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Genetic Association of *CYP1A2* Variant (rs762551) with Caffeine Induced-Hypertension Susceptibility and Subject Protein Analyses

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ABSTRACT

Hypertension affects a large proportion of Pakistan's population, with prevalence above 46% in both urban and rural areas. Caffeine has multiple cardiovascular effects. Acutely, adenosine receptor antagonism can increase blood pressure, but chronic consumption might prevent it. *CYP1A2*, a liver enzyme responsible for metabolizing more than 95% of caffeine by demethylation, has genetic variability, with the rs762551 variant (-163 C>A) affecting activity. **Objectives:** To investigate the association between the rs762551 variant and caffeine-induced hypertension susceptibility in a Pakistani population, and summarize important features of the *CYP1A2* protein. **Methods:** A case-control genotyping study was conducted with 48 participants, including 24 hypertensive and 24 controls. Genomic DNA was extracted and genotyped by ARMS-PCR. The Hardy-Weinberg Equilibrium (HWE) was calculated using chi-square and odds ratio with 95% CI. An in-silico study was also conducted to examine the structure and function of *CYP1A2* protein. **Results:** Genotypic frequencies within cases: 11 (AA), 12 (AC), and 1 (CC), controls: 5 (AA), 16 (AC), and 3 (CC). Allele frequencies within cases: A=0.71 and C=0.29, and in controls: A=0.54 and C=0.46. HWE (χ^2=2.82, p=0.093). The C-allele was not significantly associated with hypertension (OR=0.49, 95 % CI 0.21–1.13; χ^2=2.18, p=0.14). In-silico studies confirmed that the *CYP1A2* gene encodes a microsomal liver enzyme involved in caffeine demethylation. **Conclusions:** No significant association between the *CYP1A2* rs762551 was found in this cohort. More extensive studies, including lifestyle data, are needed to understand gene-environment interactions.

INTRODUCTION

According to the American Heart Association, hypertension is defined as an average systolic blood pressure of ≥ 130 mmHg or an average diastolic blood pressure of ≥ 80 mmHg [1]. An age-adjusted hypertension prevalence of 46.2 %, with similar rates in urban (44.3 %) and rural (46.8 %) populations, was reported by The National Diabetes Survey of Pakistan (2016–2017) [2]. Genetic and environmental factors play an important role in blood pressure regulation [3]. Caffeine is a popular stimulant that causes transient increases in blood pressure through the blockade of adenosine receptors, but does not cause sustained pressor responses; habitual consumption may even lower cardiovascular risk [4-6]. Caffeine is rapidly absorbed and almost entirely metabolized in the liver. The cytochrome P450 enzyme *CYP1A2* accounts for approximately 13 % of liver P450 activity and facilitates more than 90 % of caffeine demethylation [7, 8]. The rs762551 (–163 C>A) variant, or *CYP1A2**1F, is an intronic substitution associated with decreased inducibility and reduced enzyme activity, leading to slower caffeine clearance [9-11]. Individuals with the (CC) or (AC) genotypes may experience prolonged effects of caffeine and elevated risk of adverse cardiovascular outcomes, while carriers of the (AA)

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genotype clear caffeine rapidly [12]. Caffeine's cardiovascular effects are complex. Acute caffeine ingestion is an antagonist for the adenosine receptors and transiently increases blood pressure, but chronic coffee ingestion does not increase blood pressure and may have the opposite effects [13, 14] (Figure 1).

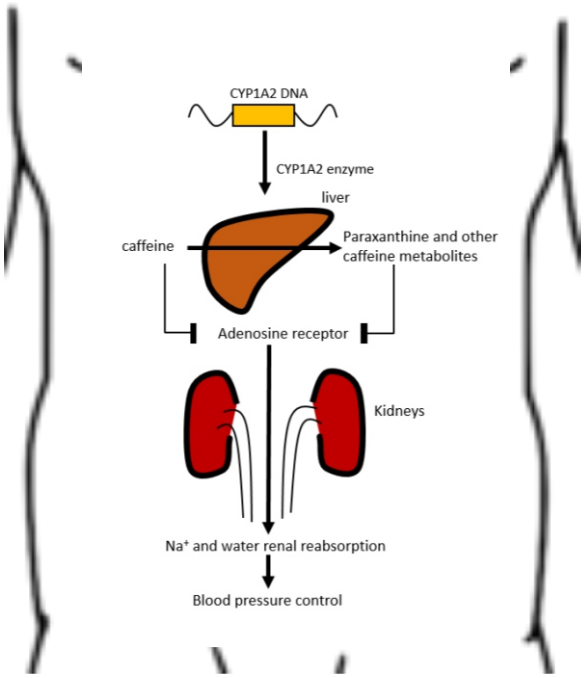

Figure 1: Pathophysiology of Caffeine Metabolism Mediated by *CYP1A2*

Observational studies have suggested that consumption of coffee may lower the risk of hypertension among C allele carriers. However, the relationship between the rs762551 polymorphism and blood pressure remains unclear, particularly in South Asian populations [15]. Hypertension is a major health concern in Pakistan, yet the role of genetic factors, particularly the *CYP1A2* rs762551 polymorphism, remains underexplored. Understanding how this enzyme variant influences hypertension could provide insights into population-specific risk. This study aims to investigate the relationship between *CYP1A2* rs762551 polymorphism and hypertension in the Pakistani population while outlining the enzyme's key functional attributes.

METHODS

Caffeine is metabolized primarily by the hepatic enzyme *CYP1A2*, which demethylates caffeine into paraxanthine. The *CYP1A2* gene is located on chromosome 15q24.1, and the rs762551 (15:74749576C>A) variant is an intronic variant (Figure 2).

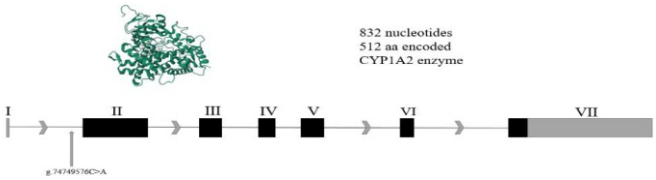

Figure 2: *CYP1A2* Gene Locus and Crystal Structure

A case-control study was started from Nov, 2022 to April 2023 at Decode Genomics Lahore, Pakistan. Forty eight unrelated individuals were selected for this resource-limited pilot-scale study, consisting of 24 cases of primary hypertension and 24 controls. Beforehand, informed consent was obtained from each individual and approval of the study was given by the ethics committee. Blood of 1mL quantity was collected in EDTA tubes and stored at 4ºC until further use. Genomic DNA was extracted using a TIANGEN Genomic DNA extraction kit following the manufacturer's protocol. Furthermore, Genotyping of the variant of interest was performed by amplification refractory mutation system (ARMS)-PCR. This specific PCR technique utilizes allele-specific primers to amplify the target allele and differentiates mutant and wild-type alleles by incorporating a mismatch at the 3′ end [16]. Primer sequences and properties are listed in table 1.

Table 1: ARMS-PCR Primer Sequences and Properties

Primer Label	Sequence (5'-3')	bp	GC (%)	TM (ºC)	Product (bp)
Forward common	CCTCTACTCCAGCCCCAGAA	20	60%	59.13ºC	151
Reverse wild	CCATCTACCATGCGTCATGG	20	55%	59.23ºC	
Reverse mutant	CTCCATCTACCATGCGTCATGT	22	50%	59.85ºC	
Forward IC	TAACCCACAGCCTCCTACAC	20	55%	60.50ºC	618
Reverse IC	TCAGCATCCTCCTCTGGAC	20	55%	60.50ºC	

The reaction mixture consisted of 5 µL of 2X master mix, 0.5 µL of the two allele-specific primers at a concentration of 0.2 µM each, 1 µL of genomic DNA (~50 ng), and nuclease-free water in the required volume. The thermal cycling conditions for the reaction are described in (Figure 3).

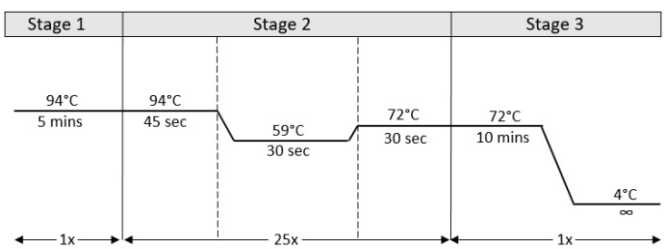

Figure 3: Thermal Cyclic Conditions of ARMS-PCR Reaction

Allele and genotype frequencies were calculated using the PLINK data analysis toolset. Hardy-Weinberg equilibrium was measured using a Chi-Square test. Differences in allele frequencies and association between cases and controls were checked by Chi-Square tests, and odds ratios (ORs) with 95 % confidence intervals (CIs) were used to estimate the association of the C allele with hypertension. All

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relevant analyses were performed using PLINK software. Statistical significance was denoted by a p-value of < 0.05. The *CYP1A2* amino acid sequence (NP_000752) was downloaded from the UniProt database. Physicochemical properties, secondary structure, motif predictions, and post-translational modification sites were examined using ProtParam, PsiPred, MotifFinder, and other tools. Protein-protein interactions were explored using the STRING tool. Only key findings relevant to enzyme activity are discussed here.

RESULTS

Genotypic and allelic frequencies of the *CYP1A2* gene variant rs762551 were calculated for all 48 study participants (Table 2).

Table 2: *CYP1A2* rs762551 Genotypes and Allele Frequencies Among Cases and Controls

Genotype Frequencies	Cases (n=24)	Controls (n=24)	Statistical Test
AA	11	5	OR=0.49 (95% CI 0.21–1.13)
AC	12	16	
CC	1	3	
Allele frequencies			
A Allele	0.71	0.54	
C Allele	0.29	0.46	

Among hypertensive cases, 11 individuals were homozygous (AA), 12 were heterozygous (AC), and 1 was homozygous (CC). In controls, 5 individuals were (AA), 16 were (AC), and 3 were (CC). Allele frequencies were 0.71 and 0.29 in cases and 0.54 and 0.46 in controls for A and C, respectively. The control group was in accordance with the Hardy–Weinberg equilibrium (χ^2=2.82, p=0.093). Differences in allele frequencies between cases and controls were not significant (χ^2=2.18, p=0.14), and the C allele showed an insignificant protective trend against hypertension (OR=0.49, 95 % CI 0.21–1.13) (Table 3).

Table 3: Hardy–Weinberg Equilibrium Assessment in the Control Group

Tests	Results
Hardy–Weinberg equilibrium (controls)	χ^2=2.82, p = 0.093
Allelic association test	χ^2=2.18, p = 0.14

The gel electrophoresis technique was utilized to resolve PCR products in the case and control groups (Figure 4).

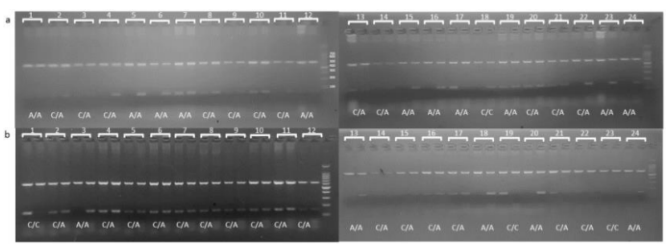

Figure 4: ARMS-PCR Products of the rs762551 Variant in 50 Samples, a) Cases, b) Controls

ProtParam analysis predicted that the *CYP1A2* protein consists of 516 amino acids, with a molecular weight of 58.4 kDa and an isoelectric point of 9.18. The protein is classified as an intracellular microsomal enzyme, anchored to the endoplasmic reticulum (Table 4).

Table 4: Physicochemical Properties of Wild-Type CYP1A2 Protein

Physicochemical Properties	Wild-Type
Amino acids	516 aa
Molecular weight	~58 kDa
Theoretical PI	9.18
Molecular formula	$C_{2652}H_{4144}N_{718}O_{763}S_{17}$
Number of atoms	8267
Negative charged residues (Asp + Glu)	49
Positive charged residues (Arg + Lys)	59

The secondary structure of the protein comprises several alpha helices and beta sheets, as is characteristic of the cytochrome P450 family. The protein consists of a P450 domain, as predicted by motif analysis (Table 5).

Table 5: Motif Prediction of *CYP1A2* Protein

pfam ID	Independent E-value (Position)	Description
P450	42..492(6.8e-104)	PF00067, Cytochrome P450

The STRING analysis is a dense interaction network with other drug metabolism enzymes, indicating an average node degree of 8.18 (PPI enrichment $p < 1 \times 10^{-11}$). The proteomic analysis was not disclosed, as it was not considered relevant to the genetic association (Figure 5).

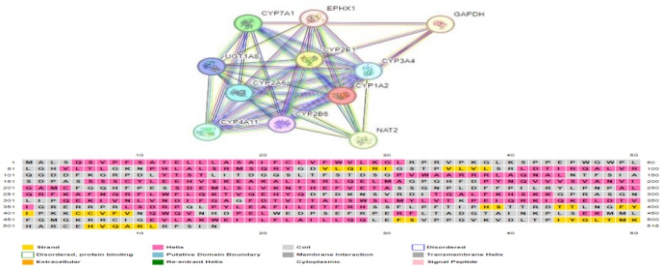

Figure 5: a) Protein-Protein interaction of *CYP1A2*, b) Transmembrane helices in *CYP1A2* protein

DISCUSSION

This study investigated the association between the *CYP1A2* rs762551 polymorphism and hypertension susceptibility in a Pakistani population. The frequencies of genotypes and alleles were similar in hypertensive patients and normotensive controls, and the C allele showed a non-significant trend towards protection. These results are consistent with a population-based study conducted in Taiwan, where an inverse relationship was observed between coffee consumption and hypertension, although rs762551 did not have a significant impact [15]. The lack of association observed in the present study may be due to the limited sample size and ethnic diversity of the cohort.

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CYP1A2 is the major liver enzyme involved in the demethylation of caffeine and is responsible for over 90% of caffeine metabolism [8]. The intronic rs762551(–163 C>A) variant affects inducibility, consequently lowering enzyme activity and caffeine clearance [17]. Thus, in individuals carrying the C-allele variant, the metabolism of caffeine is slower, whereas in homozygous AA carriers, the metabolic capacity is high [18]. Gene-environment interactions likely play a role in influencing caffeine's impact on blood pressure [19]. The nominal cohort study suggested that coffee intake is correlated with a decreased risk of hypertension, especially in individuals who possess the AC + CC genotype [12]. Other studies have found that individuals who are slow metabolizers of caffeine, who regularly take in huge quantities of coffee (more than 4 cups a day), may be at an increased risk of myocardial infarction, whereas those who are fast metabolizers may be protected [8, 20].

This present study has several limitations. Due to the exploratory nature of the study, the sample size was relatively small (24 cases and 24 controls), which may reduce the statistical power to detect modest genetic associations. Therefore, there is a possibility of a Type II error. Moreover, there was a lack of information regarding caffeine consumption, smoking habits, and environmental factors. Only one variant of the *CYP1A2* gene was investigated; however, variations in the *CYP1A2* or in the AHR and ADORA2A genes may contribute to caffeine metabolism and blood pressure [21]. All the subjects were from a single center, and therefore, the results may lack generalizability. Large-scale studies from many centers with subjects from various ethnic backgrounds are required for the validation of these results. It is proposed that in the future, a larger sample size, comprehensive lifestyle evaluations, and gene-environment interactions examinations be included in the studies. Cohort studies examining the effects of caffeine consumption, blood pressure response, and genotypes would help to elucidate whether rs762551 influences the blood pressure response to caffeine. Whole-genome studies would aid in identifying other genes in the caffeine metabolism pathway that contribute to hypertension.

CONCLUSION

In summary, the current case-control study failed to demonstrate a significant association between the *CYP1A2* rs762551 gene variant and the risk of hypertension in the Pakistani population. The effect of the C allele showed a non-significant protective effect; however, this understanding is limited by the small sample size. It is a known fact that caffeine metabolism in the body is carried out mainly by the enzyme *CYP1A2*, and genetic variability in the gene plays a significant role in the way the body responds to caffeine. Our results underscore the need for assessing the effects of gene-environment interaction and emphasize the need for further investigation into the effects of polymorphisms in the *CYP1A2* gene on hypertension.

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Authors' Contribution

Conceptualization: RS
Methodology: GA
Formal analysis: RS, MGH
Writing and Drafting: GA, RS
Review and Editing: GA, RS, MGH

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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