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Pharmacogenetic Study of Drugs affecting Coronavirus

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Abstract

A new worldwide pandemic of coronavirus disease 2019 (COVID-19) has resulted in a healthcare crisis with high mortality and morbidity. Presently, several drugs are under accelerated research without established efficacy and are being used to treat COVID-19 patients either as unapproved drug use or as clinical trials. To optimally use the drugs, several factors, such as the gene effects, drug interactions, and drug toxicity, should be considered. Genetic polymorphisms are a type of genetic diversity within a population's gene pool that constitute the basis of pharmacokinetics, which causes alteration in the drug function and response. Since there was a limited time to check individual pharmacogenomics markers, it seems population pharmacogenomics tests could be helpful in expecting drug treatment failure in COVID-19 patients. We genotyped and investigated allele frequencies of 33 Single-nucleotide polymorphism (SNPs) located on 10 pharmacogenes from 150 healthy individual samples.

A total of 32 potential pharmacogenomics variants relevant to COVID-19 treatment were identified in the Iranian population. Considering them in patients' pharmacotherapy could influence the treatment optimization and reduce severity of adverse effects.

Keywords: Corona virus, Pharmacogenetics, Single nucleotide polymorphism

1. Background

Personalized medicine, also referred to as precision medicine, is a medical treatment of a particular disease according to the individual genotypes (1). In addition, personalized medicine is used not only in the selection and utilization of appropriate drugs in the direction of targeted treatment, but also special attention has been paid to the use of genomic knowledge in the design of new drugs and the process of drug acceptance by health system authorities (2). Using patients' genomic sequences, at the time of prescribing common and used drugs, people of a population can be divided into different categories and based on that, we can predict which drugs can be helpful and effective for the present patient or at least have a higher impact on the treatment and recovery of the patient or selecting a drug in such a way that the drug has fewer side effects (3). Drugs are more likely to enter the body as prodrugs and are therefore inactive, and then prodrugs are changed to become active. Therefore, mutations in genes involved in drug metabolism can be effective in the Pharmacokinetics of the drug in the body and indicate the importance of different genetic variants that metabolize drugs (4). Pharmacogenomics is of great value because it shows abnormal drug responses, which are the major causes of pathogenesis and mortality (5). To date, the main challenge is drug diversity, as well as the extent of drug-metabolizing genes (6). However, the role of cytochrome P450 in the liver and its genes is crucial (2). Consequently, in the first step, identifying the genetic pattern of individuals in a population is very important, especially in terms of genes that play a role in drug metabolism. According to the urgent need to take effective drugs in the treatment of COVID-19, it is necessary to know the genes that affect the metabolism of drugs used and to determine their variants in each individual to prevent adverse and sometimes fatal effects of drugs (7). However, the specific conditions of the current epidemic, with its high prevalence and mortality, do not allow us to focus on the individual genetic structure of drug therapy, and what is inevitably done in many countries is to focus on population genetics (8). Accordingly, the present study aimed to investigate and determine the percentage of different variants of 15 of the most widely used genes effective in drug metabolism with a focus on the proposed coronavirus drugs in the Iranian population (9). We hope that with the results obtained from this study, we hope to find a suitable approach to deal with drugs (10). On the other hand, since the selected genes are all in the metabolism of the drugs proposed by COVID-19, it is important to determine the frequency of each gene variant in the Iranian population (10, 11).

2. Objectives

Since it is also necessary to optimize the rapid method of identifying the individual variant for each gene, the simple method of ARMS PCR or Tetra ARMS

PCR was used in this research.

3. Methods

3.1. Samples

Project number 752 was permitted by the National Institute for Genetic Engineering and Biotechnology. One hundred fifty blood samples were taken from people who were between 18-60 years old and were genetically healthy (i.e., they had no genetically proven disease). For this purpose, 2 ml of blood was taken from the participants and stored in Complete blood count (CBC) tubes containing Ethylenediaminetetraacetic acid (EDTA) at -20°C until the test was performed. Written consent was obtained from all the participants (12, 13).

3.2. DNA extraction

SinaPure TM DNA commercially available kit was used for DNA purification from fresh blood of the participants (Sinaclon, Iran) following the manufacturer's instructions (14).

3.3. Spectrophotometric analyses of DNA

Quantitative analysis of DNA-extracted (The concentration, purity absorbance) was measured (ratio at 260-280 nm) and checked with a Thermo Scientific Nanodrop™ 1000 Spectrophotometer (Thermo Scientific, Germany) using 1 µL of each sample. The spectra range obtained from samples was between 2,000-400 ng, and a totally 1,000 ng concentration was considered for each reaction.

3.4. Visualizing DNA by agarose gel electrophoresis

The quality of the extracted DNA was evaluated by agarose gel electrophoresis using 1% agarose gel (SeaKem LE agarose, Cambrex, gels for genomic and amplified DNA). Electrophoresis was done using TBE buffer (1× Tris–Borate EDTA) and a constant voltage of 80 V for 60 min. The gel was then stained using ethidium bromide. The DNA bands were visualized, and images were acquired using the Gel Doc XR+ Imaging system (Bio-Rad Laboratories Inc., Germany)(12, 15).

3.5. Design Primer

The studied rs SNP sequences were first obtained from the National Center for Biotechnology Information (NCBI) database. The Primers were designed with the oligo7 and Gene Runner software (Table 1). The specificity of the primers was then checked by the Basic Local Alignment Search Tool (BLAST) database. In general, in the Tetra ARMS method, four primers FO (forward outer), RO (reverse outer), FI (forward inner), and RI (reverse inner), were considered for each variant. The FO and RO were selected from the upstream and downstream regions of the allele change point, and the resulting band was used as a PCR control. The RI and FI were also selected in a way that the 3 'end nucleotides matched normal and mutant alleles at the allele change point, respectively. In the ARMS method, four primers, FO, RO, FN (forward normal), and FM (forward mutant), were designed so that FO and RO primers were selected from the upstream and downstream areas of the jump point, and their combination was used as PCR control. The FN was designed so that the 3 'end of the complement allele was normal, and the FM was designed so that the 3' end of the complement allele was at the allele change point. The 3' end of the FN primer was designed to complement the normal allele, and the 3' end of the FM primer was designed to complement the mutant allele.

Table 1. Genes and variants studied in the study

	Gene	The most important variants	Location	Exon count
1	CYP1A1	*1, *2B, *4	15q22-q24	7
2	CYP2B6	*1, *2, *3, *4, *5, *9	19q13.2	9
3	CYP2C8	*1, *2, *3, *3A, *4, *5, *7, *8	10q24	9
4	CYP2E1	*1, *2, *6	10q26.3	9
5	CYP3A5	*1, *2, *3, *4, *7, *8, *9, *10, *3B	7q22.1	14
6	CYP3A7	*1, *2	7q22.1	15
7	CYP3A43	*1, *1B, *2, *3	7q21.1	15
8	CES1	*25, rs202121317, rs202001817, rs71647871 (rs121912777)	16q12.2	14
9	UGT1A1	rs3064744*, rs34526305, rs34946978, rs4148323	2q37.1	13
10	ADK	rs397514452, rs397514454	10q11-q24	11

3.6. PCR amplification

The PCR amplification was carried out by PCR thermal cycler (Roche Applied Sciences, Germany). The amplification process was carried out in a 25 µL reaction volume using 0.5 µM vials containing 0.2 µM of each primer, 3 µL of DNA (as template), 15 µL master mix, and 3 µL of water. The thermal cycle program was as follows: 95°C for 5 min for the initial denaturation step, and an amplification program (95°C for 30, 60°C for 30, and 72°C for 30 seconds, respectively) repeated for 35 cycles. According to the ARMS PCR method, two vials for each DNA sample (each vial contains three primers: control primers and primer related to the desired allele) were considered. According to the Tetra ARMS PCR method, one vial for each DNA sample with four primers was considered for PCR reaction. In the current research, ARMS PCR and Tetra ARMS PCR

methods were optimized for 150 DNA samples and 33 selected variants, and then the type of variants was determined for each sample.

3.7. Confirming the results by PCR product sequencing

Randomly, a number of PCR products were selected for each SNP to ensure sequencing results. Chromas sequencing analysis software was used to evaluate the results. In this regard the status of heterozygosity or homozygosity of the test sample of the desired SNP is confirmed.

3.8. Statistical data analysis

The SNPStats for each gene was evaluated using SPSS software version 22.0 (SPSS, Inc., Chicago, IL). A $P < 0.05$ was considered statistically significant.

4. Results

4.1. Extracted DNA quality and quantity valuation

Quantitative analysis of the extracted DNA (The concentration, purity absorbance) was measured (ratio at 260-280 nm) and checked with a Thermo Scientific Nanodrop™ 1000 Spectrophotometer (Thermo Scientific, Germany) using 1 µL of each sample. The spectra range obtained from samples was between 2,000-400 ng, and a totally 1,000 ng concentration was considered for each reaction. The ratio of 260/280, which indicates protein contamination and RNA contamination, was evaluated, and the amount was evaluated in the normal range of 1.8-2, which indicates the absence of protein and RNA contamination. The ratio of 230/260, which indicates polysaccharide contamination, was evaluated, the amount of which was evaluated in the normal range of 2-2.4, which indicates no polysaccharide contamination. In this study, agarose gel electrophoresis was used to visualize DNA and check the DNA quality in 1% agarose gel. Gel electrophoresis showed a single, high molecular weight DNA band with the absence of RNA contamination (Figure 1).

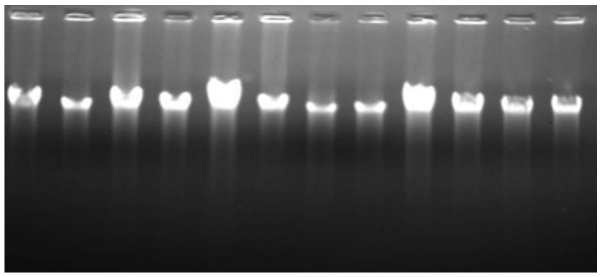

Figure 1. Results of PCR amplification of the total genomic DNA isolated from the sample in 1% agarose gel. None of the DNA samples displayed significant smearing, which shows degradation of the sample

4.2. Primer design and PCR

Appropriate primers were designed for each of the studied SNPs or ordered for synthesis. Due to the high volume of information, the results of Tetra ARMs PCR for *CYP2C19* * 2 and the results of ARMs PCR for *CYP2D6* * 10 are given as examples.

4.3. Tetra ARMS PCR

Tetra ARMs PCR technique was carried out for all the collected samples to evaluate the studied variants. The PCR products were electrophoresed on 1.5% agarose gel to observe the expected bands. For all variants, observation of the control band (combination of FO and RO) is an indication of the accuracy of PCR and should be observed in all PCR reactions. Observation of the band resulting from the combination of FO-RI primers indicates the presence of a normal allele, and observation of the band resulting from the combination of FI-RO primers indicate the presence of a modified allele. The observation of both bands for a sample is considered a state of heterozygous allele. Figure 2 shows an example of electrophoresis of Tetra ARMs PCR products (variant *CYP2C19* * 2) on 1.5% agarose gel.

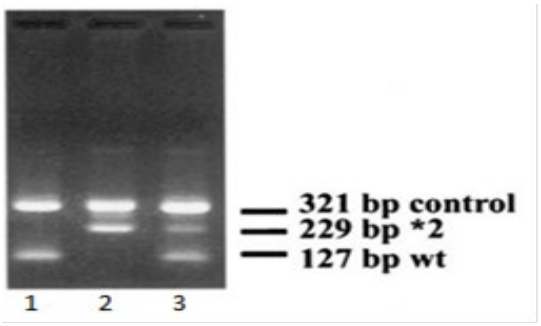

Figure 2. Electrophoresis of PCR products (variant *CYP2C19* * 2) on agarose gel: 321 base pair band (combination of FO and RO) is used as a PCR accuracy control piece. Observation of the 127 base pair fragment indicates the presence of a modified allele (combination of FO-RI primers), and the presence of a 229-base fragment on the gel indicates the presence of a normal allele (combination of FI-RO primers) in the test sample. Thus, the mutant allele, normal allele, and the heterozygous samples were considered in 1-2-3 wells, respectively

4.4. ARMS PCR

For all the extracted samples for each variant, two separate vials were considered. One vial to check for normal alleles using control primers (FO and RO) and specific allele primer (FN), and another vial to check for allele presence using a combination of control primers (FO and RO) and specific allele primer (FM). The PCR products were then electrophoresed on 1.5% agarose gel to observe the desired bands (Figure 3).

4.5. Sequencing

To ensure the bands obtained in PCR products for different alleles and to confirm their sequence, random samples were sent for sequencing each SNP. The results of sequencing several samples that confirm the accuracy of PCR steps and bands

obtained for the relevant allele are given below. Due to the high volume of information, all the sequences were omitted and only the information about the two variants is presented below (Table 2).

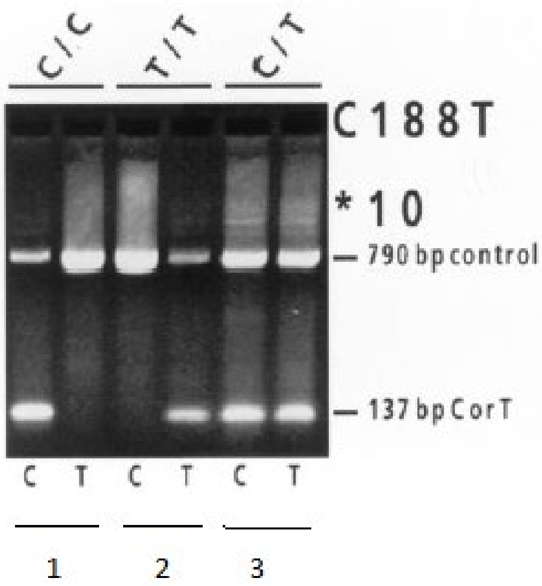

Figure 3. Electrophoresis of ARMS PCR products (*CYP2D6* * 10 variant) on agarose gel: The 790 bp fragment (combination of FO and RO) is used as a PCR accuracy control. Virtualizing 137 fragments in a vial containing an FN primer indicates the presence of a normal allele, and observation of a band of 137-base pairs in a vial containing an FM primer indicates the presence of a modified allele. Observation of the 137-band in both vials for a sample shows a heterozygous state. Accordingly, sample number 1 shows a person with a normal allele, sample number 2 of a person with an allele is changed, and sample number 3 is considered as a heterozygous person

*4.6. Sequencing results of CYP2C19 * 2 variant products*

The 2* variant of the *CYP2C19* gene has a G allele in the normal state, which changes to A at position 681, leading to splicing changes and enzyme inactivation. Figures 4-5 reveal the results of sequencing this variant in the G / A allele range (Figures 4-5).

The relationship between variants and drugs, along with possible effects regarding the use of drugs, are summarized in Table 3. Since many variants are common in the metabolism of drugs and have a similar functional effect, their repetition in tables has been avoided.

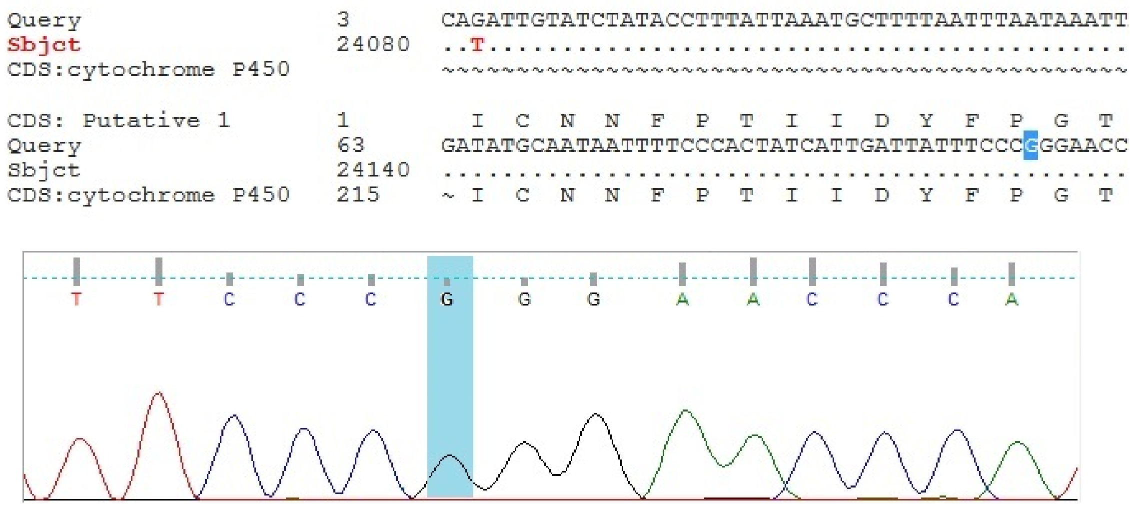

Figure 4. Sequencing of a GG homozygous sample with BLAST results. The marked part on the nucleotide shape is the location of the variant, which represents the GC nucleotide with a single peak

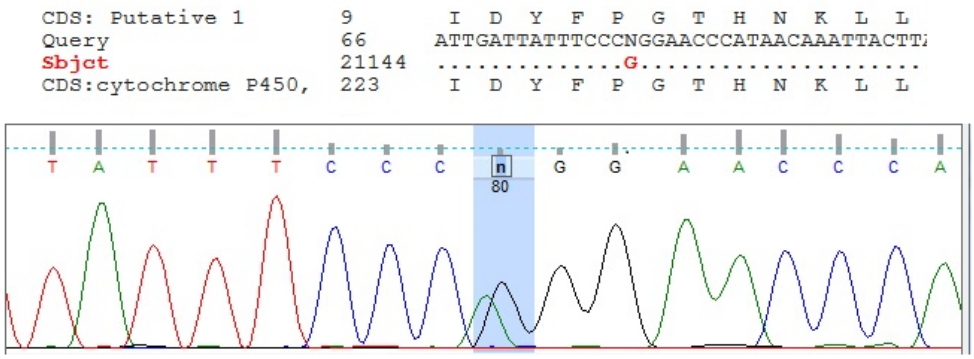

Figure 5. Sequencing of a heterozygous GA sample with BLAST results. The part marked on the nucleotide shape is the location of the variant, which indicates the two peaks of A and G

Table 2. Number and frequency results for each variant: TN=total number, WN=wild number, HN=heterozygote number, MN=monozygotic number, AN=allele number, AC=allele count, WF=wild frequency, HF=heterozygote frequency, MF= mutant frequency, AF=allele frequency, Function: increase: ↑, decreased: ↓, inactivated: –, No change: N

GENE	SNP	exon	Func.	TN	WN	HN	MN	AN	AC	WF	HF	MF	AF
CYP1A1	*2B A > G rs1048943	7	↑	150	126	23	1	300	25	0.84	0.15	0.006	0.083
	*4 C>A rs1799814	7	↑	150	132	18	0	300	18	0.88	0.12	0.0	0.06
GENE	SNP	exon	Func.	TN	WN	HN	MN	AN	AC	WF	HF	MF	AF
CYP2B6	*2 C>T rs8192709	1	↓	149	12	129	8	298	145	0.08	0.86	0.05	0.48
	*3 C>A/C>T rs45482602	5	↓	150	113	17	20	300	57	0.75	0.11	0.13	0.19
	*4 A>G rs2279343	5	↑	150	67	56	27	300	110	0.44	0.37	0.18	0.36
	*5 C>T rs3211371	9	↓	150	95	38	17	300	72	0.63	0.25	0.11	0.24
	*9 G>T rs3745274	4	↑	150	55	68	27	300	122	0.36	0.44	0.18	0.40
GENE	SNP	exon	Func.	TN	WN	HN	MN	AN	AC	WF	HF	MF	AF
CYP2C8	*2 A >T rs11572103	5	↓	150	148	2	0	300	2	0.98	0.013	0.0	0.006
	*3 A >G rs10509681	8	↓	150	127	22	1	300	24	0.84	0.14	0.006	0.08
	*3AG >A rs11572080	8	↓	150	127	21	2	300	25	0.84	0.14	0.013	0.08
	*4 C>G rs1058930	5	↓	150	142	8	0	300	8	0.94	0.053	0.0	0.053
	*5 delA rs72558196	3	-	150	150	0	0	300	0	1	0.0	0.0	0.0
	*7 C>T rs72558195	4	-	150	150	0	0	300	0	1	0.0	0.0	0.0
	*8 C>G rs72558195	4	↓	150	150	0	0	300	0	1	0.0	0.0	0.0
GENE	SNP	exon	Func.	TN	WN	HN	MN	AN	AC	WF	HF	MF	AF
CYP2E1	*2**G>A** rs72559710	2	↓	150	150	0	0	300	0	1	0.0	0.0	0.0
	*6**T>A** rs6413432	Int 6	↓	150	150	0	0	300	0	1	0.0	0.0	0.0
GENE	SNP	exon	Func.	TN	WN	HN	MN	AN	AC	WF	HF	MF	AF
CYP3A5	*2C>A rs28365083	8	-	150	150	0	0	300	0	1	0.0	0.0	0.0
	*3A>G rs776746	Int 3	-	150	150	0	0	300	0	1	0.0	0.0	0.0
	*4A>G rs56411402	8	-	150	150	0	0	300	0	1	0.0	0.0	0.0
	*7 ins A rs41303343		-	150	149	1	0	300	1	0.99	0.006	0	0.003
	*8C>T rs55817950	2	↓	150	150	0	0	300	0	1	0.0	0.0	0.0
	*9G>A rs28383479	10	↓	150	150	0	0	300	0	1	0.0	0.0	0.0
	*10T>C	12	↓	150	150	0	0	300	0	1	0.0	0.0	0.0

	rs41279854 *3B insG rs200579169	1	-	150	148	2	0	300	2	0.98	0.013	0.0	0.006
GENE	SNP	exon	Func.	TN	WN	HN	MN	AN	AC	WF	HF	MF	AF
CYP3A7	*2C>G rs2257401	11	↑	150	5	46	99	300	244	0.03	0.30	0.66	0.81
GENE	SNP	exon	Func.	TN	WN	HN	MN	AN	AC	WF	HF	MF	AF
CES1	*25 G>T rs3826190	2	↓	150	34	116	0	300	116	0.22	0.77	0.0	0.38
	rs202121317	Int5	↓	150	146	4	0	300	4	0.97	0.02	0.0	0.01
	rs202001817	6	↓	150	148	2	0	300	2	0.98	0.013	0.0	0.006
	rs71647871	4	-	150	150	0	0	300	0	1	0.0	0.0	0.0
GENE	SNP	exon	Func.	TN	WN	HN	MN	AN	AC	WF	HF	MF	AF
UGT1A1	*28insTA rs3064744	TATAA box	↓	150	150	0	0	300	0	1	0.0	0.0	0.0
	C>T rs34526305	1	N	150	148	2	0	300	2	0.98	0.013	0.0	0.006
	C>T rs34946978	4	↓	150	149	1	0	300	1	0.99	0.006	0.0	0.003
	G>A↴* rs4148323	1	↓	150	148	2	0	300	2	0.98	0.013	0.0	0.006
GENE	SNP	exon	Func.	TN	WN	HN	MN	AN	AC	WF	HF	MF	AF
ADK	C>A rs397514452	10	-	150	150	0	0	300	0	1	0.0	0.0	0.0
	G>A rs397514454	3	↓	150	150	0	0	300	0	1	0.0	0.0	0.0

5. Discussion

Coronaviruses cause acute respiratory syndrome or pneumonia, and the clinical symptoms of that are different for each person. In some people, it is asymptomatic, and in others, depending on the type of the immune system of their bodies, the symptoms of the virus can be mild to severe. Symptoms may include a sore throat and body aches and in severe cases, fever, cough, and severe respiratory distress (16-18).

Many researchers introduced personalized medicine, which is based on genetics, as a bridge between current medicine and future medicine. Since CYP gene family polymorphisms have an important role in drug metabolism, they have been extensively studied as single or multiple genes in various diseases. In 2017, Zhou et al. studied the prevalence of 176 allelic sites across CYP genes which are involved in phase I of drug metabolism, and the frequency of each allele was determined in five large populations, including African, South Asian, East Asian, and a mix of American communities (10).

Serious treatment problems in the Corona pandemic have led many researchers around the world to pay attention to the pharmacokinetics of drugs. In 2020, Stankovic et al. inspected gene variants affecting the metabolism of proposed drugs in the COVID-19 treatment and introduced 11 markers that are highly relevant in the drug treatment of COVID-19 in the Serbian population (12). In a review study, Takahashi et al. investigated the pharmacogenetics of the proposed drugs in COVID-19 in various databases, such as PubMed, PharmGKB, CPIC, and identified many relevant and effective gene markers in the drug treatment of COVID-19 patients (13-14). Deb et al. studied the potential effects of COVID-19 on drug metabolism in 2021 and emphasized the importance of drug metabolism interactions between COVID-19 and CYP (15). Furthermore, Babayeva et al., in a review study on the use of chloroquine and hydroxychloroquine in the COVID-19 treatment, stated that awareness of pharmacokinetics and pharmacogenomics of these two drugs are necessary to determine the effective and safe dose and prevent treatment failure and severe complications. Although several studies have been performed on the effect of genes on drug metabolism in Iran, only one or two gene polymorphisms have been investigated. Therefore, the present research was conducted with two aims, first focusing on the genes involved in the metabolism pathway of the proposed drugs and secondly investigating a wider range of genes and a large number of SNPs in the COVID-19 treatment (19, 20).

In the present study, according to the results obtained from the number and allelic frequency of each variant, we can have a brief view of the type of drugs used according to the gene profile of individuals in the population (21).

Chloroquine is an old anti-malarial drug, and due to its wide range of antiviral effects, it was one of the first drugs proposed to treat COVID-19 and was approved by the Food and Drug Administration (FDA) on March 28, 2020 (22). However, a few months later there was much discussion about its side effects (14). In plasma, chloroquine binds to serum albumin and glycoprotein a1, and this compound is metabolized by the genes *CYP1A1, CYP2C8, CYP2D6, CYP3A4, CYP3A5,* which are among the genes involved in the metabolism of this drug (23).

Table 3. Relationship between alleles and drugs used in Coronavirus treatment

Drug	Variant	Frequency		Probable function of the enzyme	Tips to be considered
Chloroquine	CYP1A1*2B	8.3%	↑	Chloroquine metabolism may be increased.	Increasing the speed of drug metabolism leads to a reduction in the duration of action of the drug, so it is necessary to pay attention to the time interval between drug usage.
	CYP1A1*4	6%	↑	Chloroquine metabolism may be increased.	Increasing the speed of drug metabolism leads to a reduction in the duration of action of the drug, so it is necessary to pay attention to the time interval between drug usage.
	CYP2C8*2	0.6%	↓	It may lead to decreased metabolism of chloroquine and decrease the favorable response to the drug	-The prolonged presence of high concentrations of the drug in the body may lead to unwanted side effects. -May increase the duration of treatment.
	CYP2C8*3	8%	↓	Chloroquine metabolism and the drug response may be reduced.	-The prolonged presence of high concentrations of the drug in the body may lead to unwanted side effects. -May increase the duration of treatment.
	CYP2C8*3A	8%	↓	Chloroquine metabolism and the drug response may be reduced.	-The prolonged presence of high concentrations of the drug in the body may lead to unwanted side effects. -May increase the duration of treatment.
	CYP2C8*4	5.3%	↓	Chloroquine metabolism and the drug response may be reduced.	-The prolonged presence of high concentrations of the drug in the body may lead to unwanted side effects. -May increase the duration of treatment.
	CYP2D6*2	62%	N	Has normal activity.	
	CYP2D6*4	13%	-	-Chloroquine metabolism and the drug response may be reduced. - The concentration of chloroquine and its active metabolite in plasma may be increased, which leads to increased side effects.	It may increase the concentration of chloroquine and its active metabolite in plasma andThe use of chloroquine in heterozygous and homozygous mutants of this variant may or may not have any effect Or with a very low metabolic rate, it will have little effect with many side effects -The ineffectiveness of the treatment or the increase in the duration of drug treatment lead to increased side effects -Ineffectiveness of treatment or increase the duration of drug treatment.
	CYP2D6*10	17%	-	-Chloroquine metabolism and the drug response may be reduced. -The concentration of chloroquine and its active metabolite in plasma may be increased, which leads to increased side effects.	-The use of chloroquine in heterozygous and homozygous mutants of this variant probably has no effect, or with a very low metabolic rate, will have little effect but many side effects. -Ineffectiveness of treatment or increase in the duration of drug treatment
	CYP2D6*35	4.6%	N	Has normal activity.	
	CYP2D6*41	17.6%	↓	Chloroquine metabolism and the drug response may be reduced.	-The prolonged presence of high concentrations of the drug in the body may lead to unwanted side effects. -May increase the duration of treatment.
	CYP3A4*17	1%	↓	Chloroquine metabolism and drug response may be reduced.	-The prolonged presence of high concentrations of the drug in the body may lead to unwanted side effects. -May increase the duration of treatment.
	CYP3A5*7	0.3%	-	-May reduce chloroquine metabolism and reduce drug response -May increase the concentration of chloroquine and its active metabolite in plasma and lead to increased side effects	-The use of chloroquine in heterozygous and homozygous mutants of this variant probably has no effect, or with a very low metabolic rate, will have little effect but many side effects. -Ineffectiveness of treatment or increase in the duration of drug treatment.
	CYP3A5*3B	0.6%	-	-May reduce chloroquine metabolism and reduce drug response -May increase the concentration of chloroquine and its active metabolite in	-The use of chloroquine in heterozygous and homozygous mutants of this variant probably has no effect, or with a very low metabolic rate, will have little effect but

				plasma and lead to increased side effects	many side effects. -Ineffectiveness of treatment or increase in the duration of drug treatment
Oseltamivir	CES1 rs3826190	38%	↓	-Decreased enzymatic activity may lead to a slowing of drug activation. -Prolonged presence of inactive metabolites in the body	People carrying these gene variants should be considered for the possibility of increased side effects or toxicity as a result of exposure to high concentrations of non-hydrolyzed drugs.
	CES1 rs202121317	1%	↓	-Decreased enzymatic activity may lead to a slowing of drug activation. -Prolonged presence of inactive metabolites in the body	People carrying these gene variants should be considered for the possibility of increased side effects or toxicity as a result of exposure to high concentrations of non-hydrolyzed drugs.
	CES1 rs202121317	0.6%	↓	-Decreased enzymatic activity may lead to a slowing of drug activation. -Prolonged presence of inactive metabolites in the body	People carrying these gene variants should be considered for the possibility of increased side effects or toxicity as a result of exposure to high concentrations of non-hydrolyzed drugs.
	CYP2B6*2	٪۴۸	↓	May lead to decreased lupinavir / ritonavir metabolism and decreased optimal drug response.	Possibility of increasing drug concentration in plasma and causing side effects on the central nervous system.
	CYP2B6*3	19%	↓	May lead to decreased lupinavir / ritonavir metabolism and decreased optimal drug response.	Possibility of increasing drug concentration in plasma and causing side effects on the central nervous system.
	CYP2B6*4	36%	↑	The rate of lopinavir / ritonavir metabolism may be increased.	-The possibility of creating a drug gap due to drug withdrawal in a shorter period of time than the recommended dose. -Need to pay attention to the amount and time of dose
	CYP2B6*5	24%	↓	May lead to decreased ʌopinavir / ritonavir metabolism and decreased optimal drug response.	Possibility of increasing drug concentration in plasma and causing side effects on the central nervous system.
	CYP2B6*9	40%	↑	The rate of lopinavir / ritonavir metabolism may be increased.	-Possibility of drug gap due to drug withdrawal in a shorter period than the recommended dose range. -Need to pay attention to the amount and time of dose.
	CYP2C9*2	12%	-	May lead to decreased lupinavir / ritonavir metabolism and decreased optimal drug response.	-Possibility of dysfunction of the drug. -Need to pay attention to the dose
	CYP2C9*3	9%	-	May decrease metabolism and increase plasma lupinavir / ritonavir concentrations and side effects.	-Possibility of dysfunction of the drug. -Need to pay attention to the dose -Reduced dose in case of reduced function or change of drug in case of non-function.
	CYP2C19*2	13%	-	May decrease metabolism and increase plasma lupinavir / ritonavir concentrations and increase side effects.	-The possibility of dysfunction of the drug. -Need to pay attention to the dose
	CYP2C19*3	2%	↓	May lead to decreased lupinavir / ritonavir metabolism and decreased optimal drug response.	-The possibility of dysfunction of the drug. -May increase the duration of treatment.
	CYP2C19*17	26%	↑↑	Possibility of very fast metabolism of lupinavir / ritonavir and elimination of their effectiveness in a short time	-The possibility of creating a drug gap due to drug withdrawal in a shorter period of time than the recommended dose. -Need to pay attention to the amount and time of dose.
	CYP2D6*2	62%	N	Has normal activity.	
	CYP2D6*4	13%	-	Similar to the effect of the drug chloroquine	
	CYP2D6*10	17%	-	Similar to the effect of the drug chloroquine	
	CYP2D6*35	4.6%	N	Has normal activity.	
	CYP2D6*41	17.6%	↓	Similar to the effect of the drug chloroquine	
	CYP3A4*17	1%	↓	Similar to the effect of the drug chloroquine	
	CYP3A5*7	0.3%	-	Similar to the effect of the drug chloroquine	
	CYP3A5*3B	0.6%	-	Similar to the effect of the drug chloroquine	
	CYP3A7*2C	81%	↑	May increase the rate of lopinavir / ritonavir metabolism.	-The possibility of creating a drug gap due to drug withdrawal in a shorter period of time than the recommended dose. -Need to pay attention to the amount and time of dose.
	CYP3A43*1B	2%	↓	May lead to decreased lupinavir / ritonavir metabolism and decreased optimal drug	-The possibility of dysfunction of the drug. -May increase the duration of treatment.

				response.	
	CYP3A43*2	5%	-	May lead to decreased or no lupinavir / ritonavir metabolism and increased plasma concentrations.	-Need to pay attention to the dosage. -Need to pay attention to the side effects caused by the accumulation of drug concentrations in plasma.
	CYP3A43*3	46%	↓	May lead to decreased lupinavir / ritonavir metabolism and optimal drug response.	-The possibility of dysfunction of the drug. -May increase the duration of treatment.
Atazanavir	CYP1A2*4	0.3%	↓	Similar to the effect of lupinavir	
	CYP2C8*2	0.6%	↓	Similar to the effect of chloroquine	
	CYP2C8*3	8%	↓	Similar to the effect of chloroquine	
	CYP2C8*3A	8%	↓	Similar to the effect of chloroquine	
	CYP2C8*4	5.3%	↓	Similar to the effect of chloroquine	
	CYP2C9*2	12%	-	Similar to the effect of lupinavir	
	CYP2C9*3	9%	-	Similar to the effect of lupinavir	
	CYP3A4*17	1%	↓	Similar to the effect of chloroquine	
	UGT1A1 rs34526305	0.6%	N	Has normal activity	
	UGT1A1 rs34946978	0.3%	↓	Similar to the effect of ritonavir	
	UGT1A1 rs4148323	0.6%	↓	Similar to the effect of ritonavir	

Therefore, variants of these genes can play a role in the pharmacokinetics of this drug. Two variants of * 2B and * 4 *CYP1A1* genes with an allelic frequency of 8.3% and 6% were seen in the cases, both of which increase the rate of enzymatic function, lead to faster metabolism of the drug and reduce the duration of the drug in the body. The results of the allelic frequency study in the *CYP2C8* gene showed the allelic frequency of 0.6% for variant * 2, 8% for variant * 3, 8% for variant * 3A, and 5.3% for variant * 4, all four variants lead to reduced enzymatic function; as a result, they slow down the metabolism of the target drug compared to the normal enzyme. Consequently, if a drug such as chloroquine is used in people with these alleles, the drug is metabolized slowly; therefore, the dose remains in the body for a longer period of time, and the long presence of non-metabolized drug in the body can lead to unwanted side effects (24). A significant effect of reducing the enzymatic effect of *CYP2C8* in * 2 / * * 3 / * 4 variants in treating malaria patients with chloroquine, leading to an increase in the duration of treatment, was reported in 2016 (17). In the treatment of COVID-19 in Africa, patients carrying variant * 2 experienced an adverse response to treatment with chloroquine (12). Therefore, according to the observation of these variants in Iranians, chloroquine should be used with more caution. In the *CYP2D6* gene, variant * 2 had an allele frequency of 62%, variant * 4 allele frequency of 13%, variant * 10 allele frequency of 17%, variant * 35 allele frequency of 4.6%, and variant * 41 had an allele frequency 17%. Meanwhile, although * 2 and * 35 variants have normal enzymatic activity, their association with some cancers has been observed. Variants * 4 and * 10 produce inactive enzymes, and variant * 41 produces enzymes with reduced activity. Therefore, in heterozygous and homozygous mutants of this variant, chloroquine is probably either ineffective or has little effect with very low metabolic rates and many side effects. Variant * 17 of the *CYP3A4* gene, with 1% allelic frequency, produces an enzyme with reduced activity that, by reducing the rate of drug metabolism, can lead to increased side effects of the drug in using chloroquine. Inactive enzyme production of *CYP3A5* * 7 / * 3B variants with disrupted activity can also lead to ineffective treatment with chloroquine. The effect of drug interaction due to enzymatic dysfunction of this variant has been reported in prolonging malaria treatment (17).

Oseltamivir is a neuraminidase inhibitor on the surface of influenza virus. Oseltamivir must be converted to its active state by *Carboxyterase 1* (*CES1*), i.e., Oseltamivir carboxylate (18). Since variants of this gene are effective in the activation of Oseltamivir, so this gene has a high potential to be introduced as a valid biomarker to predict the activation of this drug and optimize it in drug therapy (14). Although the most effective variant is G143E (rs71647871) with inactivated enzyme production, in this study, the frequency of this allele was 0.0%. Moreover, in a study conducted by Zhu et al. in 2009 on different populations, the frequency of this highly efficient variant in Asian populations was also announced as zero percent, which is consistent with the current results. However, the Iranian population is a small part of the Asian population; therefore, in more detailed studies of separate populations, it is possible to see a difference, which was the reason for re-studying this variant in this study focusing on the Iranian population. The other three variants of this gene were rs3826190, with a frequency of 38%, rs202121317 with a frequency of 1%, and rs202001817 with a frequency of 0.6%. All three variants lead to a decrease in enzymatic activity, consequently leading to slowing the activation rate of the drug. Therefore, the inactive drug remains in the body longer, and if the dose is not reduced, the

possibility of accumulation of this drug in the body increases. Therefore, in individuals who carry these gene variants, the possibility of increased side effects or toxicity as a result of exposure to high concentrations of non-hydrolyzed drugs should be considered (25).

Lopinavir and ritonavir are anti-retroviral compounds, and protease inhibitors are used to control human immunodeficiency virus (HIV). Observation of the inhibitory effect of this drug compound on MERS-CoV proliferation made it a promising potential for COVID-19 treatment (14). Atazanavir is also known as an anti-retroviral and protease inhibitor and has been used to inhibit HIV for many years. And its good performance in the SARS-CoV2 treatment made it a suitable option for the COVID-19 treatment. According to the results of the *CYP1A2* gene, variant * 4 has an allele frequency of 0.3%. This means that three out of every 1,000 people face a decrease in the activity of this enzyme, and they should be careful about the dose of the target drugs. These people are more likely to be allergic to medications. In the *CYP2B6* gene, five of the most important variants were examined, which confirmed the results of the presence of these five variants with different frequencies in the Iranian population. Three variants of 2 *, 3 *, and 5 *, which also showed a significant frequency in the Iranian population, lead to a decrease in enzyme activity, which leads to an increase in the concentration of the target drug in the patient's body (14). In the *CYP2C9* gene, variant * 2 had an allele frequency of 12%, and variant * 3 had an allele frequency of 9%. Both variants produce inactive enzymes that, if homozygous, interfere with the metabolism of the target drugs and may cause the drug to malfunction. Hypersensitivity to warfarin has been reported in people with a history of heart disease who have these variants as heterozygous and homozygous mutants. Therefore, genotyping of this gene is effective in determining the dose of the drug (26).

In the *CYP2C19* gene, variant * 2 leads to inactive enzyme production, variant * 3 leads to decreased enzyme activity, and variant * 17 allele frequency 26% leads to enzyme production with increased activity. Therefore, heterozygous and homozygous mutants of the first two variants in the use of these drugs face the problem of metabolic disorders. The drug is metabolized slowly or is not metabolized to the proper extent in the body, so it is possible that they do not have a good functional result from taking the drug. Variant * 17 produces an enzyme ultra-rapid metabolizer, which means that it has a much higher rate of action than the wild-type enzyme, and for example, it can metabolize the drug much faster and destroy the effectiveness of the drug in a short time (27).

The *UGT1A1* gene is one of the most important genes. It is of prime importance to pay attention to its polymorphisms when taking Atazanavir. Enzymes with reduced activity of this gene increase the risk of accumulation of bilirubin (hyperbilirubinemia), the concentration of which is lower in people carrying heterozygous alleles (23). The two variants rs34946978 and rs4148323 with allelic frequencies of 0.3% and 0.6% lead to the production of enzymes with reduced activity, which can increase the concentration of these drugs by slowing down the metabolism of the drugs if the recommended drugs of COVID-19 are used in carriers.

A summary of the relationship between alleles and their function and effect on drug metabolism is provided in separate tables in Appendix 2. The alleles with a frequency of zero, were not seen in any of the cases.

6. Conclusion

All predictions about the performance of drugs are based on the genetic behavior of enzymes and studies that have been done exclusively on genes and enzymes *in vitro*, and a very limited number of them have been studied in clinical trials. Therefore, to achieve the functional reality of drugs, clinical studies are needed, and the results of genetic and clinical studies are necessary to put together. There is no doubt that changes in enzymatic activity in the body occur as a result of SNP changes in genes, but since the metabolism of each drug is affected by several genes, how many polymorphic changes occur in the patient and what functional effect they have in total drug metabolism is a significant issue. On the other hand, each gene in a gene pathway can be affected by many upstream genes to the extent that they can cause functional deficiency of the enzyme caused by SNP and subsequently compensate for or increase the effectiveness of the drug. The cause itself needs to be investigated. Undoubtedly, what can be seen in reality is the effect of the enzymatic change predicted along with hundreds of other effective factors (e.g., interference of other genes, drug interactions). The effectiveness of the drug, all of which lead the study to personalized medicine. That is if before prescribing a drug to a person, it is possible to determine all the SNPs involved in the metabolism of the drug of choice in the individual's genome and examine all the gene pathways that affect the function of the drug in the individual's body, no doubt more power can be. In particular, he predicted the effect of the drug on the person and made a more effective decision in using the type of drug, its amount, and time of use.

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Footnotes

Conflicts of Interest: There is no conflict of interest.

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