

Association of *CYP3A5* Gene Polymorphisms and Amlodipine-Induced Peripheral Edema in Chinese Han Patients with Essential Hypertension

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Background: Amlodipine is one of the most used members of calcium channel blockers (CCB), available to treat hypertension. It is mainly metabolized by the Cytochrome P450 3A4/5 (*CYP3A4/5*) in the liver. Peripheral edema emerges as the major adverse drug reaction to amlodipine and is the primary reason for discontinuation of amlodipine therapy. However, genetic changes in *CYP3A5* may lead to changes in the tolerability of amlodipine.

Purpose: In this study, we were interested whether variants in *CYP3A5* have a role to play in amlodipine-induced peripheral edema.

Methods: A total number of 240 Chinese Han patients that have experienced hypertension were included in the study. Sixty-four patients had experienced amlodipine-induced peripheral edema, while the remaining 176 patients with no history of edema formed the control group. Twenty-four single-nucleotide polymorphisms (SNPs) of *CYP3A5* gene were sequenced by targeted region sequencing method. The relationship of these genetic variants with amlodipine-induced peripheral edema risk was assessed using logistic regression.

Results: The allele frequencies of *CYP3A5**1D (rs15524), *CYP3A5**1E (rs4646453) and *CYP3A5**3 (rs776746) were significantly different between cases and controls ($P < 0.05$). The *CYP3A5* *3/*3 (CC) or *CYP3A5* *1D/*1D (AA) carriers showed an increased risk of amlodipine-induced peripheral edema in dominant model. Meanwhile, patients carrying *CYP3A5* *1E (AC/AA) showed a reduced risk of peripheral edema. Furthermore, we found a strong linkage disequilibrium among rs15524, rs4646453 and rs776746.

Conclusion: Our study reveals for the first time that *CYP3A5* *1D, *1E and *3 were associated with amlodipine-induced peripheral edema in Chinese Han patients with hypertension. However, further studies comprising larger number of samples, more related genes and other factors are wanted.

Keywords: pharmacogenomics, amlodipine, edema, *CYP3A5*, SNP

Introduction

Calcium channel blockers (CCBs) are widely used in the treatment of hypertension. In addition to their antihypertensive effects, CCBs may also lead to many adverse reactions, including peripheral edema, dizziness, flushing, fatigue, headache, palpitations, and gingival enlargement.¹⁻⁴ Peripheral edema, particularly of lower limbs, is the most common side effect of CCBs. CCBs-induced peripheral edema is more common in women and is related to age, upright posture and duration of CCBs' therapy.^{2,5} Although it has been known for decades that CCBs could cause peripheral edema, there are still very few genetic markers that could be used in clinical treatment.

Amlodipine is a first-line antihypertensive drug and a long-acting 1,4-dihydropyridine calcium channel blocker.⁶ It became the fifth most prescribed medication in the United States in 2018.⁷ However, therapy trials report that the incidence of amlodipine-induced peripheral edema is about 19%, which also probably serves as the main reason behind the limited use of this drug.^{8,9} To our knowledge, factors that increase the serum concentration of drugs may also increase the risk of side effect. Accordingly, genetic factors affecting the metabolism of amlodipine will consequently influence its clearance, thus possibly resulting in peripheral edema. In recent years, numerous studies have focused on identifying genetic factors determining the responses to amlodipine therapy, including aspects of efficacy and safety.^{10–12} Nevertheless, the genetic predisposition to amlodipine-induced peripheral edema remains unclear. However, some studies shine light on CYP genes, which are involved in the metabolism of amlodipine.^{13,14}

CYP3A is a subfamily of cytochrome P450 and is involved in the metabolism of many compounds.^{10,15–17} CYP3A4 and CYP3A5 are the two major enzymes of CYP3A family and play important roles in the dehydrogenation of amlodipine.¹⁸ However, it has been reported that *CYP3A4*, rather than *CYP3A5*, plays a more important role in amlodipine clearance in vivo.¹⁶ However, according to our observations, almost all SNPs of *CYP3A4* are very rare in Chinese Han or East Asian.^{19–21} A recent study evaluated the influence of *CYP3A* polymorphisms on the pharmacokinetic (PK) parameters of 10 CYP3A substrates including amlodipine, and it turned out *CYP3A4* polymorphisms did not show a pronounced influence on PK of amlodipine.²² So, as far as we know, *CYP3A4* does not suffice as an explanation towards the incidence of amlodipine-induced adverse responses. Additionally, the contribution of *CYP3A5* in amlodipine metabolism is still controversial. *CYP3A5* expression varies among individuals due to gene polymorphisms that result in non-productive mRNA splicing and lower or undetectable expression of the protein.^{14,23,24} Pharmacogenomics studies have found that *CYP3A5* gene polymorphisms have significant impact on drug metabolism, efficacy, and toxicity.^{25,26} Recent studies have reported a possible relationship between *CYP3A5* and amlodipine. *CYP3A5**3/*3 genotypes might be associated with blood pressure response to amlodipine,²⁷ and *CYP3A5**3 may affect the disposition of amlodipine.¹³ In Chinese hypertensive patients who underwent renal

transplantation, researchers have demonstrated that *CYP3A5**3 polymorphism affects the antihypertensive efficacy of amlodipine. In such patients with *CYP3A5**3/*3, the reduction in diastolic blood pressure (DBP) was significantly higher than those with other genotypes.²⁸ Additionally, compared with *CYP3A5**1/*1, *CYP3A5**1/*3 patients exhibit a lower metabolic ratio of amlodipine in vivo and hypertensive patients with *CYP3A5**1/*3 genotypes show a higher decrease in blood pressure.¹⁴ These findings suggest that *CYP3A5* genetic variants are really involved in amlodipine response, having an impact on treatment effectivity of amlodipine.

Although studies have identified that *CYP3A5* polymorphisms do play a role in blood pressure control and treatment responses of amlodipine, the effect of a particular genotype in amlodipine-induced peripheral edema remains unclear. Here, we aim to test whether any genetic variants associate with amlodipine-induced peripheral edema. For this, we used the targeted region sequencing method to genotype the SNPs in all known haplotypes of *CYP3A5*, and evaluated their effect on amlodipine-induced peripheral edema in Chinese Han hypertensive patients.

Materials and Methods

Participants

We recruited a total of 240 patients with essential hypertension who have ever been treated with amlodipine or L-amlodipine for more than 4 weeks. The participants were outpatients from Beijing Chaoyang Hospital of Capital Medical University and the Second Hospital of Jilin University. The demographic characteristics and baseline data were collected, including gender, age, body mass index (BMI), smoking and drinking status, blood pressure, heart rate and use of medications. Participants were also questioned about peripheral edema symptoms (leg or ankle edema). Two trained physicians evaluated the adverse drug reactions caused by the amlodipine or L-amlodipine based on the WHO-UMC scale.²⁹ We classified the participant as a peripheral edema case if WHO-UMC causality categories were certain, probable, or possible. The others were grouped as controls.

Sample Collection and DNA Sequencing

The DNA Sampling Swabs (Taitong Gene Testing Equipments Co., Ltd., Suzhou, China) were used for collecting oral buccal mucosa cells. Genomic DNA was isolated from the swabs, using Hi-Swab DNA kit

(TIANGEN, Beijing, China) according to the manufacturer's instructions. Quantification of the DNA concentration was performed using the manufacturer's protocol with the Qubit® dsDNA HS Assay Kit (Yeasen, Shanghai, China). Methods for targeted region capture and NGS have been reported previously.³⁰ Illumina HiSeq X was used to sequence the captured DNA with paired-end reads of 150 bp length. [Table S1](#) lists the targeted regions of sequencing and the 24 SNPs of *CYP3A5*.

SNP Calling and Genotyping

High-quality sequencing reads were obtained from the raw data by removing reads that contained adapters, were with unknown bases, or had a low-quality using the Trimmomatic (v0.36)³¹ program. The high-quality reads were aligned to human reference genome hg19 using the Burrows-Wheeler Aligner (BWA, v0.7.15)³² using the default parameters. The Genome Analysis ToolKit (GATK, v3.8)³³ was used for indels realignment, quality score recalibration, variant calling, and genotyping (using Haplotype Caller).

Statistical Analysis

Demographic and clinical characteristics of different groups were compared by *t*-test or Chi-square (χ^2) test according to the data category. The associations between gene polymorphisms and the risk of peripheral edema were assessed by codominant model, dominant model, recessive model and allele model by calculating the odds ratios (ORs) and 95% confidence intervals (CIs) using logistic regression with or without adjustment by gender and alcohol status. Stratification was done by gender. Analyses above were carried out on R-4.03. PLINK 1.9³⁴ was used to calculate the minor allele frequency and assess Hardy-Weinberg equilibrium (HWE) for each SNP. Additionally, linkage disequilibrium (LD) block and haplotype were assessed by Haploview³⁵ software. The D' and r^2 values for all pairs of SNPs were calculated. P value<0.05 was considered as the significant level.

Results

General Characteristics

Two hundred and forty enrolled patients were separated into 64 cases and 176 controls. The general characteristics of the study population are summarized in [Table 1](#). In agreement with previous reports, a higher incidence of CCB-induced peripheral edema was observed in women.

A significant difference was found in the sex ratio between cases and controls ($P=0.00048$). In addition, the frequency of drinkers was 29.69% in the case group and 46.02% in the control group ($P=0.034$). There was no significant difference between the two groups regarding other characteristics.

Association of *CYP3A5* Gene Polymorphisms with the Risk of Amlodipine-Induced Peripheral Edema

All the observed SNPs and the minor allele frequencies (MAF) in two groups are listed in [Table 2](#). Except rs15524, rs4646453 and rs776746, the other SNPs were rare or not detected in the studied population. Therefore, we focused on these three SNPs (MAF>0.05) for further studies. Distributions of genotype frequencies of the SNPs did not show any deviation in Hardy-Weinberg equilibrium ($P>0.05$).

The genotype and allele allocations of the test polymorphisms differed considerably between cases and controls ([Table 3](#)). In more detail, the frequencies of alleles rs15524 G, rs4646453 A, and rs776746 T were significantly lower in cases than those in the control group (G vs A: OR=0.53, $P=0.011$; A vs C: OR=0.54, $P=0.019$; T vs C: OR=0.58, $P=0.03$; respectively). Furthermore, there was a statistically significant difference in genotype of the rs15524 and rs4646453 between the two groups in dominant model with or without adjustment by gender and alcohol status (GG+AA vs AA: OR=0.5, $P=0.021$; AA+AC vs CC: OR=0.54, $P=0.04$). As for rs776746, the

Table 1 Characteristics of the Study Population

Characteristics	Case (N=64)	Control (N=176)	P-value
Sex			
Male, n	19 (29.69%)	99 (56.25%)	0.00048
Female, n	45 (70.31%)	77 (43.75%)	0.00048
BMI, kg/m ²	25.52 ± 3.48	25.78 ± 3.70	0.614
Age, years	64.39 ± 12.09	61.09 ± 11.45	0.061
SBP, mmHg	141.48 ± 18.99	138.09 ± 12.96	0.203
DBP, mmHg	83.03 ± 10.51	82.94 ± 8.80	0.949
HR, beats/min	72.59 ± 9.33	71.68 ± 9.15	0.511
Smoking, n	10 (15.63%)	46 (26.14%)	0.120
Drinking, n	19 (29.69%)	81 (46.02%)	0.034

Notes: Categorical and continuous data were examined by Chi-square (χ^2) test and Student's *t*-test, respectively. Values are expressed as mean ± SD and n (%). Bold values are statistically significant ($P < 0.05$).

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

Table 2 Observed *CYP3A5* Variations and Frequencies

dbSNP	Substitution	Genotype	Case, n	Control, n	Total, n	MAF (Case, %)	MAF (Control, %)
rs15524	A>G	AA AG GG	40 23 1	80 81 15	120 104 16	19.53	31.53
rs4646453	C>A	CC AC AA	42 21 1	89 73 14	131 94 15	17.97	28.69
rs776746	T>C	CC CT TT	40 23 1	86 76 14	126 99 15	19.53	29.55
rs55965422	A>G	AA AG GG	63 1 0	175 1 0	238 2 0	0.78	0.28
rs28365085	A>G	AA AG GG	64 0 0	173 3 0	237 3 0	0	0.85
rs28365094	T>C	TT CT CC	64 0 0	175 1 0	239 1 0	0	0.28
rs56411402	T>C	TT CT CC	64 0 0	172 4 0	236 4 0	0	1.14
rs28371764	G>A	GG AG AA	64 0 0	175 1 0	239 1 0	0	0.28

Abbreviation: MAF, minor allele frequency.

presence of TT+CT genotype demonstrated a significantly lower risk after gender and alcohol status adjustment (TT +CT vs CC: OR=0.57, adjusted $P=0.044$).

Stratified analyses were performed to access the differential effect of gender on the association between amlodipine-induced edema and the polymorphisms. No significant differences were identified, but the relevant genotypes still showed lower risk in all subgroups. The details are presented in [Table 4](#).

Linkage Disequilibrium and Haplotype Analysis of Three *CYP3A5* Gene Polymorphisms

We employed Haploview program to assess the Linkage disequilibrium (LD) block and haplotype of the three SNPs of *CYP3A5*. The LD analysis indicated that these SNPs were in strong LD with each other ([Figure 1](#), rs15524 and

rs4646453 $D'=0.965$, $r^2=0.821$; rs15524 and rs776746 $D'=0.979$, $r^2=0.919$; rs776746 and rs4646453 $D'=0.989$, $r^2=0.898$). Frequencies of four haplotypes were found to be more than 1% in the haplotype analysis ([Table 5](#)). The most represented haplotype in the whole cohort of controls and cases was ACC, followed by GAT, GCC and GCT. Two haplotypes (ACC and GAT) were significantly associated with the risk of amlodipine-induced peripheral edema. The frequency of the ACC haplotype was higher in the cases than controls (79.7% vs 67.9%, $P=0.012$), whereas the frequency of the GAT haplotype was lower in the cases (17.2% vs 28.1%, $P=0.015$).

Discussion

Studies have demonstrated that genetic polymorphisms may influence the gene function, thus causing alterations in the pharmacokinetics (PK) and pharmacodynamics (PD)

Table 3 Genotype Frequencies of Study SNPs in Case and Control Groups

SNP	Models	Genotype	Case (n=64)	Control (n=176)	OR (95% CI)	P-value	*P-value
rs15524/CYP3A5*1D (A>G)	Co-dominant	AA	40 (62.50)	80 (45.46)	1.00		
		AG	23 (35.94)	81 (46.02)	0.57 (0.31–1.03)	0.064	0.040
		GG	1 (1.56)	15 (8.52)	0.37 (0.13–1.02)	0.055	0.038
	Dominant	AA	40 (62.50)	80 (45.46)	1.00		
		GG+AG	24 (37.50)	96 (54.54)	0.50 (0.28–0.90)	0.021	0.011
	Recessive	AA+AG	63 (98.44)	161 (91.48)	1.00		
		GG	1 (1.56)	15 (8.52)	0.17 (0.02–1.32)	0.090	0.061
	Allele	A	103 (80.47)	241 (68.47)	1.00		
		G	25 (19.53)	111 (31.53)	0.53 (0.32–0.86)	0.011	–
rs4646453/CYP3A5*1E (C>A)	Co-dominant	CC	42 (65.63)	89 (50.57)	1.00		
		AC	21 (32.81)	73 (41.48)	0.61 (0.33–1.12)	0.111	0.080
		AA	1 (1.56)	14 (7.95)	0.39 (0.14–1.09)	0.073	0.047
	Dominant	CC	42 (65.62)	89 (50.57)	1.00		
		AA+AC	22 (34.38)	87 (49.43)	0.54 (0.30–0.97)	0.040	0.025
	Recessive	CC+AC	63 (98.44)	162 (92.05)	1.00		
		AA	1 (1.56)	14 (7.95)	0.18 (0.02–1.43)	0.105	0.078
	Allele	C	105 (82.03)	251 (71.31)	1.00		
		A	23 (17.97)	101 (28.69)	0.54 (0.33–0.90)	0.019	–
rs776746/CYP3A5*3 (T>C)	Co-dominant	CC	40 (62.50)	86 (48.86)	1.00		
		CT	23 (35.94)	76 (43.19)	0.65 (0.36,1.18)	0.159	0.128
		TT	1 (1.56)	14 (7.95)	0.39 (0.14–1.10)	0.075	0.054
	Dominant	CC	40 (62.50)	86 (48.86)	1.00		
		TT+CT	24 (37.50)	90 (51.14)	0.57 (0.32,1.03)	0.063	0.044
	Recessive	CC+CT	63 (98.44)	162 (92.05)	1.00		
		TT	1 (1.56)	14 (7.95)	0.18 (0.02–1.43)	0.105	0.078
	Allele	C	103 (80.47)	248 (70.45)	1.00		
		T	25 (19.53)	104 (29.55)	0.58 (0.35–0.95)	0.030	–

Notes: Logistic regression was adopted. Bold values are statistically significant ($P < 0.05$). *Adjusting for gender and sex.

of the gene-associated drugs. Moreover, genetic variants have been confirmed for their association with appearance of adverse reactions to drugs, such as ACE inhibitors-induced cough³⁶ and rosuvastatin-induced myotoxicity.³⁷ To our knowledge, this is the first study to investigate the relationships of *CYP3A5* polymorphisms and amlodipine-induced peripheral edema by using a case–control retrospective study in the Chinese population. Our study reveals that amlodipine-induced peripheral edema may associate with genetic polymorphisms in *CYP3A5* gene. We find that the distribution of allele and genotype frequencies of the three SNPs are significantly different between cases and controls. Specifically, the alleles rs15524 G, rs4646453 A, and rs776746 T reduce the risk of amlodipine-induced peripheral edema. On the other hand, A, C, and C increase the risk and haplotype analysis also confirms this. These findings support the hypothesis

that genetic variation in *CYP3A5* is involved in the development of amlodipine-induced peripheral edema.

CYP3A5 is highly polymorphic with significant inter-individual variation in the enzyme activity contributing to the absorption, metabolism and tissue distribution of drugs.^{24,38} Genetic polymorphisms of *CYP3A5* may potentially alter its protein's expression and function, and subsequently influence the clearance of any drugs metabolized by *CYP3A5*.³⁸ The most studied SNP related to *CYP3A5* functional variation is rs776746 (also known as *CYP3A5*3* or *CYP3A5*3C*). Homozygous carriers of this SNP (*3/*3 or CC) lack functional *CYP3A5* protein because of the frame-shift mutation and truncation of the translated protein.³⁹ Previous studies have confirmed that *CYP3A5*3* is associated with drug metabolism, and *CYP3A5*3*/*3 carriers have decreased metabolism of nifedipine⁴⁰ and tacrolimus,⁴¹ compared to *CYP3A5*1*/*1 and *CYP3A5*1*/*

Table 4 Stratified Analyses Between SNPs and Risk of Amlodipine-Induced Peripheral Edema

rs15542	Genotype (Case/Control)			AG vs AA	GG vs AA	GG+AG vs AA	GG vs AA+AG
	AA	AG	GG				
Male	13/48	5/45	1/6	0.41(0.14,1.24); 0.115	0.78(0.26,2.36); 0.667	0.43(0.15,1.23); 0.118	0.86(0.1,7.58); 0.893
Female	27/32	18/36	0/9	0.59(0.28,1.27); 0.179	–	0.47(0.22,1); 0.051	–
rs4646453	Genotype (Case/Control)			AC vs CC	AA vs CC	AA+AC vs CC	AA vs CC+AC
	CC	AC	AA				
Male	14/51	4/42	1/6	0.35(0.11,1.13); 0.08	0.78(0.26,2.34); 0.656	0.38(0.13,1.13); 0.083	0.86(0.1,7.58); 0.893
Female	28/38	17/31	0/8	0.74(0.35,1.6); 0.45	–	0.59(0.28,1.25); 0.17	–
rs776746	Genotype (Case/Control)			CT vs CC	TT vs CC	TT+CT vs CC	TT vs CC+CT
	CC	CT	TT				
Male	14/45	5/44	1/6	0.43(0.14,1.3); 0.134	0.79(0.26,2.39); 0.679	0.45(0.16,1.29); 0.137	0.86(0.1,7.58); 0.893
Female	27/37	18/32	0/8	0.77(0.36,1.65); 0.503	–	0.62(0.29,1.3); 0.204	–

Notes: Logistic regression was adopted. Data are expressed as OR (95% CI); P-value.

*3 carriers. Patients with *CYP3A5**3/*3 who are treated with tacrolimus may have an increased risk of nephrotoxicity as compared to patients without it.^{25,26} These findings suggest that the *CYP3A5* *3/*3 carriers have low activity of *CYP3A5* enzyme and decreased metabolism for certain drugs dependent on it, resulting in the appearance of an adverse reaction to the drug. Conforming to these findings, our study also indicates that there is an association between *CYP3A5**3 and the occurrence of amlodipine-induced peripheral edema and that *3/*3 (CC) carriers have a higher risk of peripheral edema.

*CYP3A5**1D (rs15524) is another frequently studied SNP in *CYP3A5*, which is in the 3'-untranslated region (UTR). *CYP3A5**1D is differentially distributed among populations ranging from 77.5% in Americans to 71.4% in East Asians, 40.6% in Africans, and even rarer in Europeans (less than

8%) (GnomAD).²¹ SNPs in UTRs may influence the stability of mRNA, thus affecting the expression and activity of the

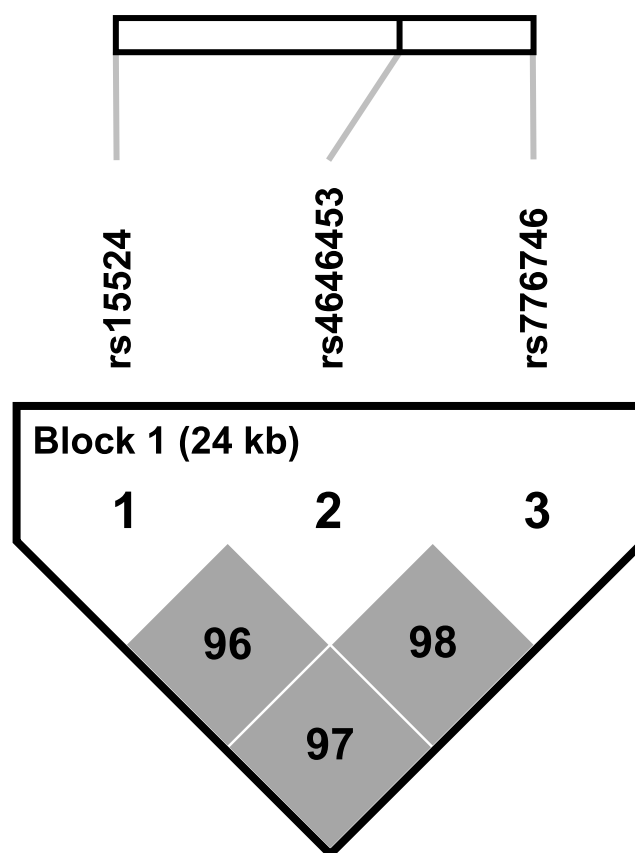


Figure 1 Linkage disequilibrium coefficients ($|D'|$) and LD block among the three polymorphisms of *CYP3A5*.

Table 5 Haplotype Frequencies of *CYP3A5* Gene and the Association with the Amlodipine-Induced Peripheral Edema Risk

Haplotype	Case (%)	Control (%)	OR (95% CI)	P-value
ACC	79.7	67.9	1.85 (1.12–3.14)	0.012
GAT	17.2	28.1	0.53 (0.30–0.90)	0.015
GCT	2.3	1.7	1.38 (0.22–6.59)	0.649
GCC	0	1.7	–	0.138

Note: Bold values are statistically significant ($P < 0.05$).

enzyme.^{42,43} According to a study identifying the potential SNPs related to miRNA, rs15524 may influence the hsa-miR-500a-5p that targets *CYP3A5*, affecting its expression.⁴⁴ In addition, studies have found that *CYP3A5*1D* influences the pharmacokinetics of many drugs, including tacrolimus⁴⁵ and carbamazepine.⁴⁶ Therefore, rs15524 may act as a genetic marker and should be considered while studying or prescribing drugs metabolized by *CYP3A5*. Here, we find that the frequencies of rs15524 G allele and GA+GG genotypes in cases are lower than controls, which indicates that this SNP is associated with reduced risk of incidence of amlodipine-induced peripheral edema. In other words, *CYP3A5*1D*/**1D* carriers may have an increased risk than others.

As for *CYP3A5*1E* (rs4646453), it is associated with a decreasing risk of amlodipine-induced peripheral edema. However, there is little information about the function of the rs4646453. To some extent, this is possible because the SNP locates in the intron region. Studies have revealed that *CYP3A5* rs4646453 is in LD with rs776746,⁴⁷ and there is a strong LD between rs15524 and rs776746.^{39,46} Our findings are consistent with these studies and further promote that the presence of LDs with rs776746 may partly explain the role of rs15524 and rs4646453 in amlodipine-induced peripheral edema.

Given all these findings, there are some limitations in our present study that we would like to acknowledge. First, the population in our study is Chinese Han, and it is known that the frequency of mutations differs among ethnic groups. Hence, our results may apply well to Chinese population but are probably not suitable for populations of other ethnic groups. Second, we have performed a retrospective study and not a prospective one. The study is also limited by the smaller number of samples. The third limitation is not involving other genes contributing to amlodipine metabolism, such as *CYP3A4*. As we mentioned before, polymorphisms of *CYP3A4* are rare in Chinese, and the evidence so far that *CYP3A4* polymorphisms influence amlodipine metabolism is scarce. So, we took no account of them currently. But it would be better for future studies to include these related genes to better explain the connection between genetic factors and amlodipine-induced peripheral edema. Hence, more case-control studies with large number of polyethnic samples and involvement of diversified factors are necessary.

None the less, our study does investigate the novel relationship between the genetic variants and amlodipine-induced peripheral edema. In conclusion, we provide evidence that *CYP3A5* polymorphisms are involved in the

occurrence of amlodipine-induced peripheral edema, and the three genetic variants of *CYP3A5* have the potential to serve as novel biomarkers for amlodipine-induced adverse reactions. Our findings thus provide new insights into amlodipine-induced peripheral edema and are of importance in developing and prescribing personalized and precise medicine for hypertension.

Data Sharing Statement

The raw data are available on reasonable request to the correspondence author Songnian Hu.

Ethics and Consent Statement

All the participants have signed the written informed consent, and approval was obtained from the Research Ethics Board of Beijing Chaoyang Hospital of Capital Medical University and the Research Ethics Board of the Second Hospital of Jilin University. This study has been conducted in accordance with the World Medical Association Declaration of Helsinki.

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Disclosure

All authors declare that they have no conflicts of interest for this work.

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