Polymorphism impact on *AGTR1* gene in Covid-19 patients in Babylon Province

Zainab A. Tolaifeh [(1)], Nada naji shaalan[(2)],Abeer Fauzi Al-Rubaye[(1)], Maani seher Abid AL-Kafaji[(3)] , Kawther M. A. Hasan [(1)], and Zaidan K. Imran [(1)]

(1) University of Babylon, College of Science for Women

(2) University of Kerbala, College of Education for pure Sciences

(3) University of Babylon, College of Medicine

Corresponded author email: zaidanomran62@gmail .com

ABSTRCT:

Infection by the Covid-19 produces in people an illness of profoundly factor and erratic seriousness. The presence of continuous hereditary single nucleotide polymorphisms (SNPs) in the population could prompt a more prominent weakness to infections or an overstated provocative reaction. We concentrated on the relationship between SNP from *AGTR1* gene and the seriousness of the infection created by the SARS-Cov-2 infection.

Methods: 30 (18 of male and 12 of Female) Covid-19 patients with age range from 55-60 years were assembled in light of the seriousness of side effects.

Results: one SNP rs5186 in *AGTR1* gene were related with the seriousness of illness. Our results were showed that the rs5186 (C) allele in *AGTR1* gene is associated with increased risk for Covid 19 based on OR value: homozygous allele CC has 2.5(0.7-8.5), allele C with allele frequency 20 more than control group, and with OR=2.5(1.5-5.9) with significant P values (P=0.03).

Key words: Covid-19, *AGTR1* gene, SNP rs5186. Allele frequency.

DOINumber:10.14704/nq.2022.20.8.NQ44644 **NeuroQuantology2022;20(8):6196-6202**

Introduction:

A new coronavirus strain called SARS-CoV-2 caused a terrible epidemic that spread quickly from China (Zhu et al., 2020; Wang et al., 2020).Coronavirus disease 19 (COVID-19) was brought on by SARS-CoV-2, and the severity of the disease was influenced by risk factors such age, cardiovascular disease, metabolic disorders (diabetes, obesity), etc. (Lauc and Sinclair, 2020; Wang et al., 2020). On the other hand, viral infections accompanied human evolution through host/virus interaction, and at least some interindividual variations were linked to the host genetic profile (Albright et al., 2008; Fauci and Morens, 2012). Parallel to this, the range of Covid-19 phenotypes prompted the inquiry of how much the varying response to SARS-CoV-2 is impacted by the varying genetic make-up of the hosts. (Di Maria *et al*., 2020).

Angiotensin II type I receptor gene, or AGTR1, is thought to be a significant risk factor associated with Covid-19 infection. This gene, which went by the names AT1, AG2S, AT1B, AT1R, AT1AR, AT1BR, AT2R1, HAT1R, and AGTR1B, had a variety of roles, including the principal regulation of aldosterone release by the powerful vasopressor hormone angiotensin II. It is a crucial factor in the cardiovascular system's volume and blood pressure regulation. It operates on at least two different receptor

types. The type 1 receptor, which is encoded by this gene, is considered to mediate the majority of angiotensin II's cardiovascular effects.

This gene may contribute to the development of reperfusion arrhythmias once the ischemic or infarcted heart receives blood flow again. In contrast to earlier theories, it is currently considered that humans only have one gene for the type 1 receptor. This gene is known as AGTR1B.

(https://www.ncbi.nlm.nih.gov/gene?Db=gene &Cmd=DetailsSearch&Term=185).

Most often, but not exclusively, in those individuals with severe prior conditions, the SARS-CoV-2 infection results in a severe and fatal sickness. One of the most reliable markers of death is hypertension (Wang et al.,2020). However, at the most severe stages of the sickness, perhaps these comparable drugs will be helpful. It is known that long-term administration of angiotensin framework inhibitors alters the expression of ACE, ACE2, and AGTR1 (Li et al., 2017).

A few studies have suggested blocking ACE2 as a potential method to reduce SARS-CoV-2 viral load in lung illnesses and prevent the virus from spreading to other organs (Abassi et al.,2020).

In reality, ACE2 polymorphism is present in patients with Covid-19 weakness. (Mehrabadi*et al*.,2021). The purpose of this study was to further explore the relationship between the AGRT1 polymorphic variations and Covid-19 severity and susceptibility in the Babylon community. We examined the correlation of the AGTR1 gene polymorphism with COVID-19 in an effort to better understand its function.

Material and Methods:

A total of 30 COVID-19 subjects with positive polymerase chain reaction (PCR) test for SARS-Cov-2 virus were included in the study. 300ul of peripheral blood was dropped in 1.5 microtubes, and subjected to protocol of extraction. The kit used for extraction was Favorgen manufactured by Korea. The participants were grouped into 2 groups: cases and healthy. Patients were expected to have begun of lung complication.

To complete the investigation of polymorphisms, DNA was removed from 200µl of fringe blood. Primer pair was designed in this study AGF: GGCCATGCCTATCACCATTTG, AGR: GACATTGTTCTTCGAGCAGCC, the investigation of the polymorphism in *AGTR1*gene was completed by PCR convention utilizing an underlying denaturation at 93°C for 5 min; 30 patterns of denaturation at 93°C for 30Sec. min, strengthening at 58°C for 30 sec, and prolongation at 72°C for 1 min. PCR product:476bp, the last cycle was trailed likewise at 72°C for 3 min and electrophoresis in agarose (1%) gel. The excess chose single nucleotide polymorphisms (SNPs) in *AGTR1*gene were broke down by Sanger sequencing methodology. Patients' Informed composed assent was gotten from all patients, and the neighborhood morals council of each partaking focus endorsed the review convention, which was directed as per the standards of the Declaration of Helsinki.

Statistical and bioinformatics analysis

The allele and genotype frequencies were dictated by direct counting. The Chi χ2 test was utilized to analyze allele and genotype conveyance in patients with Covid-19 and sound control subjects. The Odd Ratio (ORs) and 95% confidence intervals (CIs) were determined with SPSS. The Hardy-Weinberg harmony of every polymorphism was dictated by the program HWE. All calculations were finished utilizing P-value 0.005.Since the AGTR1 gene is found on all three chromosomes, the SNPs from the AGTR1 gene were examined independently in females and males. The genotypes of all patients were used to compute allele frequencies.

Results:

Target amplified in patients Covid-19:

The results showed success the primer pair efficiency to amplification target DNA region of *AGTR1* gene, the amplification region with flanking primers, PCR product 476 bp for patients Covid-19 infection group (Figure 1).

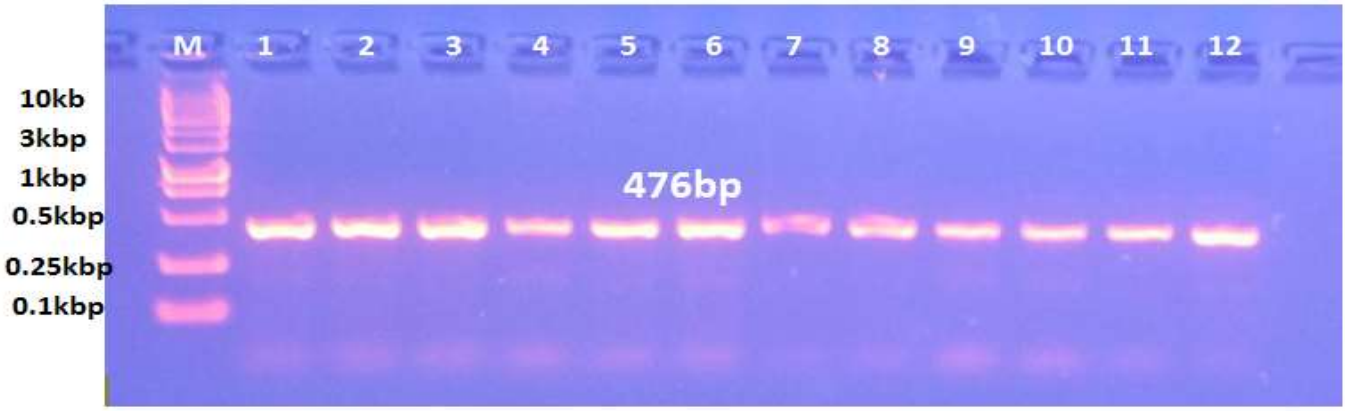

Figure 1: Profile of PCR products of target of *AGTR1*gene shown PCR products 476 bp for 12 PCR products for patients without fungal. 1-12 patient samples, M=molecular marker first step100bp. 1.5% agarose -TBE gel, pre-staining with 0.5ul Ethidium, bromide, 45min., 100 voltages.

Polymorphism screening and genotyping:

By directly sequencing the genomic DNA of 15 covid-19 patients and 5 healthy controls, AGTR1 gene polymorphisms were screened. One primer pair was created in this work to be used in the polymerase chain reaction (PCR) to amplify the AGTR1 gene's incomplete sequence. (Table1). Direct sequencing was conducted by send 18-20 ul of PCR of samples of three groups for each. After reserved sequence charts from Microgen, the sequences were checked they familiarity to *AGTR1* gene intergenic region based pairwise alignment for each. The text file was used for pair wise alignment with deposited NCBI database. The Chromatogram file in rare which out of 4 files enclosed were import to genius software for alignment.

Multiple alignments of 15 sequences of AGTR1 gene in covid-19:

The multiple alignments of chromatograms data show the genotyping findings for patients with covid-19 showing the SNP: rs5186 were (AA, AC &CC) Figure (2)

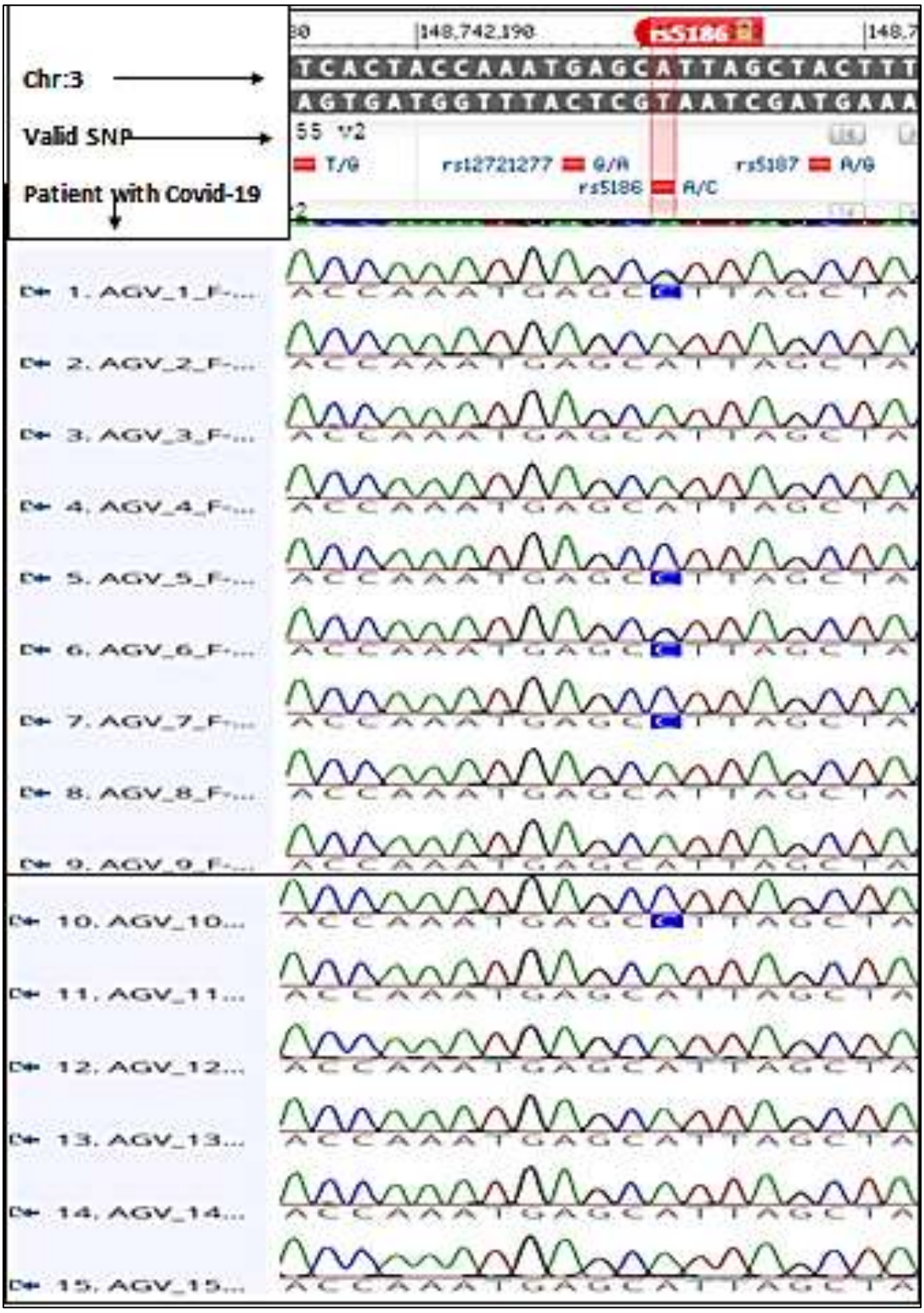

Figure 2: The multiple alignment of chromatograms of targeted region of *AGTR1*gene as shown three SNP: re5186. (Covid-19 patients) alignment performed by Geneious prime software.

***AGTR1* gene rs5186A>C polymorphism:**

The detail information was clearest in Table (1). The genotyping findings for COVID-19 patients and control group, showing the three genotypes (AA, AC &CC), (67.7%, 0%, 33.3%) respectively and allele frequency (A 66.7%, C 33.3%) respectively for control subjects. A significant appeared in OR of homozygous allele (CC)=2.5(0.7-8.5) and allele frequency in case group 20 opposite 10 in control group and OR of Mutant allele was 2.5(1.5-5.9). Many previous studies referred to the relation of this SNP with Covid-19. This study proven the validity of this SNP in studied population under interest for the first time at the same time this SNP:rs5186 A>C.

Table (1) Genotype and allele frequency of *AGRT1* rs5186 A>C*)* associated with/without covid-19 patients and control.

Rs5186 A>C		**Covid-19infected N=30**	**Control N=30**	**OR (95%CI)**	**P-value**
genotypes	**AA**	20(66.7%)	25(83.3%)	Reference group	
	AC	0(0%)	0(0%)	1	1
	CC	10(33.3%)	5(16.7%)	**2.5(0.7-8.5)**	0.14
Allele Frequency	**A**	40(66.7%)	50(83.3%)	0.4(0.16- 0.9)	0.03
	C	**20(33.3%)**	10(16.7%)	**2.5(1.5-5.9)**	0.03

Discussion:

Our results were showed that the rs5186 (C) allele in *AGTR1* is associated with increased risk for Covid 19 based on OR value: homozygous allele CC has 2.5(0.7-8.5), allele C with allele frequency 20 more than control group, and with OR=2.5(1.5-5.9) with significant P values (P=0.03), these results consistent with result of Musso *et al*., (2019) and Parchwani *et al*., (2018) they referred to the role of many SNPs such as: rs5183, rs5185 and rs5186 SNPs also might affect the *AGTR1* gene function.

The AGTR1 gene's function can alter as a result of any mutation. This gene, which may be found on chromosome 3q24, codes for the angiotensin type 1 receptor. Vasopressor hormone angiotensin II controls pro-inflammatory gene expression, vascular cell migration,and hypertrophy/hyperplasia. It primarily promotes vascular muscle constriction through the AGTR1 gene. It controls blood pressure and the cardiovascular system's homeostasis in a significant way.

The etiology of hypertension, cardiovascular disorders (myocardial infarction and arteriosclerosis), and renal disease may all be affected by elevated tissue levels of angiotensin II. This is suggested by the description of these states in various pathological circumstances (Duan, and Wang 2016, Feng et al.,2014).

Our findings revealed considerable variations in genotype frequencies for the AGTR1 SNP, but no correlation with illness severity was seen in the patients group. However, substantial variations in A/A genotype frequencies at AGTR1 rs5186 were discovered regarding the probability of hospitalization between the groups with and without comorbidities; this suggests that hypertension in patients with a certain genotype may worsen COVID-19 illness. The severity of COVID-19 may be correlated with the AGTR1 gene allele CC (rs5186), according to our results. We speculate that COVID-19 severity and allele C may be related (Mehta and Griendling, 2007). During COVID-19, these angiotensin II side effects might be serious (Miesbach, 2020).

According to research looking at the relationship between the methylation of the AGTR1 promoter and the risk of essential

hypertension, CpG1 hypomethylation in the promoter is probably related to that risk, with males having much lower CpG1 methylation levels than females (Fan et al., 2015).

In the present investigation, we demonstrated that male sex was a risk factor for CHD and found that exclusively male CHD patients had hypermethylation of the AGTR1 gene. As a result, variations in lifestyle and sex hormone levels may be reflected in the sex disparities in AGTR1 methylation levels in CHD patients.

Conclusions:

Our research found a correlation between the AGTR1 polymorphism and Covid-19 severity in the Babylon population. Our results were showed that the rs5186 (C) allele in *AGTR1* gene is associated with increased risk for Covid 19 greater than in A allele carriers, probably as a result of cardiovascular diseases.

Ethical approval:

A formal informed consent form was signed by the patient. Statement of Conflicting Interests

The authors affirm that they have no known financial or interpersonal conflicts that would have seemed to have an impact on the research presented in this study.

References:

- **Abbasi,** M.S., Ahmed, N. Sajjad,B et al.,2020.E-Learning perception and satisfaction among health sciences students amid the COVID-19 pandemic. Work 67(3):549-556.
- **Albright,** F.S., Orlando, P., Pavia, A.T., Jackson, G.G., Cannon Albright, L.A., 2008 Jan 1. Evidence for a heritable predisposition to death due to influenza. J. Infect. Dis. 197 (1), 18–24.
- **Di Maria,** E., Latini, A., Borgiani, P., Novelli, G., 2020 Sep 11. Genetic variants of the human host influencing the coronavirus-associated phenotypes (SARS, MERS and COVID-19): rapid systematic review and field synopsis. Hum Genom. 14 (1), 30.
- **Duan** LJ, Wang XD. Analysis of correlations between coronary heart disease and haplotypes of the angiotensin II receptor type 1 (AGTR1) gene. *Genet Mol Res*. 2016;15(1).
- **Fan,** R., Mao, S., Zhong, F., Gong, M., Yin, F. , Hao, L. (2015). *Association of AGTR1 promoter methylation levels with essential hypertension risk:a matched case-control study.* Cytogenet Genome Res.
- **Fauci**, A.S., Morens, D.M., 2012 Feb 2. The perpetual challenge of infectious diseases. N. Engl. J. Med. 366 (5), 454–461
- **Feng,** X., Zheng, B.S., Shi, J.J., Qian, J., He, W., Zhou, H.F., 2014 Sep. A systematic review and meta-analysis of the association between angiotensin II type 1 receptor A1166C gene polymorphism and myocardial infarction susceptibility. J. Renin- Angiotensin-Aldosterone Syst. 15 (3): 307–315.
- **Lauc,** G., Sinclair, D., 2020 Apr 8. Biomarkers of biological age as predictors of COVID-19 disease severity. Aging (Albany NY) 12 (8), 6490–6491.
- **Li,** X.C., Zhang, J., Zhuo, J.L., 2017 Nov. The vasoprotective axes of the renin-angiotensin system: Physiological relevance and therapeutic implications in cardiovascular, hypertensive and kidney diseases. Pharmacol. Res. 125 (Pt A),21–38
- **Mehrabadi** ME, Hemmati R, Tashakor A, Homaei A, Yousefzadeh M, Hemati K, and Hosseinkhani S (2021) Induced dysregulation of ACE2 by SARS-CoV-2 plays a key role in COVID-19 severity. Biomed Pharmacother 137:111363.
- **Mehta,** P.K., Griendling, K.K., 2007 Jan. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. Am. J. Phys. Cell Phys. 292 (1), C82–C97
- **Miesbach,** W., 2020 Jun 26. Pathological role of angiotensin II in severe COVID-19. TH Open. 4 (2), e138–e14.
- **Musso** G, Saba F, Cassader M, Paschetta E, De Michieli F, Pinach S, et al. Angiotensin II Type 1 Receptor rs5186 Gene Variant Predicts Incident NAFLD and Associated

*e*ISSN1303-5150

Hypertension: Role of Dietary Fat-Induced Pro-Inflammatory Cell Activation. *Am J Gastroenterol*. 2019;114(4):607–619.

- **Parchwani** DN, Patel DD, Rawtani J, Yadav D. Analysis of Association of Angiotensin II Type 1 Receptor Gene A1166C Gene Polymorphism with Essential Hypertension. *Indian J Clin Biochem*. 2018;33(1):53–60.
- **Wang**, C., Horby, P.W., Hayden, F.G., Gao, G.F., 2020 Feb 15. A novel coronavirus outbreak of global health concern. Lancet. 395 (10223), 470–47.
- **Zhu,** N., Zhang, D., Wang, W., et al.,2019 China Novel Coronavirus Investigating and Research Team, 2020 Feb 20. A novel coronavirus from patients with pneumonia in China, N. Engl. J. Med. 382 (8), 727–733.