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Pharmacogenomic Study of Selected Genes Affecting Amlodipine Blood Pressure Response in Patients with Hypertension

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Introduction: Despite the availability of various antihypertensive medications, the response to these medications varies among individuals. Understanding how individual genetic variations affect drugs treatment outcomes is a key area of focus in precision medicine. This study investigated the correlation between single nucleotide polymorphisms (SNPs) in selected genes (CACNA1C, CACNA1D, ABCB1, ACE, ADBR2, and NOS1AP) and the blood pressure (BP) control by amlodipine.

Methods: Four hundred individuals of Pashtun ethnicity undergoing amlodipine treatment for hypertension were included in the present study and divided into the controlled (BP less than 140/90 mmHg) and uncontrolled (BP greater than 140/90 mmHg) hypertension groups. Blood samples (3 mL) were collected from each participant, and DNA was extracted using the Kit method. Ten SNPs in amlodipine pharmacogenes were selected and genotyped using real-time PCR with the TaqMan® system. Logistic regression model was used to determine the association between SNPs and the amlodipine BP response.

Results: Notable association were observed between SNP rs2239050/CACNA1C and amlodipine blood pressure response, with GG genotype carriers demonstrating a better response (P=0.004) than individuals carrying CC or CG genotypes. SNP rs312481/CACNA1D also exhibited a positive pharmacogenetic association, Individuals with the GG genotype showing a considerable reduction in BP (P=0.021) compared to participants with AA or GA genotypes. In case of SNP rs429/ACE individuals carrying TA genotype were less likely to achieve BP control (P=0.002) than AA genotype carriers.

Conclusion: Our finding suggests that the SNPs rs2239050/CACNA1C, rs312481/CACNA1D and rs429/ACE influence amlodipine blood pressure response in patients with hypertension. It is recommended that prior knowledge of amlodipine associated pharmacogenetic variants is important that could improve its treatment outcomes in hypertensive patients.

Keywords: pharmacogenomics, hypertension, genetic markers, personalized medicine, amlodipine, Pashtun, Pakistan

Introduction

Hypertension (HTN) is a major public health issue, when left untreated leads to the development of various disorders like cardiovascular diseases, renal failure and premature death.[1] The global burden of HTN has rapidly increased over the past decades, especially in low- and middle-income countries.[2,3] According to the World Health Organization (WHO) global report on hypertension (1990–2019) the HTN prevalence and incidence among adults has increased twofold, rising from 650 million in 1990 to 1.3 billion in 2019. The surge in the number of hypertensive cases is more frequent in the South Asian and Western Pacific regions than in the European and American populations.[4,5] The prevalence of hypertension in Pakistan, a developing, middle-income south Asian nation, is alarmingly high and requires immediate

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medical attention. National Health Survey of Pakistan on hypertension prevalence reported that 46.2% of adult Pakistani population is affected by hypertension and this number is escalating rapidly.[6] Because of its growing mortality rate and lack of its initial sign and symptoms, it is recognized as one of the high prevalent chronic non-communicable diseases globally, earning the moniker of a "silent killer".[7] The key players contributing to high prevalence of hypertension are lack of exercise/physical activity, smoking, increased sodium intake, air pollution and rapid urbanization.[4] In addition to factors such as poor lifestyle and various environmental factors, it has been documented that genetic factors (like Gene polymorphisms/ mutation in core genes) also play a significant role in both the onset of HTN and treatment response to anti-hypertensive medications.[8,9] Pharmacogenomic research studies as of the present study aim to elucidate how variations in different genes influence the response to antihypertensive medications. The ultimate goal is to develop pharmacotherapy based on individual genetic makeup aiming for personalized treatment strategies.

Different medications are used in blood pressure regulation; these include Diuretics, Beta Receptor Blocker (B-blockers), Calcium Channel Blockers (CCBs) Angiotensin Receptor Blockers (ARBs), direct vasodilators and Angiotensin-Converting Enzymes Inhibitors (ACEIs).[10,11] Calcium-Channel Blockers (CCBs) are commonly prescribed for hypertension treatment because CCBs controls high blood pressure effectively compared to other classes of antihypertensive medications.[12] Amlodipine, a third-generation long-acting calcium channel blocker has demonstrated good efficacy in regulating elevated (high) blood pressure (BP) and has shown decreased risk of cardiovascular diseases in individuals with hypertension.[13] Nonetheless, the BP lowering response to amlodipine varies significantly and several pharmacogenomic studies explored potential genetic polymorphisms that explain the observed differences among individuals and populations.[14–16] These investigations analyzed polymorphisms in genes directly implicated in the Pharmacokinetics and Pharmacodynamics of amlodipine. Genetic polymorphisms in genes that encode ion channels (such as CACNA1C, CACNA1D, GNB3, TANC2, ADRB2, and ADRA1A) have been documented to alter the response to CCBs (such as amlodipine) by affecting their transport.[17] For instance, a significant association between the CACNA1C variant rs2238032 and amlodipine treatment outcomes has been observed. Patients carrying the TT genotype showed positive treatment outcomes and those carrying the G allele showed negative treatment outcomes with amlodipine. Likewise in case rs2239050/ CACNA1C, individuals with GG genotype had better BP control with amlodipine compared to the individuals with CG genotype.[18]

Polymorphisms in the ABCB1 (a member of ATP-binding cassette (ABC) transporters) and CYP3A5 (a family member of cytochrome P450 enzyme) genes have been documented to influence the elimination and/or clearance of amlodipine.[19] In the case of ABCB1, subjects carrying the TT genotype have an increased rate of amlodipine clearance compared to those carrying the CT or CC genotype.[20] Whereas in case of CYP3A5 gene, CYP3A5*3/*3 genotype carriers showed reduced plasma amlodipine concentrations compared to CYP3A5*1 genotype carriers.[21] Moreover, single nucleotide polymorphisms located within genes indirectly associated with the pharmacokinetics and antihypertensive action of amlodipine, such as AGT (Angiotensinogen) and ACE (angiotensin-converting enzyme) genes have been investigated.[22] Both Angiotensinogen and angiotensin-converting enzyme play pivotal roles as an integral part of the renin-angiotensin system (RAS), which regulates BP by modulating fluid volume within the body.[23] The variant rs4291 (of ACE gene) showed a significant correlation with the development of high BP;[22] nevertheless, its direct link to the BP-lowering response of amlodipine remains unclear. A study conducted in an African American population documented that, in patients and subjects receiving amlodipine therapy, the presence of rs11122576 in the AGT gene demonstrated a lower risk of coronary heart disease. However, its association with BP regulation by amlodipine has not been documented/established.[24] In addition, SNP rs10494366, located at the NOS1AP (nitric oxide synthase-1-adaptor protein) gene, was found to be associated with an increased risk of developing cardiovascular events in amlodipine users. Additionally, if rs1042713/ADRB2 SNP is present, cardiovascular drugs may show limited effectiveness.[25–27]

Given the multifaceted nature of HTN and the intricate physiological regulatory systems affecting its severity and management, it is crucial to study polymorphisms that are either directly or indirectly involved in pathways linked to the antihypertensive effects of pharmacological drugs. This will improve our understanding of the complex physiology of drug response outcomes in hypertensive individuals. Currently, there is limited information and data on specific SNPs that could affect the outcomes of hypertensive therapy in the Pakistani population. The present pharmacogenomic study in the Pashtun population of Pakistan aimed to explore polymorphisms in amlodipine-related genes and to evaluate their

relationship with BP regulation. The Pakistani population is categorized into five major tribes/ethnic groups: the Sindhis, Baluchis, Punjabis, Muhajirs, and Pashtuns. Among these, Pashtuns have unique genetic makeup and distinct cultural practices, societal beliefs, and behaviors owing to these characteristics, making them the most suitable for such a pharmacogenomic study.

Materials and Methods

Study Subject Enrolment

A total of four hundred individuals of Pashtun ethnicity, taking amlodipine (for last 12 months) for hypertension treatment were included in the study. The enrolled participants were categorized into two groups: a) Un-controlled hypertensive patients (un-controlled HTN, n=200); and b) Controlled hypertensive patients (controlled HTN, n=200). Controlled hypertensive patients were marked/identified as individuals who were prescribed amlodipine (alone or in combination with any other antihypertensive medication) and maintained a mean arterial blood pressure of < 140/90 mmHg. Uncontrolled hypertensive patients included those who were prescribed amlodipine (alone or in combination with any other antihypertensive medication) but had a mean arterial blood pressure of ≥ 140/90 mmHg.

Study participants were selected from different districts in Khyber Pakhtunkhwa (KP), including Peshawar, Swabi, Charsadda, Bannu, Mardan, Nowshera, Dir, Swat, and Kohat. The study participants were enrolled from the cardiac care units of three large teaching- and research-based (tertiary care) hospitals (HMC), the Lady Reading Hospital (LRH), and Khyber Teaching Hospital (KTH). These hospitals offer special care and treatment for individuals with hypertension and other cardiovascular conditions. Written informed consent or an agreement form (that shows the wellness to be included in the study) was signed and obtained from all study subjects. For uneducated patients, the agreement form (or consent form) was verbally explained to them in local Pashtu language for ease of understanding. After agreeing to participate in the study and adhering to the terms and conditions, the agreement form was either signed by patients or by their attendant/relative on their behalf.

Demographics and Clinical Data Collection

The demographic and detailed clinical data of the study participants were gathered using a proforma specially designed for this study. The information collected included: sex; age; district from which the patients belonged; smoking status; exercise level; presence or absence of comorbidities; socioeconomic status; drug and dietary compliance; prescribed medications for hypertension comprised amlodipine alone or in combination other antihypertensive medications like enalapril, losartan, and hydrochlorothiazide, or any other drug; glomerular filtration rate; triglyceride levels; BMI; urea and creatinine levels; blood glucose levels; and some other variables with potential influence on hypertension development.

Inclusion and Exclusion Criteria

Study participants of Pashtun ethnicity, age between 20 to 80 years, using calcium channel blocker (the amlodipine) for blood pressure control from the past 12 months were included in the study whereas individuals who were bedridden, pregnant, or experiencing mental illness, chronic diseases such as HIV or Hepatitis were excluded from the study.

Blood Samples Collection

With the help of a skilled and trained nurse, whole blood samples were collected from all the study participants using aseptic procedures. Blood samples were taken from the median cubital vein. Each participant contributed three millilitres (3 mL) of whole blood drawn into EDTA tubes that were carefully labeled to facilitate proper tracking and identification of the samples. Following blood collection and labelling, EDTA tubes (filled with 3 mL blood) were stored at −10 °C, until further testing or analysis.

DNA Extraction and Quantification

For DNA extraction, two hundred (200) microliters (μL) of whole blood was used. The Wizard Genomic DNA Extraction Kit (model no. W64120) was utilized for this purpose, carefully following the manufacturer's instructions provided with the kit. Subsequent to the successful extraction of DNA, DNA was quantified using a Qubit™3 (Cat. No. Q34860). Finally DNA concentration was adjusted to 5 ng/μL.

Selection of Amlodipine Pharmacogenes

Ten known pharmacogenetic variants previously linked to blood pressure response to amlodipine, including rs2239050/CACNA1C, rs2238032/CACNA1C, rs312481/CACNA1D, rs3774425/CACNA1D, rs3774426/CACNA1D, rs2032582/ABCB1, rs4291/ACE, rs1799752/ACE, rs1042713/ADBR2, and rs10494366/NOS1AP, were carefully selected. The selection process involved consulting reliable sources, such as the Pharmacogenomics Knowledge Base (PharmGKB) and PharmacoGenomic Mutation Database (PGMD)[28,29] and conducting a comprehensive review of recent literature. Our focus was directed towards genes situated within pathways, either directly or indirectly, affecting the mechanism through which amlodipine lowers blood pressure.

Genotyping

Genetic polymorphism analysis (genotyping) of the selected pharmacogenetic variants was carried out using Real-Time Polymerase Chain Reaction (real-time PCR) with the TaqMan® SNP Genotyping Assay system (Applied Biosystems - Thermo Fisher Scientific). In brief, the reaction mixture consisted of 5 microlitre of TaqMan® Master Mix and 0.5 microlitre of working reagent (primer/probe), resulting in a total of 5.5 microlitre of reagent per well. DNA samples along with controls were diluted with Nuclease-free water to attain a concentration of 10 ng/μL per well. Subsequently, 5.5 microlitre of the previously prepared reagent and 4.5 microlitre of the diluted sample were added to each well of the MicroAmp™ 96-well optical reaction plate, resulting in a total volume of 10 μL per well. Next the plate was sealed with adhesive tape and centrifuged at 1000 rpm before processing on real–time PCR machine. Allelic discrimination data analysis was conducted using Applied Bio-systems/Thermo Scientific 7500 v2.3 software.

Statistical Analysis

All statistical analyses and tests were performed using the IBM SPSS version 25 (Statistical Package for Social Sciences, V25). The key variables considered for analysis included age, sex, triglyceride levels, BMI, co-administered or current medications, smoking status, districts from where the study subjects belonged, occupation, exercise level, lifestyle, diet, and specific genetic variants in CACNA1, CACNA1D, ABCB1, ACE, ADBR2, and NOS1AP. To identify genetic variants confirmatory of the Hardy-Weinberg equilibrium (HWE), the chi-square (χ^2) test was employed. To examine the allelic and genotypic frequencies differences between the uncontrolled and controlled AH groups, the χ^2 test was utilized. The correlation or association between alleles, genotypes, and BP response to amlodipine was tested using a logistic regression analysis. The effects of confounding factors on this association were assessed using an adjusted logistic regression model. Statistical significance was set at $p < 0.05$.

Results

Description of the Study Cohort

Sociodemographic characteristics, biochemical parameters of the study subjects, and the prevalence of comorbid conditions are given in Tables 1–3. The present study recruited 400 individuals age > 20 years, consisting of 200 individuals with controlled hypertension and 200 individuals with uncontrolled hypertension. Both the groups were age- and weight-matched.

In the controlled-HTN group, 75.5% of patients were males and 23.5% were females. District-wise, 25.5% of individuals were from district Peshawar, 22.5% from district Charsadda, 17.5% from district nowshera, 13 individuals (6.5%) from district Mardan, and a similar number of individuals from district Mardan were from district Karak. The occupation-wise majority (27.5%) was attached to farming. Moreover 85.0% showed family history of HTN whereas

Table 1 Sociodemographic Features of Study Subjects Under Investigation

Variables	Controlled HTN n(f)	Uncontrolled HTN n(f)	P-value
Gender			0.221
Male (M)	153 (76.5%)	145 (72.5%)	
Female (F)	47 (23.5%)	55(27.5%)	
Mean age (yrs)	54 ± 13:43	56 ± 13:40	0.805
Mean weight (Kg)	60.55 ± 8:32	62.64 ± 6:07	0.913
Address			0.328
Peshawar	50 (25.0%)	55 (27.5%)	
Charsadda	44 (22.0%)	34 (17.0%)	
Mardan	13 (6.5%)	22 (11.0%)	
Kohat	11 (5.5%)	12 (6.0%)	
Swabi	4 (2.0%)	19 (9.5%)	
Nowshera	35 (17.5%)	17 (8.5%)	
Bannu	10 (5.0%)	18 (9.0%)	
Karak	13 (6.5%)	05 (2.5%)	
Dir	10 (5.0%)	06 (3.0%)	
Swat	10 (5.0%)	12 (6.0%)	
Occupation			0.098
Business	16 (8.0%)	20 (10.0%)	
Govt. servant	28 (14.0%)	37 (18.5%)	
Retired	30 (15.0%)	35 (17.5.0%)	
Farming	55 (27.5%)	35 (17.5%)	
House wife	35 (17.5%)	40 (20.0%)	
Labor	36 (18.0%)	33 (16.5%)	
Family Hx of HTN			0.031
Yes	170 (85.0%)	135 (67.5%)	
No	30 (15.0%)	65 (32.5%)	
Marital status			0.138
Single	43 (21.5%)	61 (30.5%)	
Married	157 (78.6%)	139 (69.5%)	
Smoking			0.073
Yes	140 (70.0%)	104 (52.0%)	
No	60 (30.0%)	96 (48.0%)	

(*Continued*)

Table 1 (Continued).

Variables	Controlled HTN n(f)	Uncontrolled HTN n(f)	P-value
Naswar (smokeless tobacco product)			0.061
Yes	163 (81.5%)	130 (65.0%)	
No	47 (18.5%)	70 (35.0%	
Socioeconomic status			0.524
Good	24 (12.5%)	22 (11.0%)	
Average	153 (76.5%)	132 (66.0%)	
Below	23 (11.5%)	46 (23%)	

Table 2 Prevalence of Other Diseases Among Study Participants in Addition to HTN

Name of Co Morbid Disease	Frequency (f)		P-value
	Controlled HTN	Uncontrolled HTN	
Type 2 diabetes	12.5%	22.0%	0.051
IHD	19.0%	21.0%	0.611
Kidney Failure	4.00%	6.00%	0.912
Retinopathy	9.1%	23.0%	0.012
HBV	0.00%	0.00%	NA
HCV	0.00%	0.00%	NA

Abbreviations: HCV, Hepatitis C virus; HBV: Hepatitis B virus; IHD: Ischemic heart disease.

Table 3 Clinical Features/Characteristics of the Participants Under Investigation

Variables	Controlled HTN n(f)	Uncontrolled HTN n(f)
Total cholesterol (mg/dL)		
Normal	90 (45.0%)	94 (47.0%)
Changed	110 (55.0)	106 (53.0%)
LDL- cholesterol (mg/dL)		
Normal	36 (18.0%)	30 (15.0%)
Changed	164 (82.5%)	170(85.0%)
HDL- cholesterol (mg/dL)		
Normal	133 (66.5%)	148 (74.0%)
Changed	67 (33.5%)	52 (26%)
Triglycerol (mg/dL)		
Normal	102 (51.0%)	96 (48.0%)
Changed	98 (49.0%)	104 (52.0%)

(*Continued*)

Table 3 (Continued).

Variables	Controlled HTN n(f)	Uncontrolled HTN n(f)
Urea (mg/dL)		
Normal	183 (91.5%)	173 (86.5%)
Changed	17 (8.5%)	27 (13.5%)
Creatinine (mg/dl)		
Normal	187 (93.5%)	180 (90.0%)
Changed	13 (6.5%)	20 (10.0%)
HBA1C (%)		
Normal	179 (89.5%)	182 (91.0%)
Changed	21 (10.5%)	18 (9.0%)
Amlodipine alone	28 (14.0%)	42 (21.0%)
Amlodipine + 1 drug	44 (22.0%)	58 (29.0%)
Amlodipine + 2 drugs	91 (45.5%)	62 (31.0%)
Amlodipine + 3 drugs	36 (18.0%)	22 (11.0%)

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein; mg/dL, milligrams per deciliter; mg, milligrams.

15.0% replied "NO" when asked for family history of HTN. Seventy percent (70%) were smokers and 30% were non-smokers. Drug and diet compliance was good in the majority (71.0%) of individuals, whereas 29.0% of the study participants showed poor drug and dietary compliance. Considering the socioeconomic status majority (76.5%) were from average-income families and 11.5% were from poor families.

In case of uncontrolled-HTN group, 72.5% of the patients were males and 27.5% were females. Fifty-five participants (27.5%) were from Peshawar district, 17.0% were from Charsadda district, and a few participants (2.5%) were from Karak district. Occupation-wise, 18.5% were government servants, 20.0% among the female candidates were housewives, 10.0% were attached to business, and thirty five participants (67.5%) had a family history of hypertension, whereas the remaining 32.5% were documented to have no family history of hypertension. The majority (66.0%) of the patients belonged to middle-income families, whereas 23.0% had poor family backgrounds.

Comorbidities (Type 2 diabetes, renal failure, hypercholesterolemia, and retinopathy) were more prevalent in the uncontrolled HTN group compared to control group (Table 2). Similarly, lipid profiles (including triglycerides, total cholesterol, HDL-cholesterol, and LDL-cholesterol), renal function markers (urea and creatinine), and glycemic profiles were assessed in both groups. The detailed results are provided in Table 3.

Expression of Selected Pharmacogenetic Variants in the Study Participants

The study participants were screened for absence or presence of n=10 single nucleotide polymorphism associated with amlodipine response namely rs2239050, rs2238032, rs312481, rs3774425, rs3774426, rs2032582, rs4291, rs1799752, rs1042713, and rs10494366 using Real time PCR machine. Among the selected SNPs, seven variants (rs2239050, rs312481, rs3774425, rs3774426, rs4291, rs1042713, and rs10494366) were identified in the target population whereas the other three SNPs were not found in the study population. SNPs identified in the target population were in Hardy–Weinberg equilibrium (HWE). Details of the expressed and unexpressed SNPs in the study population are listed in Table 4.

Table 4 Expressed/Un-Expressed Selected Single Nucleotide Polymorphisms (SNPs) in the Study Participants

Gene	SNP	Status	Controlled HTN n(f)	Uncontrolled HTN n(f)
CACNA1C	rs2239050	Present	175 (87.5%)	135 (67.5%)
		Absent	35(17.5%)	65(32.5%)
CACNA1C	rs2238032	Present	–	–
		Absent	200(100%)	200(100%)
CACNA1D	rs312481	Present	172(86%)	180 (90%)
		Absent	28 (14%)	20 (10%)
CACNA1D	rs3774425	Present	98 (49%)	121 (60.5%)
		Absent	102 (51%)	79(39.5%)
CACNA1D	rs3774426	Present	105(52.5%)	112(56%)
		Absent	95(47.5%)	88 (44%)
ABCB1	rs2032582	Present	–	–
		Absent	200 (100%)	200 (100%)
ACE	rs4291	Present	165 (82.5%)	146 (73%)
		Absent	35 (17.5%)	54 (27%)
ACE	rs1799752	Present	–	–
		Absent	200 (100%)	200 (100%)
ADBR2	rs1042713	Present	112(56%)	132 (66%)
		Absent	88 (44%)	68 (34%)
NOS1AP	rs10494366	Present	116(58%)	98 (49%)
		Absent	84(42%)	102 (51%)

SNPs and Their Association with Amlodipine Response

The influence of alleles and genotypes on the amlodipine-induced blood pressure control was observed using unadjusted and adjusted logistic regression models. A strong association between amlodipine response and the SNP rs2239050/ CACNA1C was observed in the study participants. Participants carrying the GG genotype had better response/outcomes (P=0.004) when treated with amlodipine than carriers of CC or CG genotypes. After adjusting for confounding factors (age, sex, drug and diet compliance, etc)., no observable changes were noticed in the degree, level, or magnitude of the association. In the case of rs312481/CACNA1D, crude logistic regression analysis showed a positive pharmacogenetic association between SNP rs312481 and amlodipine response; individuals carrying the GG genotype showed a notable reduction in blood pressure (p=0.021). When confounding factors were considered, the association between SNP rs312481 and blood pressure regulation by amlodipine remained consistent, that is, the covariates/confounding factors showed no additive or opposing effect on the association. In the case of SNP rs429/ACE, individuals with the TA genotype were less likely to achieve blood pressure control (or have un-controlled hypertension) than those with the AA genotype. The remaining SNPs (rs3774425/ CACNA1D, rs3774426/ CACNA1D, rs1042713/ ADBR2, and rs10494366/ NOS1AP) showed no considerable influence on amlodipine response. The detailed results are presented in Table 5.

Table 5 The Influence of Alleles and Genotypes on Amlodipine Produced Blood Pressure Response Using Unadjusted and Adjusted Regression Models

Genotypes	Controlled HTN (n=200) n(f)	Un-controlled HTN (n=200) n(f)	Unadjusted Odds Ratios (95% CI)	p-value	Adjusted Odds Ratios (95% CI)	p-value
rs2239050/ CACNA1C						
CC	67 (33.5%)	70 (35%)	1	–	1	–
CG	27 (13.5%)	33 (16.5%)	1.12 (0.61–2.96)	0.163	1.37 (0.64–2.01)	0.831
GG	**106 (53%)**	**97 (48.5%)**	**4.69 (2.51–25.01)**	**0.004**	**3.05 (2.76–20.08)**	**0.006**
Allele C	86 (43%)	75(37.5%)	1	–	1	–
Allele G	114(57%)	125(62.5%)	1.5 (0.60–3.76)	0.431	1.5 (0.53–4.34)	0.122
rs312481/ CACNA1D						
AA	117(58.5%)	129(64.5%)	1	–	1	–
AG	26(13%)	36(18%)	1.3 (0.57–4.01)	0.155	1.5 (0.71–4.96)	0.093
GG	**57(28.5%)**	**35(17.5%)**	**2.91(1.34–4.97)**	**0.021**	**2.01 (1.12–5.01)**	**0.024**
Allele A	98(49%)	105(52.5%)				
Allele C	102(51%)	95(47.5%)	0.32 (0.08–1.31)	0.282	0.92 (0.42–1.44)	0.227
rs3774425/ CACNA1D						
GG	91(45.5%)	89(44.5%)	1	–	1	–
AG	44(22%)	38(19%)	2.4 (0.95–6.10)	0.341	2.9 (1.96–7.08)	0.812
AA	65(32.5%)	73(36.5%)	0.8 (0.31–1.81)	0.213	0.7 (0.37–1.99)	0.191
Allele G	102(51%)	110(55%)	1	–		–
Allele A	98(49%)	90(45%)	0.9 (1.10–3.05)	0.086	1.4 (0.52–2.13)	0.761
rs3774426/ CACNA1D						
CC	132(66%)	140(70%)	1	–	1	–
CT	22(11%)	16(8.0%)	0.3 (0.36–2.44)	0.113	0.5 (0.33–2.74)	0.101
TT	46(23%)	44(22%)	0.29 (0.19–1.92)	0.314	0.82 (0.29–1.33)	0.411
Allele C	135(67.5%)	141(70.5%)	1	–	1	–
Allele T	65(32.5%)	59(29.5%)	0.63 (0.16–2.06)	0.112	0.57 (0.34–1.43)	0.081
rs4291 /ACE						
AA	72(36%)	65(32.5%)	1	–	1	–
TA	**82(41%)**	**102(51%)**	**3.27 (1.15–5.72)**	**0.002**	**2.99 (1.17–5.81)**	**0.005**
TT	46(23%)	33(16.5%)	0.93 (0.46–1.09)	0.272	0.35 (0.25–1.46)	0.281

(*Continued*)

Table 5 (Continued).

Genotypes	Controlled HTN (n=200) n(f)	Un-controlled HTN (n=200) n(f)	Unadjusted Odds Ratios (95% CI)	p-value	Adjusted Odds Ratios (95% CI)	p-value
Allele A	122(61%)	114(57%)	1	–	1	–
Allele T	78(39%)	86(43%)	1.12 (0.73–2.41)	0.601	1.02 (0.72–4.32)	0.420
rs1042713/ ADBR2						
GG	88(44%)	79(39.5%)	1	–	1	–
GA	97(48.5%)	91(45.5%)	1.58 (0.91–4.01)	0.514	1.26 (0.66–2.24)	0.332
AA	15(7.5%)	30(15%)	1.44 (0.36–6.14	0.726	0.83 (0.36–3.91)	0.644
Allele G	56(28%)	76(38%)	1	–	1	–
Allele A	144(72%)	124(62%)	1.71 (0.79–3.11)	0.862	1.01 (0.27–3.42)	0.566
rs10494366/ NOS1AP						
TT	66(33%)	70(5%)	1	–	1	–
GT	102(51%)	114(57%)	1.55 (1.02–5.01)	0.661	0.99 (0.52–4.28)	0.472
GG	32(16%)	16(8.0%)	1.63 (0.250–3.44)	0.540	1.24 (0.26–4.36)	0.391
T	101(50.5%)	110(55%)	1	–	1	–
G	99(49.5%)	90(45%)	1.29 (0.55–2.72)	0.326	1.09 (0.58–2.95)	0.227

Discussion

Amlodipine (a calcium channel blocker) is commonly prescribed for hypertension treatment. However, the outcomes of amlodipine treatment vary significantly from person to person, primarily due to genetic differences.[13,30] Considering genetic factors while prescribing anti-hypertensive medications would lead to the concept of personalized medicine that would help to address the rising incidence of hypertension. Pharmacogenes associated with amlodipine responses have been understudied in the Pakistani population. Owing to the high prevalence of HTN in Pakistan and the lack of pharmacogenomic data regarding anti-hypertensive drugs, the present study is carefully designed to examine the co-relation between ten selected genetic biomarkers/variants and blood pressure response to amlodipine therapy. Among the studies SNPs (n=10), seven SNPs (rs2239050, rs312481, rs3774425, rs3774426, rs4291, rs1042713, and rs10494366) were detected or reported or expressed in the Pashtun ethnic population whereas SNP rs1799752, rs1042713, and rs2238032 were not expressed within the study population. This may be attributed to the limited number of participants enrolled in the study. Studies with larger sample sizes tend to exhibit higher statistical power, which enables the detection of rare and common variants. The identified SNPs within the study population adhered to the Hardy–Weinberg equilibrium (HWE).

We reported a significant correlation between amlodipine response and SNP rs2239050 (located in the intron region of CACNA1C). Troy et al reported same association (SNP rs2239050×amlodipine treatment response) previously in Caucasian subjects.[18] Our study findings suggest that individuals with GG (homozygous) genotype had significantly better response (un-adjusted odd-ratio (95% CI) = 4.69 (2.51–25.01 and crude P=0.004) to amlodipine treatment compared to the individuals with CC or CG genotypes. When adjusted for confounding factors/covariates, no observable changes (adjusted odds ratio (95% CI) = 3.05 (2.76–20.08), P=0.006) were observed in the degree, level, or magnitude of the association. No considerable changes in unadjusted, adjusted Odd ratios (ORs), confidence intervals (Cl), and P-values suggest that the study subjects (individuals with uncontrolled hypertension and individuals with controlled

hypertension) are closely matched in terms of age, sex, and other related confounding factors. CACNA1C encodes a protein that forms the alpha-1C subunit of the L-type voltage-gated calcium channel, which plays an integral role in regulating calcium influx into cardiac and vascular smooth muscle cells.[31,32] Amlodipine (a calcium channel blocker), acts on this channel to lower blood pressure.[33,34] Studies have identified connections between CACNA1C gene variants and amlodipine response.[18,35] These findings imply that CACNA1C gene variants may affect an individual's reaction to amlodipine, potentially influencing its effectiveness and side-effect profile. Thus, CACNA1C holds promise as a pharmacogenetic biomarker for tailored approaches to hypertension treatment.

Regarding rs312481/CACNA1D, the initial crude logistic regression analysis indicated a positive pharmacogenetic association between rs312481 and amlodipine treatment response. Individuals with the GG genotype demonstrated a significant independent reduction in blood pressure (unadjusted odds ratio (95% CI)=2.91(1.34–4.97), p=0.021). After adjusting for confounding variables, the association between SNP rs312481 and blood pressure regulation by amlodipine remained consistent (adjusted odds ratio (95% CI)= 2.01 (1.12–5.01), P=0.024). This suggests that the covariates and confounding factors have neither an additive nor opposing effect on the observed association. However, further research is needed to gain a comprehensive understanding of the relationship between rs312481 and amlodipine response. The SNP rs312481 is located within the CACNA1D gene, which encodes the alpha-1D subunit of the L-type voltage-gated calcium channel in humans.[36,37] Amlodipine, a calcium channel blocker, interacts with this subunit as a part of its mechanism of action. The rs312481 polymorphism involves a C to T substitution and is located in intron 3 of CACNA1D. Studies have identified the rs312481G>A polymorphism as being associated with variations in response to amlodipine, suggesting potential implications for pharmacokinetics or pharmacodynamics that could explain these response differences.[12,16]

In the case of SNP rs4291/ACE, the TA genotype (rs4291) was independently and potentially linked to uncontrolled hypertension (unadjusted odds ratio(95% CI)= 3.27 (1.15–5.72, P=0.002) during amlodipine treatment. When adjusted for confounding factors (like age, gender and diet/drug compliance) the magnitude and direction of this association/correlation remained consistent (adjusted odd ratio (95% CI) = 2.99 (1.17–5.81), P=0.005). Individuals carrying the TA genotype (rs4291) showed a decreased response to amlodipine compared with individuals with the AA genotype. A study conducted in South African Adult population with Hypertension documented similar findings/results as we noticed in the present study.[35] The ACE gene and renin-angiotensin-aldosterone system (RAAS) are intricately linked components of body regulatory mechanisms, particularly in the maintenance of blood pressure and electrolyte balance. ACE encodes the angiotensin-converting enzyme (ACE), which plays a pivotal role in the RAAS pathway. This pathway regulates diverse physiological processes; for example, it regulates the BP, inflow, and outflow of electrolytes, and maintains fluid homeostasis.[38,39] Overall, ACE is an important biomarker for hypertension and related cardiovascular diseases.[23,40,41] Pharmacogenomic study of this ACE gene will help devise anti-hypertensive therapies according to the genetic makeup of the individual.

Furthermore, the present study finds no association between rs3774425/CACNA1D, rs3774426/CACNA1D, rs1042713/ADBR2, and rs10494366/NOS1AP and the blood pressure response to amlodipine. These nucleotide polymorphisms were investigated to determine their potential roles in the therapeutic outcomes of amlodipine. Despite the biological plausibility that variations in these genes may affect calcium channel function (CACNA1D), beta-adrenergic receptor activity (ADBR2), and nitric oxide signaling (NOS1AP), our findings did not demonstrate any significant correlation. This lack of association suggests that these genetic variants do not modify the efficacy of amlodipine in lowering blood pressure in hypertensive individuals. Our results indicate that the rs3774425, rs3774426, rs1042713, and rs10494366 genotypes are not reliable biomarkers for predicting patient responses to amlodipine therapy. This insight is crucial for clinicians and researchers focusing on personalized medicine and pharmacogenomics, as it highlights the need to explore other genetic factors or mechanisms that may contribute to variability in drug responses, thereby improving the precision and effectiveness of hypertension treatment strategies.

Conclusion

In this Pharmacogenomic study, we examined the influence of various alleles and genotypes on blood pressure regulation by amlodipine using unadjusted and adjusted logistic regression models. Our findings revealed a potential postive

association between the SNP rs2239050/CACNA1C and the efficacy of amlodipine in lowering blood pressure. Specifically, participants with the GG genotype demonstrated good response (P=0.004) compared with those carrying the CC or CG genotypes.

Similarly, the SNP rs312481/CACNA1D showed a positive pharmacogenetic association with the amlodipine response. Individuals with the GG genotype exhibited a lowering in blood pressure (P=0.021), and this association persisted after controlling for potential confounding variables, suggesting a stable genetic influence on drug response.

Conversely, for SNP rs429/ACE, individuals with the TA genotype were less likely to achieve blood pressure control than those with the AA genotype, highlighting a potential negative impact on treatment efficacy. In contrast, the SNPs rs3774425/CACNA1D, rs3774426/CACNA1D, rs1042713/ADBR2, and rs10494366/NOS1AP did not significantly affect the blood pressure response to amlodipine.

These findings underscore the importance of considering genetic biomarkers to predict patient response to amlodipine treatment. The observed associations suggested that genetic testing for these SNPs could potentially guide personalized hypertension treatment strategies and enhance therapeutic outcomes. Further research is warranted to validate these results and uncover the underlying pathways/mechanisms involved in these associations.

Study Limitation

The study limitation in includes small sample size and targeting only Pashtun ethnic population. We adjusted the results for major confounding factors however other factors like lifestyle and non-hypertensive may have affect on the study outcomes. Future studies with larger cohorts and exploration of gene-environment interactions are needed to provide deeper insights into amlodipine pharmacogenomics.

Data Sharing Statement

All the necessary data and information are provided in the manuscript. The corresponding author can be contacted for any additional data or information related to this article.

Ethical Statement

This study was approved by the members of institutional research board District Head Quarter Hospital (approval number 247/Cl.Phar, dated 17/10/2021). All experiments and procedures were performed in accordance with the ethical guidelines established by the Declaration of Helsinki (1975).

Informed Consent Statement

Informed consent was obtained from all the participants.

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Disclosure

The authors report no conflicts of interest in this work.

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