 ± 6.9 kcal/mol) was higher than that for the G allele–HIF-1 complex (–94.6 ± 14.1 kcal/mol). This difference suggested that the G allele–HIF-1 complex is more favorable than the A allele–HIF-1 complex.

The specific amino acid–nucleotide contacts were analyzed using NUCPLOT (**Figure 7**) and LIGPLOT, and a representation was made using LigPlot+ (version 2.1) [28] (**Figure 8**). **Table 1** summarizes the atoms implied as donor or acceptor atoms and the distance in Å in the HIF-1 model between the

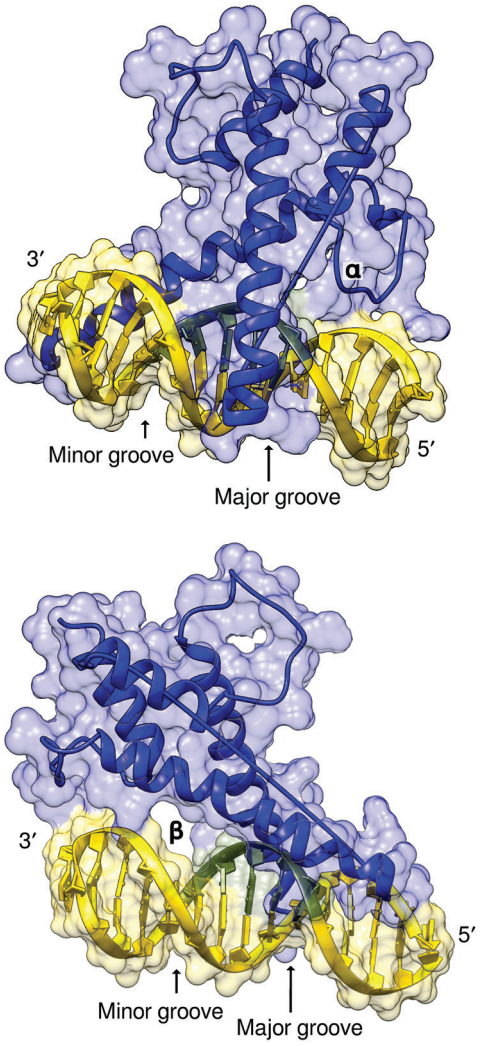

Figure 6. Differences in the binding pattern between G allele–hypoxia-inducible factor 1 (HIF-1) (left) and A allele–HIF-1 (right) interactions; molecular graphics were created using the Chimera package (version 1.11.2) [20] from the present docking results using High Ambiguity Driven protein-protein DOCKing (HADDOCK) [24] between the structures shown in **Figures 1** and **3**. HIF-1 protein (blue) with DNA (gold); hypoxia-response element (HRE) recognized (olive green).

Table 1. Protein–DNA contacts (hydrogen bonds) in the hypoxia-inducible factor 1 (HIF-1) DNA-binding domain complex observed in LIGPLOT [27] and NUCPLOT [26].

Donor	Acceptor	Distance (Å)
Hydrogen bonds – G allele		
Arg 63 NH1	A5 O2P	2.82
Arg 63 NH2	A5 O2P	2.82
Ser 67 OG	G6 O2P	2.70
Lys 42 NZ	C7 O1P	2.86
Arg 74 NH2	C7 O5′	2.65

Table 1. Protein–DNA contacts (hydrogen bonds) in the hypoxia-inducible factor 1 (HIF-1) DNA-binding domain complex observed in LIGPLOT [27] and NUCPLOT [26] (*Continued*).

Donor	Acceptor	Distance (Å)
Arg 74 NH2	G8 O1P	2.65
Arg 75 NH2	G8 O2P	2.68
Arg 75 NH1	G8 O1P	2.98
Arg 16 NH1	T9 O5′	2.94
His 8 NE2	G10 O2P	2.88
Arg 16 NH2	G10 O2P	2.73
Arg 5 NH1	A11 O2P	2.74
Arg 2 NH1	G12 O2P	2.77
Arg 5 NH2	G12 N7	2.87
Glu 1N	A16 O2P	2.79
Glu 1N	A16 O1P	2.72
Arg 68 NH2	T20 O5′	3.00
Arg 68 NE	C21 O2P	2.68
Lys 64 NZ	C21 O5′	2.80
Lys 39 NZ	G27 O5′	2.83
Hydrogen bonds – A allele		
Arg 68 NH2	G6 O2P	2.94
Ser 67 OG	C7 O1P	2.87
Arg 72 NH1	C7 O2P	2.75
Arg 72 NH2	A8 O2P	2.64
Arg 75 NH2	A8 O1P	2.66
Arg 75 NH1	A8 O2P	2.72
His 8 NE2	G10 O1P	2.77
Arg 15 NH2	G10 O2P	2.72
Tyr 83 OH	C19 O3′	2.86
Arg 2 NH2	C25 O2P	2.75
Arg 2 NH1	C25 O5′	2.67
Arg 5 NH2	T26 O3′	2.86
Lys 64 NZ	G28 O1P	2.64
Ser 60 OG	G29 O5′	2.84

We analyzed how HIF-1 recognizes the sequence of the hypoxia-response element nucleotides: 6–10. The aryl receptor nuclear translocator subunit corresponds to amino acid residues 1–59 and the HIF-1α subunit to amino acid residues 60–116 (or 1–57, arbitrary numbering) [21]. Typical observed contact in known structures is as follows. *G allele*: OG of serine (Ser) 67 with O2P of guanine (G) 6 = 2.70 Å (*favorable contact*), NZ of lysine (Lys) 42 with O1P of cytosine (C) 7 = 2.86 Å, NH_2 of arginine (Arg) 74 with O5′ of C7 = 2.65 Å, NH_2 of Arg 74 with O1P of G8 = 2.65 Å, NH_2 of Arg 75 with O2P of G8 = 2.68 Å (*favorable contact*), NH_1 of Arg 75 with O1P of G8 = 2.98 Å (*favorable contact*), NH_1 of Arg 16 with O5′ of thymine (T) 9 = 2.94 Å, Nε2 of histidine (His) 8 with O2P of G10 = 2.88 Å (*favorable contact*), and NH_2 of Arg 16 with O2P of G10 = 2.73 Å (*favorable contact*). *A allele*: NH_2 of Arg 68 with O2P of G6 = 2.94 Å, OG of Ser 67 with O1P of C7 = 2.87 Å (*favorable contact*), NH_1 of Arg 72 with O2P of C7 = 2.75 Å, NH_2 of Arg 72 with O2P of adenine (A) 8 = 2.64 Å, NH_2 of Arg 75 with O1P of A8 = 2.66 Å, NH_1 of Arg 75 with O2P of A8 = 2.72 Å, Nε2 of His 8 with O1P of G10 = 2.77 Å (*favorable contact*), and NH_2 of Arg 15 with O2P of G10 = 2.72 Å.

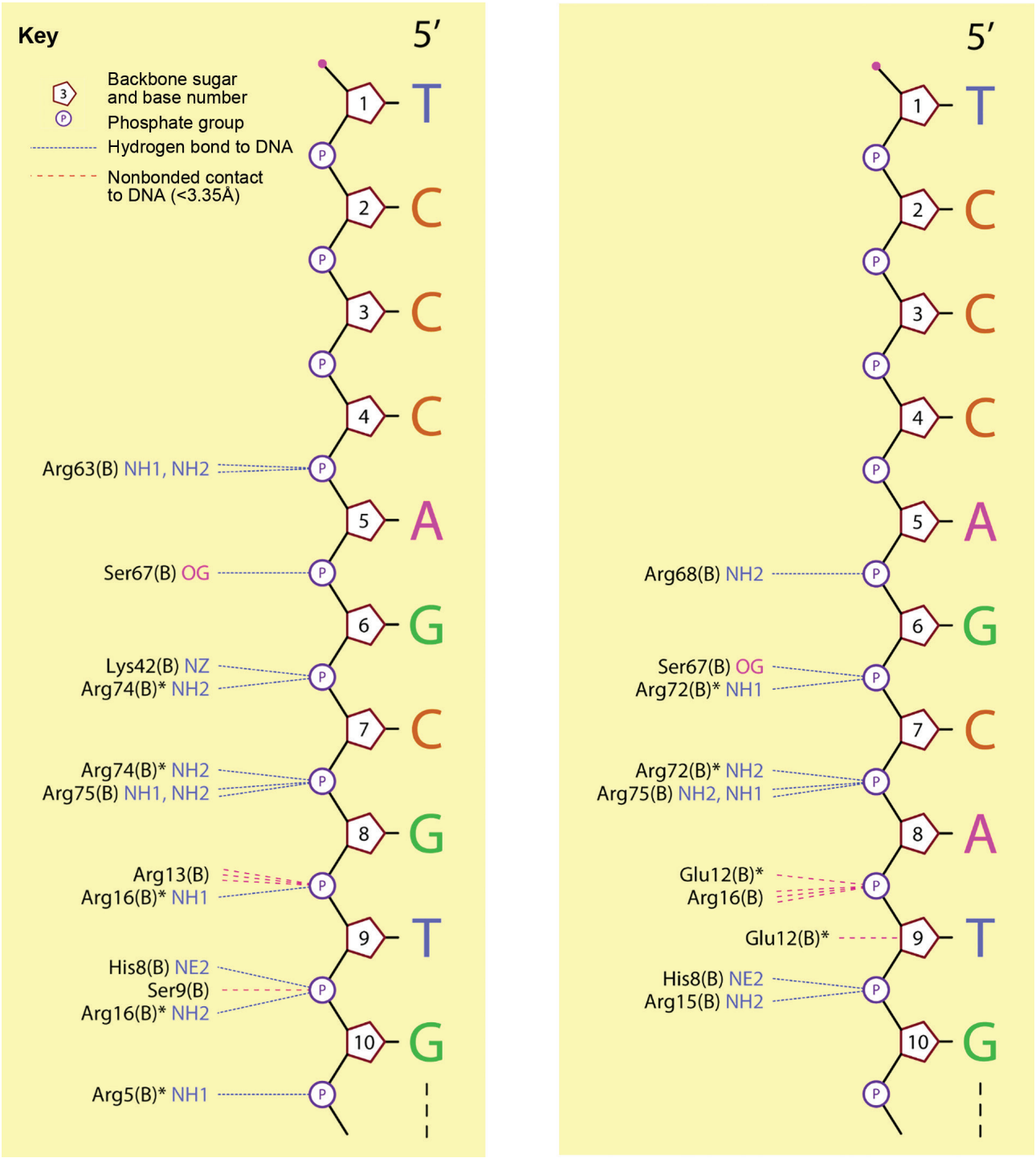

Figure 7. 2D schematic representation of DNA–protein contacts observed in the hypoxia-inducible factor 1 (HIF-1) DNA-binding domain using NUCPLOT (version 1.0) [26]. The aryl receptor nuclear translocator (ARNT) subunit corresponds to amino acid residues 1–59, and HIF-1α subunit corresponds to residues 60–116 (or 1–57, arbitrary numbering). Left, G allele–HIF-1 contacts; right, A allele–HIF-1 contacts. C, cytosine brown; A, adenine magenta; T, thymine blue; G, guanine green. (B) indicates the amino acid acting as a ligand, and * indicates strong bonding between amino acids and nucleotides (either favorable or unfavorable contact).

G or A alleles. We only analyzed hydrogen bonding between the amino acids and nucleotides that was within 3.0 Å. When HIF-1 bound to the A allele, it formed only 14 H bonds between the HIF-1α/ARNT subunits and the nucleotides. By contrast, the G allele–HIF-1 complex binding included 20 H bonds between HIF-1α/ARNT subunits and the nucleotides (**Table 1**).

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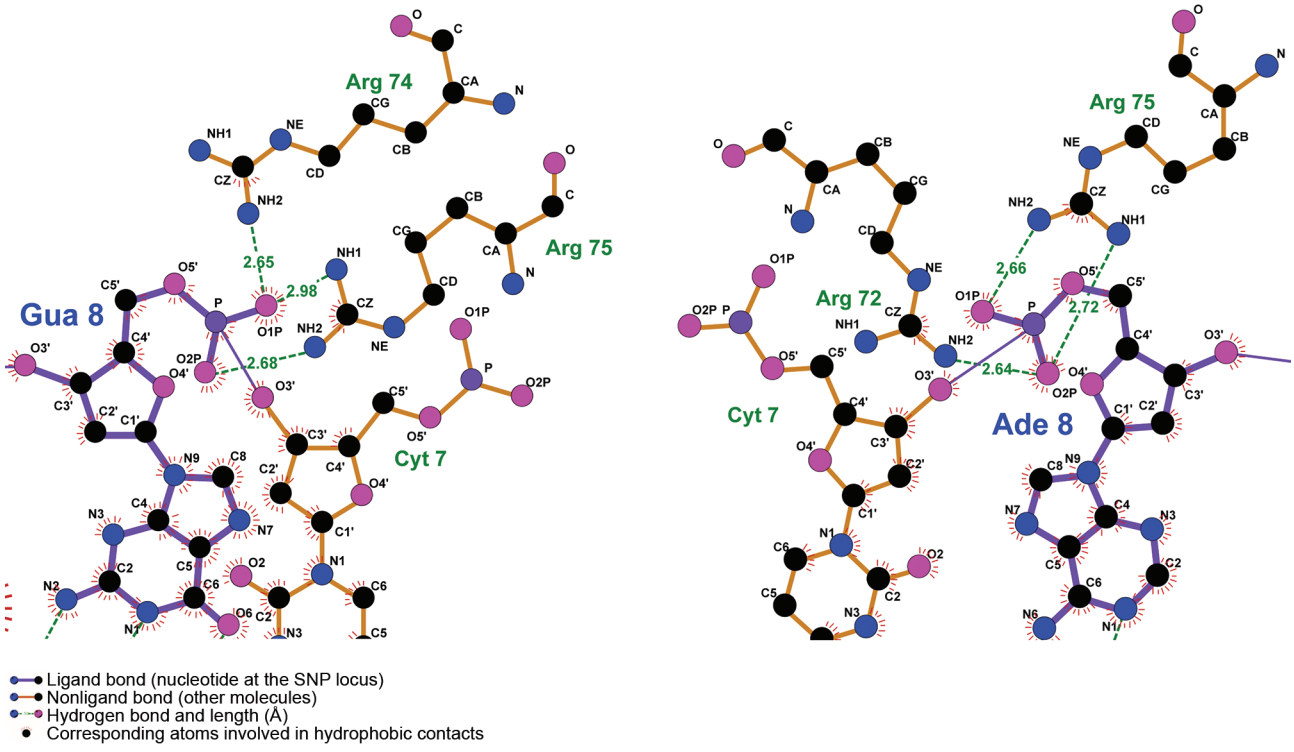

Figure 8. Visualization of hypoxia-inducible factor 1 (HIF-1)–hypoxia-response element DNA at the 8th nucleotide, guanine in G allele (A) and adenine in A allele (B) using LIGPLOT [27] with LigPlot+ (version 2.1) [28]. Here, the G allele (guanine) contacts arginine (Arg) 75 favorably. By contrast, the A allele (adenine) makes less *favorable contact* with Arg 75. Color key to atoms: carbon, black; nitrogen, blue; oxygen, magenta; phosphorous, purple.

Thus, HIF-1 formed a stronger contact with the G allele than it did with the A allele, and therefore, binding with the G allele was likely to stimulate stronger transcription activity of *AGT*.

Discussion

We searched for all available 3D structures for the HIF-1 bHLH domain in the Research Collaboratory for Structural Bioinformatics PDB [31], but most were nondimeric structures determined by either NMR or X-ray crystallographic methods (e.g., PDB entries IH2K, IL3E, and 5I9V). We only found pure HIF-1α and HIF-1α combined with other molecules in model structures. Because no experimental structure was yet available for the bHLH domain of HIF-1, we used the model proposed by Michel et al. [21]. This model, shown in **Figure 2**, was obtained by a combination of different modeling approaches and validated through Ramachandran plots, physicochemical properties, and energetic profiles. Sequence comparison has shown that the bHLH motif is present in many proteins. Michel et al. aligned the sequence of each HIF-1α and ARNT subunit with the partial sequences of other bHLH factors of known 3D structures, using ClustalW [32]. Such a multiple alignment was designed to detect a portion of the query sequence that would correspond to a similar secondary and tertiary structure (in this case, the bHLH motif). Various tools were used to analyze the structure. The HINT (Hydropathic INTeractions) molecular modeling system (hint! version 3.1.2; eduSoft) [33] was used to detect and estimate the strength of the hydrophobic and polar interactions, and ProSA [34] was used to determine errors in protein 3D structures [21]. In addition, to confirm HLH domain, available X-ray crystallographic and NMR structures have shown that the HLH domain dimerizes in the form of a parallel 4-bundle left-handed helix, where the contacts occur within a hydrophobic core region [35].

In our present study, we found that *AGT* contains HRE in its promoter region as a target directly regulated by HIF-1 through transcriptional activity at the –152 site. HIF-1 contributes to the development of hypoxia by affecting *AGT* levels directly and induces hypertension. Here, we present a prediction of the binding pattern between DNA models (G–152A) and HIF-1 protein. HIF-1 is most favorably bound to DNA in the HRE sequence (of the G allele) and is directly in contact with the major groove. α-Helices in the DBD of eukaryotic transcription factors orient in the major groove of DNA, in which the atoms of the protein form specific hydrogen bonds

and van der Waals interactions with nucleotides in the DNA. The protein–DNA-binding sequence interacts more preferentially with the major groove of B-DNA than the minor groove, because the protein has more functional groups that are able to recognize the consensus sequence [36].

The genetic variant G–152A changes the binding pattern of HIF-1 to the *AGT* promoter. The pattern could be explained by favorable interactions between amino acids and nucleotides. Lustig and Jernigan showed that the most favorable interactions are observed between guanine and either arginine or lysine. Interactions between histidine and guanine, between glutamate and cytosine, and between serine with cytosine are also favorable [21, 37]. However, paired interactions between arginine and adenine or cytosine are unfavorable.

We analyzed how HIF-1 recognizes the HRE sequence (nucleotides: 6–10) (**Table 1**). In the present study, most of the HRE consensus sequences of the G allele formed direct contact with the active sites of the HIF-1 subunits (HIF-1α and ARNT) with strong and favorable bonds. By contrast, most of the contacts of HIF-1 and the A allele were unfavorable. This unfavorable association with the A allele is likely to reduce the ability of HIF-1 to stimulate the expression of *AGT* during hypoxia, and downregulation of *AGT* production by HIF-1 is implied. The occurrence of SNP G–152A in the *AGT* promoter of Indonesian patients with essential hypertension suggests that the G allele is a genetic susceptibility factor in hypertension as a compensatory mechanism regulated by HIF-1.

Michel et al. suggested a model that predicts a pattern of interactions between the amino acids of HIF-1 and HRE DNA bases. In the ARNT subunit, His 8 (H), Glu 12 (E), and Arg 16 (R) were the only conserved residues in ARNT that made specific contacts with HRE [21]. By contrast, only the Arg residue was conserved in HIF-1α (in our present study, Arg 75). Thus, Arg 75 (E) likely plays a key part in the control of initiating HIF-1 transcription. The present study showed that Arg 75 (residue of HIF-1α) makes a direct and favorable contact with the G allele, but not with the A allele (**Figure 8**). This is likely to influence the ability of HIF-1 to stimulate expression of *AGT*.

HIF-1 functions as a major regulator of oxygen homeostasis. HIF-1 regulates oxygen delivery by regulating angiogenesis and vascular remodeling, and oxygen use by regulating glucose metabolism and redox homeostasis [14]. Secretion of *AGT* into the blood plasma is proposed as a compensative response to hypoxia; this causes pronounced vasoconstriction throughout the body, which can lead to a rapid rise in arterial blood pressure during a shortage in oxygen supply. Our present findings suggest that this compensatory mechanism is more responsive in individuals with the –152G SNP than it is in those with a –152A SNP. Although it is likely an adaptive response to hypoxia, the HIF-1α-mediated upregulation of inflammatory mediators also initiates inflammatory processes [38]. A previous study in a Taiwanese population showed a potentially important association between the G–152A polymorphism of *AGT* and systolic heart failure. Specifically, the investigators concluded that the GG genotype at G–152A was more strongly associated with the presence of systolic heart failure than the AG or AA genotypes [39]. Thus, HIF-1 may play a role in a self-perpetuating cycle of damage. More recently, a study in an eastern Indian population found an association of SNPs of *AGT* with a risk of hypertension, which is consistent with studies of other ethnic groups. The investigators attributed most of the associations to epigenetic modulation by DNA methylation. However, their analysis of sequence nearby the G–152A polymorphism (rs11568020) did not support this hypothesis, suggesting some other mechanism for the association of this SNP with essential hypertension [40] as proposed in the present article. Further studies are needed to validate the findings in silico showing how the transcription factor (HIF-1) directly regulates (–152) sites in the *AGT* promoter in both G and A SNP alleles, and the impact of these SNPs on protein products and the occurrence of essential hypertension.

Conclusions

The *AGT* promoter (5′-UTR locus) in our Indonesian patients with hypertension had genetic variants –152G and –152A in the ratio 19:1. HIF-1 binds favorably to the DNA of the G allele of the HRE region. The SNP G–152A reduces the favorability of the binding of HIF-1 to the *AGT* promoter.

Author contributions. AHS, W, and MSR contributed substantially to the conception and design of the study; AHS, DHU, and ML acquired the data; and AHS, MSR, and DHU analyzed and interpreted it. AHS, W, and DHU drafted the manuscript, and AHS, W, MSR, and ML critically revised it. All authors approved the final version submitted for publication and take responsibility for the statements made in the published article.

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Conflict of interest statement. The authors have each completed and submitted an International Committee of Medical Journal Editors Uniform Disclosure Form for Potential Conflicts of Interest. None of the authors has any conflict of interest to disclose.

Data sharing statement. The pdb files to show the docking in silico interactions between HIF-1 and the G allele (MODEL1810260001) and A allele (MODEL1810260002) are available at: https://www.ebi.ac.uk/biomodels/. The SNP has been submitted to ClinVar https://www.ncbi.nlm.nih.gov/clinvar/ submission ID SUB4739030. Other data will be made available to other investigators wishing to use them for non-commercial purposes, upon reasonable request to the corresponding author.

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