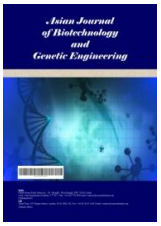

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Determination of Allele Distribution of *CYP1A2* Gene rs762551 Polymorphism on Caffeine Metabolism in Healthy Individuals

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Authors' contributions

This work was carried out in collaboration among all authors. Authors FNC, ED, EP and ÖÖÖ designed the study, managed the literature searches, the analyses of the study and wrote the manuscript. Authors TP, CSD, KU and MK managed the literature searches, performed the statistical analysis and the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the study was to determinate the *CYP1A2* gene rs762551 polymorphism responsible for caffeine in healthy individuals.
Study Design: DNA was isolated from saliva samples taken from healthy individuals. Analysis of A and C allele distribution of *CYP1A2* gene rs762551 polymorphism was performed by amplifying DNA regions from individuals.
Place and Duration of Study: It was carried out between February 2019 and April 2020 in Üsküdar University Medical Genetics and Molecular Diagnosis Laboratory.
Methodology: Thirty healthy individuals without age, gender, height and weight restrictions were included in our study. DNA analysis was performed on the Real-Time PCR device by taking saliva samples from individuals.

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Results: The genotype distribution of this study was 13 people (43.33%) had AA, 9 people had AC (30%) and 8 people have a CC genotype (26.67%) respectively. According to the results of the study, individuals with the AA genotype are in the majority, but since there are more individuals with the C allele, those who metabolize caffeine slowly are in the majority. In our study, statistical analysis was not performed because it was aimed only to determine the allele gene distribution.
Conclusion: Studies show an association between caffeine and disease. However, the genetic reasons for this relationship have not been fully understood yet. Therefore, more studies are needed on larger samples of genes that metabolize caffeine. Caffeine-related diseases can be prevented by detecting variations on caffeine genes of healthy individuals with more studies in the future.

Keywords: Caffeine intake; Genotyping; CYP1A2; Rs76255; Healthy individual.

ABBREVIATIONS

The Cytochrome P450 (CYP1A2)
Single nucleotide polymorphism (SNP)
Real Time Polymerase Chain Reaction (RT-PCR)

1. INTRODUCTION

Approximately 80% of the world's population consume caffeine (1,3,7-trimethylxanthin) daily. Caffeine is found in a variety of foods and beverages such as cola, tea, coffee, chocolate and energy drinks [1]. The caffeine content of 240 ml of food and drink is indicated in Table 1 in mg form. <400 mg / day caffeine intake is recommended for healthy individuals, but it is difficult to adjust the amount since it is found in most foods [2]. 200-500 mg of caffeine can cause headache, irritability and tachycardia. 750 mg of caffeine can cause serious metabolic effects [3]. The lethal dose is 5000 mg, which corresponds to 41 cups of coffee [4]. Chlorogenic acid in coffee may cause a decrease in glycemic response as a result of high caffeine intake in a single dose [5]. Especially, in pregnancy caffeine intake is a condition that needs the most attention. It is recommended not to intake much caffeine during the pregnancy. Maximum 200 mg of caffeine per day is suitable for pregnant women [6].

Caffeine is metabolized in the liver [7]. The Cytochrome P450 (*CYP1A2*) enzyme is responsible for > 90% of caffeine metabolism. The metabolism of a cup of coffee reaches its peak in 30-60 minutes. The effect takes 3-5 hours to complete [8]. However, studies show that this period may vary according to individuals.

The metabolic rate caused by genetic variations is usually not known. It can be taken higher than the dose of caffeine that can be metabolized and many chronic diseases can be triggered. The most common single nucleotide polymorphism (SNP) for caffeine metabolism rate is *CYP1A2* gene rs762551 polymorphism [10] which has two allele genes such as A (fast variable) and C (slow variable) allele. Caffeine is over-metabolized when the 'A' allele is taken from parents. Caffeine is slowly metabolized when the 'C' allele is taken from parents an in Table 2. Gene distribution in the general population is on average 40% AA, 50% AC and 10% CC [11]. While moderate caffeine intake provides protection against disease in individuals with AA genotype, it can cause diseases in individuals with AC and CC genotypes. People with AC and CC genotype may experience diabetes, heart attacks, and high blood pressure due to caffeine intake [7,11]. The incidence of these diseases is high in Turkey. Caffeine intake with tea and coffee has been high in Turkey. Therefore, high caffeine intake is thought to be associated with an increase in disease [12]. AC and CC allele carrier have the risk of diseases in Fig. 1 as a result of high caffeine intake than recommendations.

Caffeine replaces the adenosine receptor to prevent sleep and performance degradation. The adenosine receptor stimulates the central nervous system, causing fatigue and sleep [13]. *CYP1A2* gene is located in the 15q24.1 chromosome region of DNA, contains seven exons and six intron regions [14]. The *CYP1A2* gene rs762551 polymorphism shows a high correlation with caffeine metabolism, and individuals with this polymorphism may be exposed to the adverse effects of caffeine [15]. The incidence of hypertension is increasing in all over the world. Hypertension, which was observed approximately 2 times more than women compared to men, was 15.8% in general in 2016, and reached 16.4% in women and men according to 2019 results. The prevalence of

diabetes has been increasing in the world population since many years. Diabetes, which is observed more frequently in women compared to men, reached 10.2 by almost doubling in 2019, while it was 5.9% in 2008. Unlike hypertension, diabetes and myocardial infarction (heart attack) which has been observed more in men, has increased in recent years, but its incidence is not as high as other diseases mentioned. When the results of the last 10 years are evaluated, it was seen at 1.9% in the society in 2008, while it increased to 2.1% in 2019 [16].

The purpose of this cohort study is to determine *CYP1A2* gene rs762551 polymorphism in healthy individuals. We suggest more genotyping studies to help presentation of diseases that may develop due to caffeine intake in healthy individuals.

Table 1. Caffeine content of food and beverages

Caffeinated products (240 ml)	Caffeine (mg)	Reference
Decaffeinated	3	[9]
Instant coffee	75	[9]
Brewed coffee	85	[9]
Espresso	320	[9]
Green tea	40	[9]
Black tea	40	[9]
Coca-Cola	24	[9]
Red Bull	77	[9]

Table 2. Effect of allele genes

Allele Gene	Effect	Reference
AA	It rapidly metabolizes caffeine. It improves performance and provides moderate intake, disease prevention.	[7,11]
AC CC	It slows metabolizes caffeine. There are studies that show that it reduces performance. It can cause diabetes, heart attacks, and high blood pressure.	[7,11]

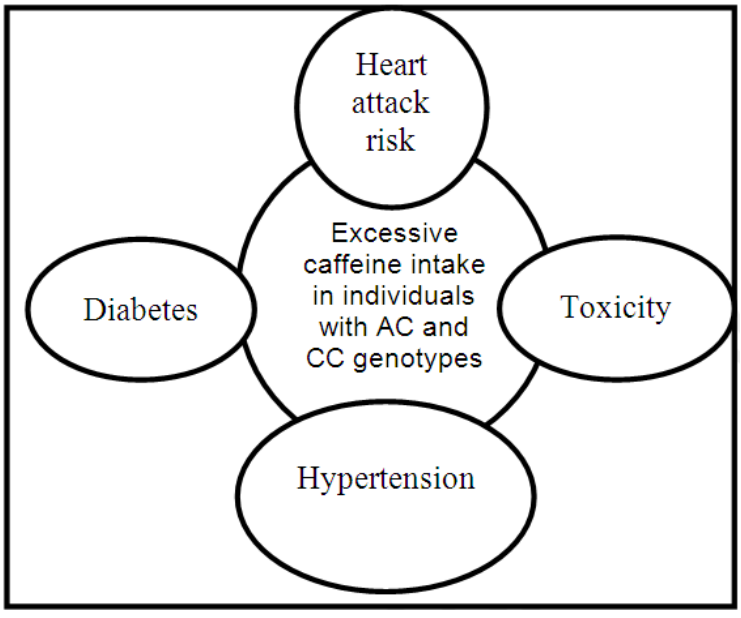

Fig. 1. Diseases that may occur as a result of excessive caffeine intake in individuals with AC and CC alleles [7,11]

2. MATERIALS AND METHODS

2.1 Study

The limitation of our study is 30 people. It was conducted with 30 healthy individuals without age, gender, weight and height limitation.

2.2 Genotype Analysis

DNA samples were collected by DNA collector swap and isolated with DNA purification kit (Thermo Fisher Invitrogen), by following manufacturer's instructions. The rs762551 genotyping was carried out by Real-Time Polymerase Chain Reaction (RT-PCR) by using commercially provided Taqman Genotyping Assay (Catalog no: #4362691 Thermo Fisher, USA), by using specific primers (Table 3).

A 10 µl mixture of 4.2 µl distilled water, 5 µl Master Mix kit and 0.5 µl template DNA, in an 0.5 mL Eppendorf tube was used in reproducing the *CYP1A2* rs762551 allele region. Performed during Real Time- PCR and the pre-denaturation process was performed for 10 minutes at 95 °C.

It was followed by the denaturation process at 95 °C for 15 seconds to ensure that the DNA strands separated from each other. The reading was performed in 60 seconds by bringing the temperature to 60 °C. Fluorescein Amidity (FAM ™) and HEX probes in a Master Mix were used to determine genotype distributions. For the Rs762551 allele region, HEX luminescence identified the 'A' allele, and FAM luminescence identified the 'C' allele.

3. RESULTS AND DISCUSSION

In our study group, 13 people (43.33%) with the AA genotype, 9 people with AC genotype (30%) and 8 people with CC genotype (26.67%) were detected in rs762551 polymorphism. Allele frequencies are 35 (58.33%) with the A allele and 25 (41.57%) with the C allele. Table 4 shows the effect of genotypes on caffeine metabolism. In our study, statistical analysis was not performed because it was aimed only to determine the allele gene distribution.

In our study, 13 people metabolize caffeine faster, while 17 people metabolize caffeine slowly. Genotype distribution in *CYP1A2* gene rs762551 polymorphism was 40% of AA, 50% of AC and 10% of CC carriers [14]. It was focused on rs762551 polymorphism studies due to there was not only caffeine genotype study in the literature. AC and CC genotypes have been shown together in studies (C allele). However, the limitation of our study is 30 people. Therefore, more studies are needed for healthy individuals.

The individuals with AC genotype according to coffee intake had high levels of glucose release after meals and decreased insulin sensitivity [17] which can lead to type 2 diabetes mellitus (type 2 DM) [18]. Although the increase in glucose was not observed much in the AA genotype, it was found that glucose levels returned to normal two hours after the meal. Robertson et al. applied a liquid mixed meal tolerance test for people who consume chronic coffee after caffeine intake. As a result of the study, individuals with the AA genotype had a high postprandial glucose response and low free fatty acid concentration. Also, Robertson et al. conducted a liquid meal tolerance test on people who consumed coffee for 12 weeks for insulin levels too. There was no change in insulin levels in individuals with the AA genotype [17].

Guest et al. found that AA genotype was 49%, AC genotype was 43% and CC genotype was 8% in 101 cyclists *CYP1A2* gene rs762551 polymorphism. As a result, individuals with the AA genotype improved their performance, cyclists in the AC and CC genotypes decreased in performance after a certain amount of caffeine [11]. Genotyping studies are partly similar to our study. Klein et al. reported that the distribution of tennis players genotyping did not affect tennis performance [19]. Yücesoy studied the *CYP1A2* gene rs762551 polymorphism in a study involving 20 short and long runners. As a result of the study, AA, AC and CC genotype distribution is 20%, 35% and 45%, respectively [7]. Güneş et al. conducted studies with 146 people from investigate the difference of *CYP1A2* gene rs7622551 polymorphism activity by smoking, gender, age. 13% of the participants had the AA genotype and 87% had the C allele [20]. Arıcı and Özhan investigated *CYP1A2* gene rs7622551 polymorphism activity in smokers and non-smokers. 48% of the participants in the study have AA and 52% C alleles. As a result, *CYP1A2* enzyme activity was higher in smokers than non-smokers [21]. Song et al. investigated the relationship between bladder cancer and *CYP1A2* gene rs762551 polymorphism. Song et al. investigated the relationship between bladder cancer and *CYP1A2* gene rs762551 polymorphism. 212 bladder cancer patients and

200 healthy groups participated in the study. The cancer group had 23.11% of the AA genotype and 76.89% of the C allele. In the healthy group, there were 8.5% people with AA genotype and 77.75% people with C allele. According to the results, there was a significant relationship between having AA genotype and bladder cancer risk, while those carrying the C allele were not found [22]. Contrary to the study of Song et al., Altaylı et al. reported that 20% with AA genotype and 80% with C allele were found in bladder cancer patients [23].

Studies have been conducted between caffeine and various diseases. For instance, cardiovascular disease rises with artery lipid and calcium accumulation. When the relationship between cardiovascular disease and caffeine was examined, it has been found that 40-60% of those who drink at least 5 cups of coffee a day compared to those who do not drink cardiovascular disease risk [24]. Chlorogenic acid in coffee prevents the formation of fibrils, which are frequently seen in type 2 DM. Studies have shown that coffee consumed after a meal prevents fibril formation [25].

Caffeine is useful for metabolic syndrome [26]. The reason may be related to the fact that caffeine lowers triglyceride levels [27]. Studies on fasting glucose causing metabolic syndrome attract attention. The relationship between caffeine consumption with *CYP1A2* gene and impaired fasting, glucose risk has been studied in individuals with hypertension aged 18-45 years. A total of 1180 non-diabetic patients participated.

Table 3. Primers used for the genotyping of rs762551

Genomic DNA Region	DNA Sequence (5'→3')
CYP1A2 (rs762551)	TGCTCAAAGGGTGAGCTCTGTGGGC (C Allele)
	CAGGACGCATGGTAGATGGAGCTTA (A Allele)

Table 4. The effect of genotypes on caffeine metabolism

Participant	Rs762551	The Speed of Caffeine Metabolizing
1	AA	Rapid
2	AA	Rapid
3	AA	Rapid
4	AA	Rapid
5	AA	Rapid
6	AA	Rapid
7	AA	Rapid
8	AA	Rapid
9	AA	Rapid
10	AA	Rapid
11	AA	Rapid
12	AA	Rapid
13	AA	Rapid
14	AC	Heterozygous Slow
15	AC	Heterozygous Slow
16	AC	Heterozygous Slow
17	AC	Heterozygous Slow
18	AC	Heterozygous Slow
19	AC	Heterozygous Slow
20	AC	Heterozygous Slow
21	AC	Heterozygous Slow
22	AC	Heterozygous Slow
23	CC	Homozygous Slow
24	CC	Homozygous Slow
25	CC	Homozygous Slow
26	CC	Homozygous Slow
27	CC	Homozygous Slow
28	CC	Homozygous Slow
29	CC	Homozygous Slow
30	CC	Homozygous Slow

74% of the participants consumed low coffee and 87% consumed moderate level (1-3 cups / day) and 13% of high caffeine consumer (3 cups / day). Considering the results, having C allele and drinking high coffee was more risk of fasting glucose than other groups [28]. Ohnaka et al. compared the group that consumed coffee for sixteen weeks and the group did not consume coffee. As a result, the postprandial glucose response of coffee consumers decreased [29]. In a study conducted with type 2 DM patients, the group consuming 4-6 cups of coffee per day and individuals drinking 6 cups of coffee per day were compared. Six cups of coffee consumers had the lowest risk for developing Tip2 DM [27]. It is stated that overdose caffeine intake causes diseases [30]. Consequently, the common point in caffeine intake in relation with the disease was the caffeine dose and gene polymorphism [30,31].

4. CONCLUSION

In conclusion, the C allele carrier, which is associated with diseases, metabolizes caffeine slowly, was found to be more in our study. The *CYP1A2* gene rs762551 polymorphism has generally been investigated in patients and athletes, so it should contribute to the prevention of diseases by increasing the number of studies conducted in healthy individuals.

CONSENT

As per international standard or university standard, respondents' written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

All procedures performed in studies involving human participants complied with the ethical standards of the institutional and / or national research committee and the 1964 Helsinki Declaration and its subsequent amendments or comparable ethical standards. The study was approved by Üsküdar University Ethics Committee (No. 61351342- / 2019-115) with the Permission of the Non-Interventional Ethics Committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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