



# **TRIB3 rs6037475 is a potential biomarker for predicting felodipine drug response in Chinese patients with hypertension**

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**Background:** Our previous studies have found that single nucleotide polymorphisms (SNPs) of *tribbles homolog 3* (*TRIB3*) are related to the hypotensive effects of calcium-channel blockers (CCBs) and angiotensin-converting enzyme (ACE) inhibitors. In this study, we aimed at exploring and validating the effect of *TRIB3* polymorphism on antihypertensive drugs responses.

**Methods:** A total of 830 hypertensive patients, who were administered with open-labeled hydrochlorothiazide (12.5 mg once daily) and randomly assigned to off-labeled felodipine (5 mg) or a matched placebo combination treatment (1:1), were selected from the Felodipine Event Reduction (FEVER) study. A strategy of screening 259 samples and validating the remaining 531 samples was implemented. Four functional SNPs were selected (rs2295490, rs11470129, rs4815567 and rs6037475 in *TRIB3*). A mixed linear model was performed to analyze the effects of *TRIB3* SNPs on antihypertensive drugs responses.

**Results:** We found that *TRIB3* rs6037475 CC genotype was associated with a reduction of diastolic blood pressure (DBP) ( $P=6.3 \times 10^{-3}$ ) in the felodipine treatment group of screening set, and was also associated with a reduction of systolic blood pressure (SBP) ( $P=0.021$ ), DBP ( $P=6.0 \times 10^{-3}$ ) and mean arterial pressure (MAP) ( $P=0.021$ ) in the felodipine treatment group of the validation set. As for the reductions influenced by the rs2295490, rs11470129 and rs4815567 genetic variations, however, the adjusted P-value did not reach statistical significance. Combined screening and validation set analysis found that patients with *TRIB3* rs6037475 CC genotype had a significant higher mean SBP, DBP and MAP than those with TT genotype in the felodipine treatment group (CC vs. TT  $-10.2 \pm 0.74$  vs.  $-17.8 \pm 0.21$ ,  $P=7.8 \times 10^{-3}$ ;  $-4.6 \pm 0.50$  vs.  $-10.2 \pm 0.23$ ,  $P=3.0 \times 10^{-4}$ ;  $-6.5 \pm 0.54$  vs.  $-12.7 \pm 0.14$ ,  $P=3.0 \times 10^{-4}$ , respectively).

**Conclusions:** These results suggest that *TRIB3* rs6037475 genetic variation can be useful as a bio-marker for predicting felodipine drug response in Chinese patients with hypertension.

**Keywords:** *Tribbles homolog 3* (*TRIB3*); hypertension; felodipine; pharmacogenetics; individualized drug therapy

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## Introduction

Hypertension is a major preventable risk-factor that results in cardio-cerebrovascular disease, chronic kidney disease, disability and premature mortality (1). The latest epidemiological data have shown that an added 244.5 million adults in China have suffered from hypertension. Half of them were aware of their conditions but fewer than 40% received treatment. Moreover, less than 15% of the treated patients achieved their blood pressure control goals (2,3). In general, inadequate blood pressure control is one of the leading causes of end-organ damage and the subsequent increase of social and economic cost burden (4). However, whether all patients benefit from treatment according to the existing primary guidelines is still controversial. The pathogenesis of hypertension is a complex process that is influenced by both genetic and environmental factors (5). Substantial inter-individual variation, especially the genetic polymorphisms, can be the leading causes of the low effective control rate in blood pressure response to antihypertensive therapy (6).

*Tribbles homolog 3 (TRIB3)* interacts with a host of proteins to control many aspects of eukaryotic cell biology. Emerging evidence has revealed that the primary function of *TRIB3* is to control the transduction of insulin signal and endothelial vascular function (7). Our previous studies found that single nucleotide polymorphism (SNP) of *TRIB3* (rs2295490) could significantly affect the responses of calcium-channel blockers (CCBs) and ACE-inhibitors (8-10). However, these results were limited by the small sample size or the combination of other drug treatments, such as hypoglycemic, lipid-lowering and anticoagulant drugs. Therefore, it is essential to confirm the effect of *TRIB3* gene polymorphism on antihypertensive drug sensitivity in a large and long-term follow-up clinical trial cohort.

The European Society of Hypertension (ESH) and European Society of Cardiology (ESC) guidelines emphasized that most patients need the combination of two or more drugs to achieve better blood pressure control (11). Meanwhile, surveys in China showed that CCBs monotherapy were the most commonly used treatment (57.1%), and only those with diuretics monotherapy were able to increase the overall rate of blood pressure control rate by 11% compared to those with CCBs monotherapy (12). These results suggest that the combination of CCBs and diuretics may be more beneficial for Chinese hypertensive patients. FEVER is a double-blind, randomized controlled

clinical trial of Chinese hypertensives and was designed to compare the effect of a low-dose felodipine and a low-dose hydrochlorothiazide (HCTZ) combination therapy with that of the matched placebo treatment (13). Using this clinical trial and our previous work as a basis (10), we designed and conducted this study to explore and validate the effect of functional *TRIB3* gene polymorphisms on the responses of antihypertensive drugs.

## Methods

### *Patients and treatment*

This is a retrospective survey on the FEVER study. Details on the FEVER study design and organization have been published previously (13). In brief, FEVER study is a double-blind, randomized and multi-center clinical trial that was approved by local ethics committees (registered on [www.clinicaltrials.gov](http://www.clinicaltrials.gov), No. NCT01136863), and the trial was conducted following the Declaration of Helsinki. All patients were self-reported as Han Chinese and provided written consent. Eligible patients were treated with an open-labeled hydrochlorothiazide 12.5 mg once a day for 6 weeks. After a comprehensive assessment, they were randomly assigned to the felodipine (intensive) and the matched placebo (less intensive) treatment groups. For the intensive treatment group, patients received a combination therapy of a low dose of diuretic (HCTZ: 12.5 mg q.d.) and a low dose of calcium antagonist (felodipine: 5 mg q.d.). For the less intensive treatment group, patients were given a low dose of diuretic (HCTZ: 12.5 mg) combined with the matched placebo therapy. Randomized double-blind treatment was maintained for at least 36 months. Follow-up was conducted at 1-month intervals during the first 6 months, then at 3-month intervals thereafter. In this study, 858 patients' DNA samples and the matched clinical trial data in the FEVER study cohort were graciously provided by the Beijing Fu Wai Hospital with the collaboration of the Chinese Hypertension League.

### *DNA isolation*

Peripheral venous blood was collected from Chinese patients with hypertension. Genomic DNA was extracted from peripheral venous blood using E.Z.N.A.<sup>®</sup> SQ blood DNA Kit II (Omega Bio-Tek company, USA) according to the manufacturer's instructions. Extracted genomic DNA

was stored at  $-80^{\circ}\text{C}$  until use.

### **Pharmacogenetics study protocol**

The pipeline of this pharmacogenetics study protocol is provided in detail in *Figure 1*. The candidate SNPs selection and functional prediction in *TRIB3* used the Encyclopedia of Deoxyribonucleic Acid (DNA) Elements (ENCODE) database. The ENCODE database is an ongoing international cooperation project that has systematically listed functional elements, chromatin annotations and variation annotations in human genome, intuitively showing whether a SNP is located in any potential functional region, such as transcription factor binding sites, open chromatin regions, micro-ribonucleic acid (miRNA) and long non-coding RNA (lncRNA) transcription regions, miRNA target sites, and DNA methylation sites. For the candidate SNPs, a minimal allele frequency (MAF) of more than 10% of the Chinese population in 1000 Genomes Project was required. To explore the relationship between SNPs and antihypertensive drug response, we implemented a strategy of screening small samples and validating in the remaining large samples. By using computer-generated random numbers, patients were assigned (1:1) to each treatment group according to the study protocol. Candidate SNPs were genotyped for screening by Bioyong Technologies Inc. using a Sequenom MassARRAY<sup>®</sup> SNP system. The significant SNPs were then genotyped for validation by using the TaqMan fluorescent probe typing method on the ABI-based AppliedBiosystems7500 Instrument platform. Primers information are shown in *Table S1*. The reaction mixture (20  $\mu\text{L}$ ) contained the following: TaqMan genotyping master mix (2 $\times$ ) 10.0  $\mu\text{L}$ , TaqMan genotyping assay mix (2 $\times$ ) 9.0  $\mu\text{L}$ , and g-DNA 1.0  $\mu\text{L}$ . Temperature cycling proceeded as follows: (I) initial denaturation for 10 min at  $95^{\circ}\text{C}$ ; (II) 40 cycles of 5 s at  $95^{\circ}\text{C}$ ; (III) elongation at  $60^{\circ}\text{C}$  for 1 min. Finally, 5% of the participants were randomly selected for validation via Sanger sequencing.

### **Statistical analysis**

All the statistical analyses were performed using SAS software (version 9.4, SAS Institute) for windows. Allele frequencies were determined by the genotypes of all the participants. Hardy-Weinberg equilibrium analysis was carried out by using the chi-squared or Fisher's exact test. Baseline characteristics among different phenotypes were assessed by independent-samples T-test or Wilcoxon

rank-sum test, as appropriate. A mixed linear model, with adjustment for age, body mass index (BMI) and gender, was used to analyze the effect of SNPs on the antihypertensive drug response during the whole follow-up period. The quantitative data described in the text and figures are presented as means  $\pm$  standard deviation (SD) and percentages for categorical data.  $P < 0.05$  (2-tailed) was considered a statistically significant value.

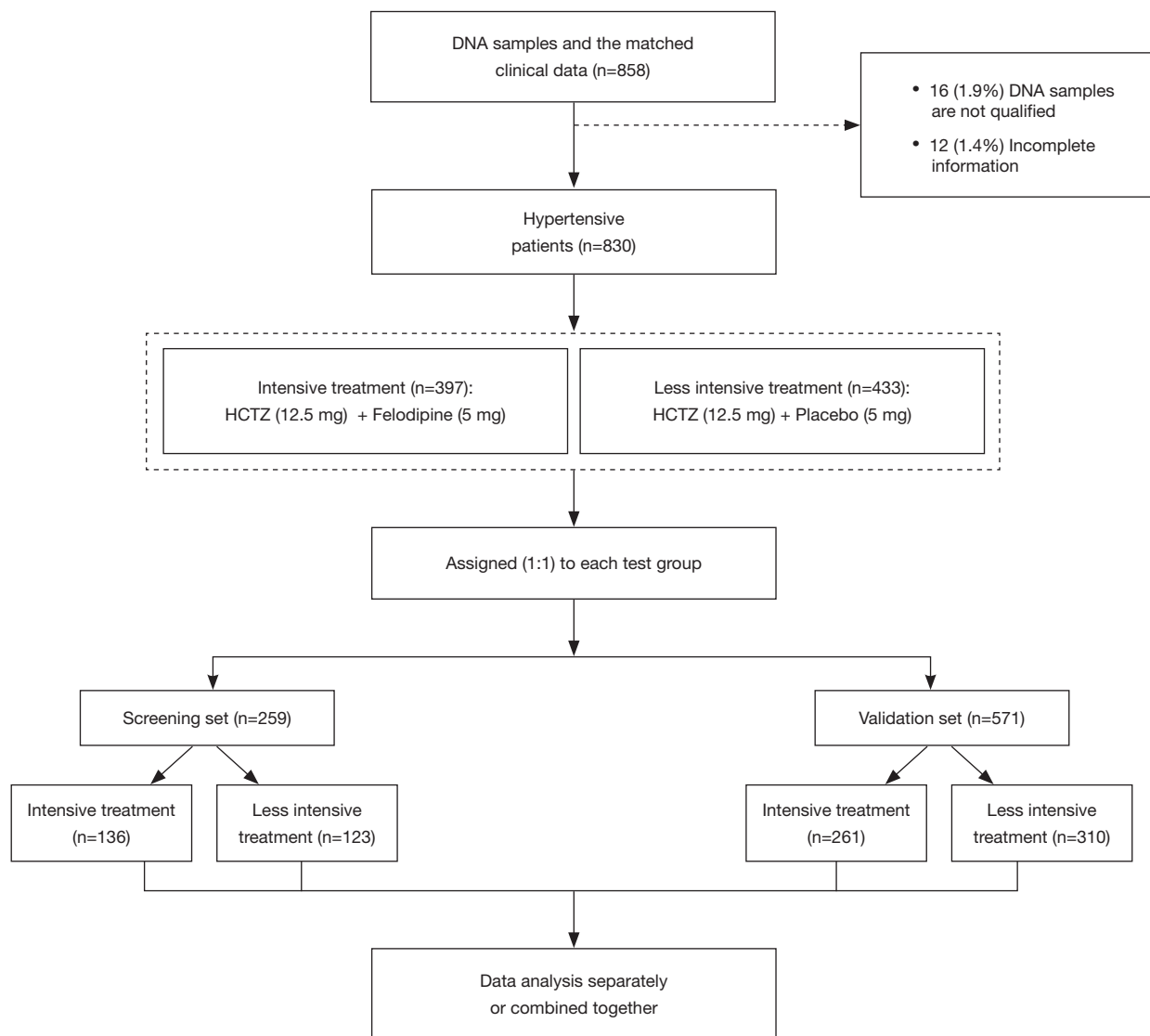
## **Results**

### **Baseline characteristics and genotyping results**

A total of 858 Chinese patients with hypertension provided clinical data and matched DNA samples. However, 16 (1.9%) DNA samples that did not qualify for genotyping, and 12 (1.4%) patients with incomplete clinical information were excluded. Finally, 830 patients were included in our study. Among them, 397 (47.8%) were assigned to the intensive treatment group, and 433 (52.2%) were assigned to the less intensive treatment group (*Figure 1*). Except for heart rate, the clinical baseline characteristics between the two groups (age, sex, and BMI, etc.) did not observe a significant difference (*Table 1*). According to our study protocol, 4 candidate SNPs (rs2295490, rs11470129, rs4815567, and rs6037475) in *TRIB3* gene were included and successfully identified. The detailed results are shown in *Table S2*.

### **Association analysis of the candidate SNPs**

In the screening stage, 259 patients were included in our study. Among them 136 (52.5%) were assigned to the intensive treatment group, and 123 (47.5%) were assigned to the less intensive treatment group. A mixed linear regression model was used for analyzing the influence of rs2295490, rs11470129, rs4815567 and rs6037475 on antihypertensive efficacy of the two afore-mentioned regimens. This revealed that compared with the placebo treatment group, rs6037475 genetic variation could significantly influence the effect of felodipine on diastolic blood pressure (DBP) reduction ( $P = 5.5 \times 10^{-3}$ ) (*Table 2*). As for the reductions influenced by the rs2295490, rs11470129 and rs4815567 genetic variations, however, the adjusted P-values did not reach statistical significance. Further analysis, as shown in *Figure 2*, confirmed that *TRIB3* rs6037475 CC genotype was associated with the lower DBP ( $P = 6.3 \times 10^{-3}$ ) compared with TT genotype in the



**Figure 1** Outline of the study protocol.

felodipine treatment group during the follow-up period. Collectively, these results suggest that *TRIB3* rs6037475 may be associated with the effects of felodipine. Therefore, we carried out further research focused on this genetic variation locus.

#### **Validation and combined analysis of *TRIB3* rs6037475**

To validate whether rs6037475 is a biomarker for predicting felodipine drug response, a validation study that included 261 cases of HCTZ combined with the felodipine therapy and 310 HCTZ combined with the

matched placebo controls was performed. As it is shown in *Table S3*, rs6037475 genetic variation was still significantly associated with DBP reduction ( $P=0.021$ ) in the intensive treatment group and exhibited a marginal association with systolic blood pressure (SBP) ( $P=0.062$ ) and mean arterial pressure (MAP) ( $P=0.061$ ) reduction. In the less intensive treatment group, however, no correlation was observed between rs6037475 genetic variation with SBP, DBP and MAP reduction. We also found that *TRIB3* rs6037475 CC genotype was significantly associated with the reduction of SBP ( $P=0.021$ ), DBP ( $P=6.0 \times 10^{-3}$ ) and MAP ( $P=0.021$ ) in the felodipine treatment group (*Figure 3*).

**Table 1** Clinical baseline characteristics of the study cohort

Characteristics	HCTZ + felodipine (N=397)	HCTZ + placebo (N=433)	P value
Sex (male, n%)	198 (49.8)	217 (50.2)	0.15
Age (years)	61.6±7.0	61.3±7.0	0.58
BMI (kg/m <sup>2</sup> )	26.0±3.8	26.2±3.9	0.55
Randomization SBP (mmHg)	155±11	155±12	0.34
Randomization DBP (mmHg)	91±8	91±8	0.97
Randomization MAP (mmHg)	112±7	112±7	0.54
Randomization HR (bpm)	78±9	77±8	0.012

MAP was calculated as MBP = DBP + (SBP-DBP)/3. HCTZ, hydrochlorothiazide; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure, HR, heart rate; MAP, mean arterial pressure.

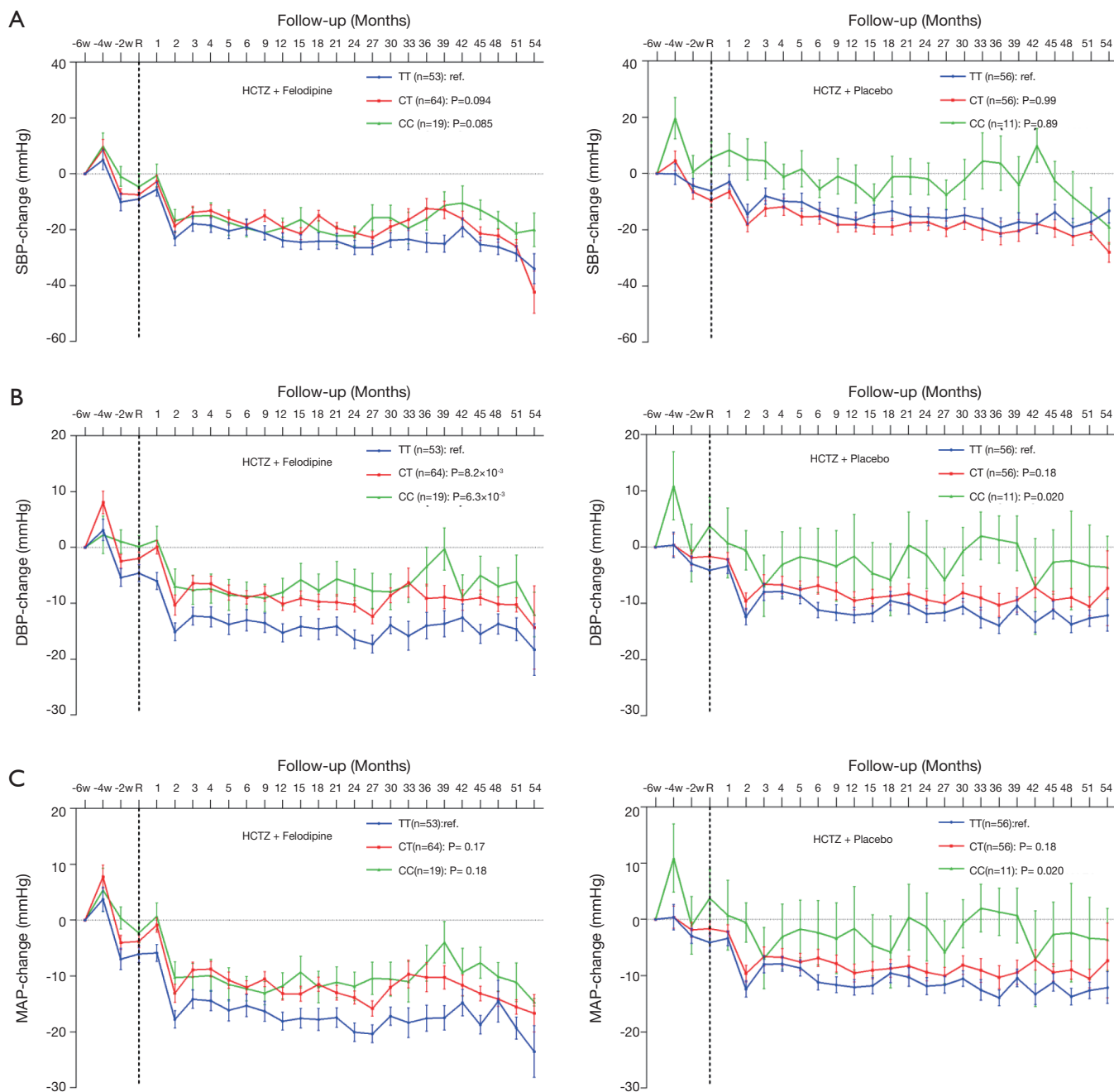
**Table 2** Association of testing for the candidate SNPs on blood pressure reduction in patients with hypertension after the felodipine or placebo treatment

SNP_ID	P <sub>F</sub> (n=136)	P <sub>P</sub> (n=123)	P <sub>all</sub> (n=259)
SBP			
rs2295490 (A>G)	0.62	0.38	0.34
rs11470129 (-/AA)	0.61	0.24	0.29
rs4815567 (G>A)	0.38	0.53	0.76
rs6037475 (T>C)	0.12	0.99	0.71
DBP			
rs2295490 (A>G)	0.20	0.66	0.15
rs11470129 (-/AA)	0.056	0.44	0.16
rs4815567 (G>A)	0.36	0.38	0.44
rs6037475 (T>C)	5.5×10 <sup>-3</sup>	0.051	7.0×10 <sup>-4</sup>
MAP			
rs2295490 (A>G)	0.37	0.41	0.17
rs11470129 (-/AA)	0.99	0.45	0.42
rs4815567 (G>A)	0.98	0.68	0.66
rs6037475 (T>C)	0.26	0.37	0.46

P<sub>F</sub> present as HCTZ + felodipine treatment group; P<sub>P</sub> present as HCTZ + placebo treatment group. MAP was calculated as MBP = DBP+(SBP-DBP)/3. SNP, single nucleotide polymorphism; DBP, diastolic blood pressure; SBP, systolic blood pressure; MAP, mean arterial pressure.

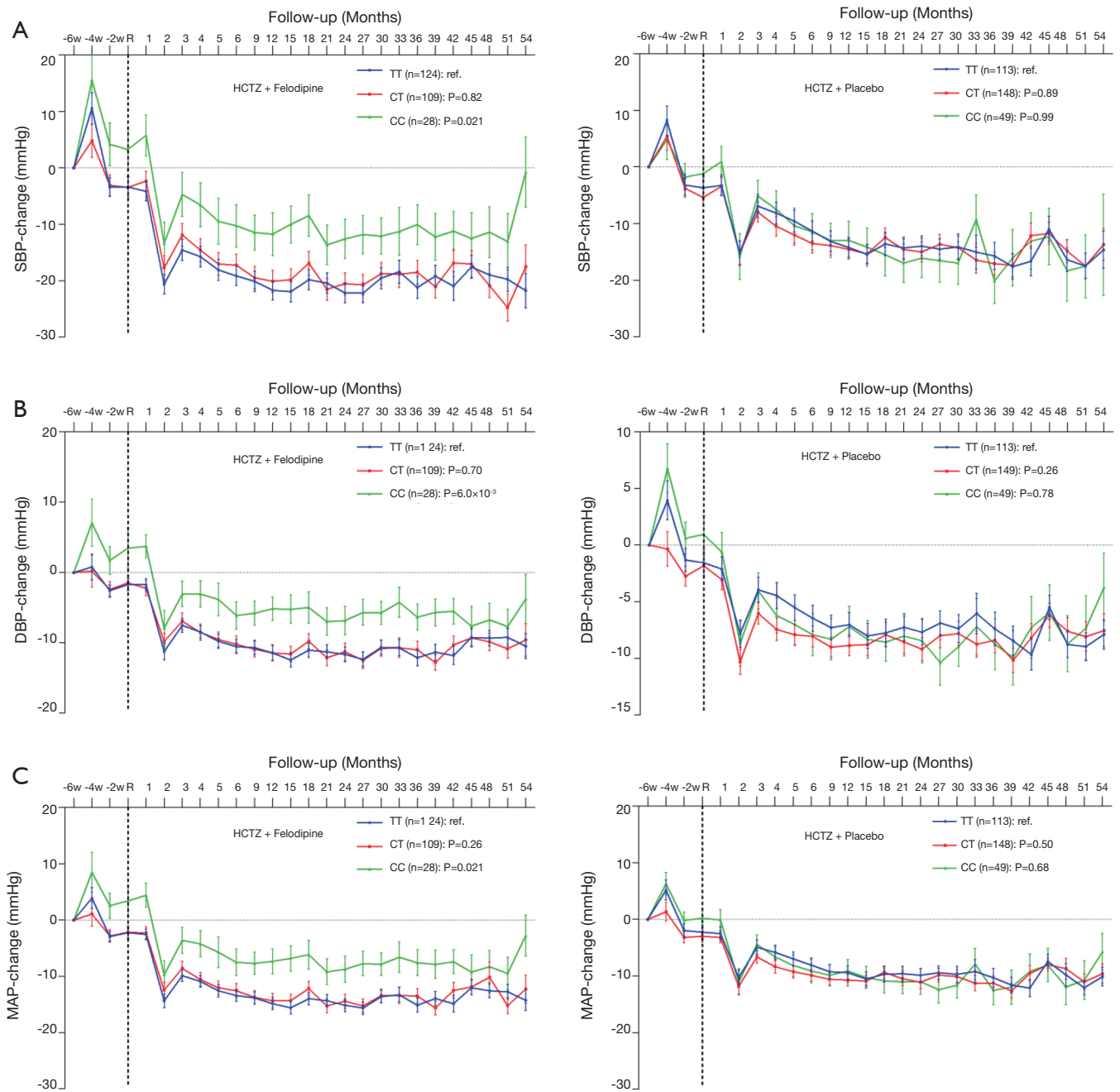
Combined screening and validation set analysis found that *TRIB3* rs6037475 genetic variation significantly influenced the effect of felodipine on SBP (P=0.028), DBP (P=2.1×10<sup>-3</sup>) and MAP (P=1.3×10<sup>-3</sup>), but a significant difference was not observed in the matched placebo treatment group (Table 3). Further analysis found that patients with *TRIB3* rs6037475

CC genotype had a significant lower reduction of mean SBP (CC vs. TT -10.2±0.74 vs. -17.8±0.21), DBP (CC vs. TT -4.6±0.50 vs. -10.2±0.23) and MAP (CC vs. TT -6.5±0.54 vs. -12.7±0.14) than those with TT genotype in the felodipine treatment group, but not in the matched placebo group (Table 4). Meanwhile, compared with the



**Figure 2** Preliminary screening test of *TRIB3* rs6037475 genetic variation on blood pressure response during the follow-up between felodipine and placebo treatment groups. Data are shown as mean  $\pm$  SEM. P values were estimated from a mixed linear regression model with adjustments for sex, age and BMI. Panels (A-C) respectively illustrate that BP-changes from the baseline in patients carrying the *TRIB3* rs6037475 genotype after felodipine or placebo treatment. *TRIB3*, *tribbles homolog 3*; BMI, body mass index.





**Figure 3** Validation testing of *TRIB3* rs6037475 genetic variation on blood pressure response during the follow-up between felodipine and placebo treatment groups. Data are shown as mean ± SEM, P values were estimated from a mixed linear regression model with adjustments for sex, age and BMI. Panels (A-C) respectively illustrate that BP-changes from the baseline in patients carrying the *TRIB3* rs6037475 genotype after felodipine or placebo treatment. *TRIB3*, *tribbles homolog 3*; BMI, body mass index.

**Table 3** The effect of *TRIB3* rs6037475 on blood pressure reduction in patients with hypertension after the felodipine or placebo treatment

SNP_ID	P <sub>f</sub> (n=397)	P <sub>p</sub> (n=433)	P <sub>all</sub> (n=830)
SBP			
rs6037475 (T>C)	0.028	0.35	0.019
DBP			
rs6037475 (T>C)	2.1×10 <sup>-3</sup>	0.43	2.7×10 <sup>-3</sup>
MAP			
rs6037475 (T>C)	1.3×10 <sup>-3</sup>	0.41	3.2×10 <sup>-3</sup>

P<sub>f</sub> present as HCTZ + felodipine treatment group; P<sub>p</sub> present as HCTZ + placebo treatment group. MAP was calculated as MBP = DBP + (SBP-DBP)/3. *TRIB3*, *tribbles homolog 3*; SNP, single nucleotide polymorphism; DBP, diastolic blood pressure; SBP, systolic blood pressure; MAP, mean arterial pressure.

**Table 4** Association of *TRIB3* rs6037475 genetic variation on blood pressure reduction in patients with hypertension after the felodipine or placebo treatment

Variables	Genotype	HCTZ + felodipine (n=397)		HCTZ + placebo (n=433)	
		Mean (mmHg)	P	Mean (mmHg)	P
<i>TRIB3</i> rs6037475 (SBP-changes)	TT (ref)	-17.8±0.21		-12.1±0.22	
	CT	-15.9±0.22	0.30	-13.1±0.18	0.63
	CC	-10.2±0.74	7.8×10 <sup>-3</sup>	-9.6±0.50	0.28
<i>TRIB3</i> rs6037475 (DBP-changes)	TT (ref)	-10.2±0.23		-7.4±0.14	
	CT	-8.5±0.17	0.081	-7.3±0.11	0.99
	CC	-4.6±0.50	3.0×10 <sup>-4</sup>	-5.3±0.32	0.23
<i>TRIB3</i> rs6037475 (MAP-changes)	TT (ref)	-12.7±0.14		-8.9±0.22	
	CT	-10.9±0.16	0.11	-9.2±0.18	0.81
	CC	-6.5±0.54	3.0×10 <sup>-4</sup>	-6.7±0.50	0.34

HCTZ, hydrochlorothiazide; *TRIB3*, *tribbles homolog 3*.

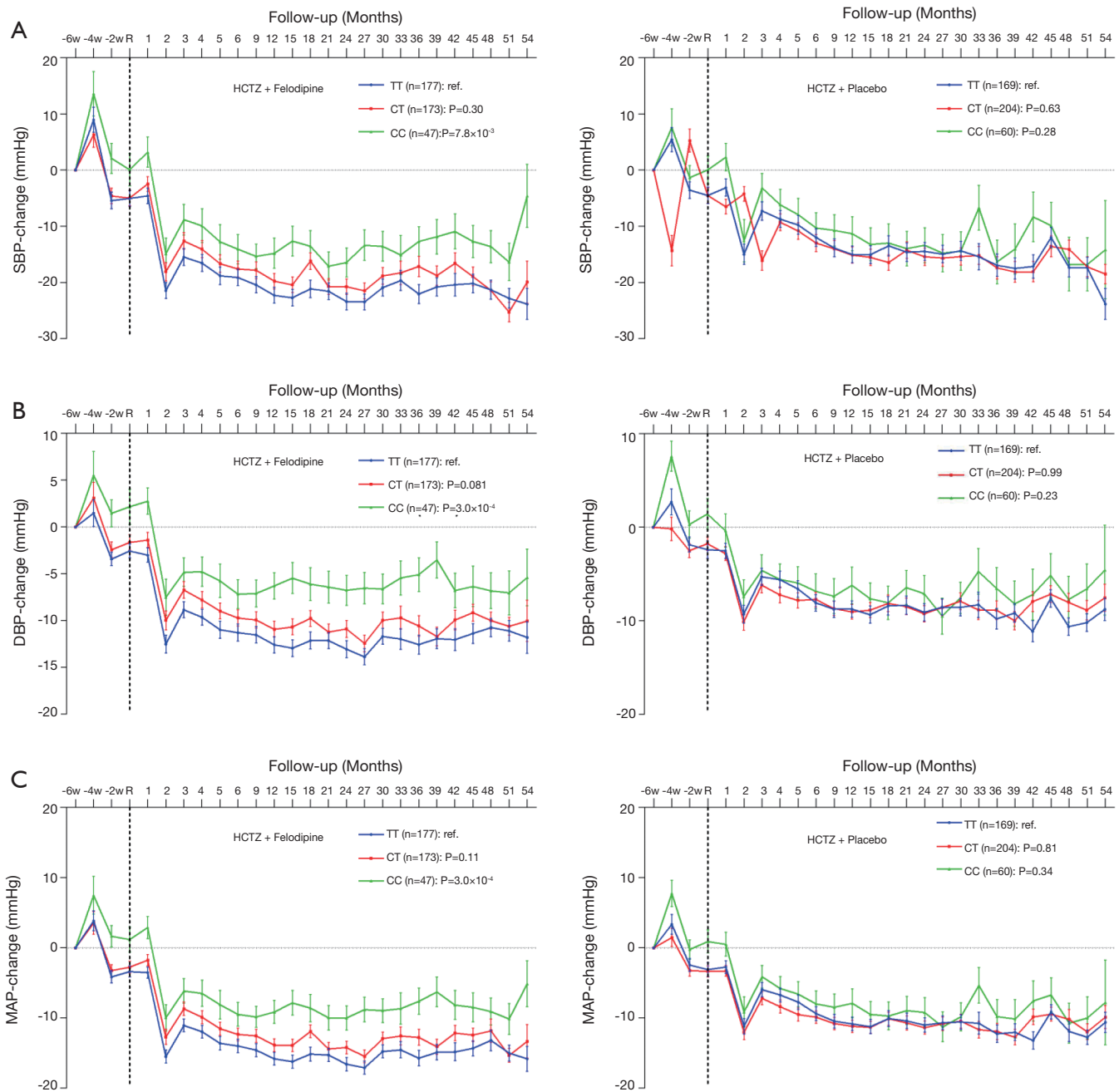
TT genotype carriers, patients with *TRIB3* rs6037475 CC genotype had a significant higher mean SBP (P=7.8×10<sup>-3</sup>), DBP (P=3.0×10<sup>-4</sup>) and MAP (P=3.0×10<sup>-4</sup>) than those with TT genotype in the felodipine treatment group (Figure 4).

## Discussion

The results from our previous studies, which were based on a well-controlled drug clinical trial, suggested that *TRIB3* (rs2295490, A>G) genetic variation was closely related to blood pressure regulation and antihypertensive drugs responses (8-10). Our data indicated that a novel variant of *TRIB3* rs6037475, which was located 2 kb upstream of the gene, was significantly associated with the felodipine effect

on DBP-lowering. However, in this study, *TRIB3* rs2295490 genetic variation did not affect the antihypertensive efficacy of felodipine or hydrochlorothiazide. A systematic review elaborated that the abnormal expression of *TRIB3* is related to insulin resistance, impaired insulin secretion, endothelial dysfunction, and eventually led to type 2 diabetes mellitus (T2DM) and cardiovascular disease (7). Andreozzi *et al.* reported that *TRIB3* rs2295490 mutation altered the structure rather than the expression of *TRIB3*, and enhanced the regulatory effect of *TRIB3* and *Akt* (14). Another study found that *TRIB3* rs2295490 mutation resulted in a significant reduction in insulin-induced nitric oxide (NO) release compared with the wild type *in vitro* (15). In view of this, *TRIB3* rs6037475 may regulate felodipine





**Figure 4** Data of all combined patients with *TRIB3* rs6037475 genetic variation for blood pressure lowering during the follow-up between felodipine and placebo treatment groups. Data are shown as mean ± SEM. P values were estimated from a mixed linear regression model with adjustments for sex, age and BMI. Panels (A-C) respectively illustrate that BP-changes from the baseline in patients carrying the *TRIB3* rs6037475 genotype after felodipine or placebo treatment. *TRIB3*, *tribbles homolog 3*; BMI, body mass index.

response by affecting the expression of *TRIB3* or mediating NO release. Further investigations are warranted to confirm these possible mechanisms. Our previous studies also found that patients with *TRIB3* rs2295490 G allele had a weaker DBP response to calcium channel blockers (azelnidipine and compound nitrendipine), which was inverted by angiotensin receptor blocker (candesartan and irbesartan) or ACE-inhibitors (imidapril) prescription (8-10). In short, these findings suggested that *TRIB3* genetic variations were involved in the regulation of blood pressure. Furthermore, *TRIB3* genetic variations could affect hypotensive process, due to *TRIB3*'s unique pseudokinase plastic domains (16).

At present, no report on the regulatory effect of felodipine and hydrochlorothiazide on *TRIB3* exists. However, Ding *et al.* revealed that felodipine could significantly increase NO production, Ca<sup>2+</sup>-dependent nitric oxide synthase (NOS) activity and endothelial nitric oxide synthase (eNOS) protein expression by a NO-cGMP mediated mechanism in human umbilical vein endothelial cells (HUVEC) cells (17). Emerging data also demonstrated that a CCB potentiates the vascular protective effects of angiotensin receptor blockers (ARBs) in salt-sensitive hypertension as compared with a diuretic. For instance, olmesartan combined with azelnidipine resulted in a more significant reversal of the decrease in p-eNOS, total eNOS and p-Akt than olmesartan combined with hydrochlorothiazide (18). However, monotherapy of diuretics, such as hydrochlorothiazide and indapamide, did not affect NOS activity, eNOS and inducible nitric oxide synthase (iNOS) protein expressions (18,19). Jiang *et al.* reported tag-SNPs in four insulin resistance genes (*ADIPOQ*, *LEPR*, *RETN* and *TRIB3*). Among them, gene polymorphisms in *LEPR* and *ADIPOQ* were significantly associated with hypertension, while the other two (*RETN* and *TRIB3*) were not (20). However, the tag-SNPs study was limited, because it did not contain the most classical mutation of *TRIB3*, such as rs2295490. Therefore, these results may indirectly favor our findings that *TRIB3* rs6037475 genetic variation was significantly associated with DBP-lowering in the HCTZ therapy combined with felodipine, but not in the place-combined treatment.

Studies have found that when a 10 mmHg is decreased in SBP, major cardiovascular disease events can be markedly reduced by about 10–35% (21-23). In the A Coronary disease Trial Investigating Outcome with Nifedipine GITS (ACTION), a blood pressure reduction of 14.6/7.6 mmHg in the nifedipine administration group was associated with a 38% reduction in the incidence of heart failure as compared

with the matched placebo treatment group (24,25). In the FEVER trial, for the felodipine treated group, in which blood pressure achieved slightly lower values than in the placebo group, a blood pressure reduction of 3.5/1.5 mmHg was found to significantly reduce the incidence of all cardiovascular events by about 28% (13). In our study, there was no significant difference in SBP, DBP and MAP reduction between the two groups in patients who carried *TRIB3* rs6037475 TC and CC genotypes (shown in *Table S4*). A significant antihypertensive benefit after felodipine treatment was observed in those patients with TT genotype. Therefore, we believe that *TRIB3* rs6037475 genetic variation may be a potential biomarker for predicting the efficacy of felodipine in patients with hypertension.

The strength of our study is that the FEVER is a well-controlled clinical drug trial with long-term follow-up. Nevertheless, certain limitations should be considered when interpreting our findings. First, due to the quality of peripheral blood samples, we did not obtain enough qualified DNA samples. Therefore, sampling errors might have occurred that were factored into the statistical analysis. Second, other SNPs (e.g., rs4813620 variant), which may also be involved in the interaction between *TRIB3* expression and drug efficacy or affect vascular function (26), were not examined in our study. Besides, the *TRIB3* rs6037475 genetic variation in the promoter region of *TRIB3* may have a functional effect on transcriptional activity, but this speculation was only derived from database prediction. Overall, our results strongly suggest that although felodipine is more effective in the treatment of patients with *TRIB3* rs6037475 TT genotype than those with CC genotype, its clinical application merits further investigation.

## Conclusions

The results we present here reveal that *TRIB3* rs6037475 genetic variation is significantly associated with blood pressure reduction in patients with felodipine treatment and can be useful as a potential biomarker for predicting felodipine drug response in Chinese hypertensive patients.

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## Footnote

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/atm.2020.03.176>). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study protocol was approved by local ethics committees (registered on [www.clinicaltrials.gov](http://www.clinicaltrials.gov), No. NCT01136863), and all patients provided written consent.

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**Table S1** Primer sequences of *TRIB3* SNPs

SNPs	Primer sequences
rs11470129	F: ACGTTGGATGCATGTCCACTTCTCCTAAAG R: ACGTTGGATGAAACAGTGACTGTTGTCGGG
rs4815567	F: ACGTTGGATGCACCTGTGTCTCAGGAGATG R: ACGTTGGATGTGTCCCCTGCACCGCCAAC
rs2295490	F: ACGTTGGATGCGTTTCGGGAGGTTTCTTGG R: ACGTTGGATGAGGCTGTAGAATACCTTCTC
rs6037475	F: ACGTTGGATGACTCAGCAGTGACCAGAATG R: ACGTTGGATGTTCTGATAATGGTCTATC

SNP, single nucleotide polymorphism; *TRIB3*, *tribbles homolog 3*.

**Table S2** Predicted common SNPs with potential functional significance in *TRIB3* gene based on ENCODE bioinformatics tools

SNP_ID	Chr	Gene	Function	MAF <sup>a</sup> (%)	MAF <sup>b</sup> (%)	Pb-HWD
rs2295490 (A>G)	chr20:368905	TRIB3	Missense	19.2	20.7	0.31
rs11470129 (-/AA)	chr20:360678	TRIB3	Neargene-5	31.7	23.0	0.035
rs4815567 (G>A)	chr20:362078	TRIB3	Neargene-5	21.6	27.1	0.42
rs6037475 (T>C)	chr20:358685	TRIB3	Upstream	32.4	35.5	0.88

<sup>a</sup>, data from 1,000 Genomes Project (CHS + CHB), <sup>b</sup>, data from our study cohort (screening patients or all patients). SNP, single nucleotide polymorphism; *TRIB3*, *tribbles pseudokinase 3*; MAF, minimum allele frequency; HWD, Hardy-Weinberg disequilibrium.

**Table S3** Validation testing for *TRIB3* rs6037475 on blood pressure reduction in patients with hypertension after the felodipine or placebo treatment

SNP_ID	P <sub>F</sub> (n=261)	P <sub>P</sub> (n=310)	P <sub>all</sub> (n=571)
SBP			
rs6037475 (T>C)	0.062	0.99	0.17
DBP			
rs6037475 (T>C)	0.021	0.52	0.12
MAP			
rs6037475 (T>C)	0.061	0.79	0.33

P<sub>F</sub> present as HCTZ + felodipine treatment group; P<sub>P</sub> present as HCTZ + placebo treatment group. MAP was calculated as MBP = DBP+(SBP-DBP)/3. *TRIB3*, *tribbles homolog 3*; SNP, single nucleotide polymorphism; DBP, diastolic blood pressure; SBP, systolic blood pressure; MAP, mean arterial pressure.

**Table S4** Blood pressure response between the felodipine or placebo treatment groups according to *TRIB3* rs6037475 genotype

Variables	Genotype	HCTZ + felodipine (n=397), mean (mmHg)	HCTZ + placebo (n=433), mean (mmHg)	P
TRIB3 rs6037475 (SBP-changes)	TT (ref)	-17.8±0.21	-12.1±0.22	0.005
	TC	-15.9±0.22	-13.1±0.18	0.13
	CC	-10.2±0.74	-9.6±0.50	0.73
TRIB3 rs6037475 (DBP-changes)	TT (ref)	-10.2±0.23	-7.4±0.14	0.019
	TC	-8.5±0.17	-7.3±0.11	0.19
	CC	-4.6±0.50	-5.3±0.32	0.54
TRIB3 rs6037475 (MAP-changes)	TT (ref)	-12.7±0.14	-8.9±0.22	0.006
	TC	-10.9±0.16	-9.2±0.18	0.15
	CC	-6.5±0.54	-6.7±0.50	0.90

MAP was calculated as  $MBP = DBP + (SBP - DBP) / 3$ . *TRIB3*, *tribbles homolog 3*; HCTZ, hydrochlorothiazide; DBP, diastolic blood pressure; SBP, systolic blood pressure; MAP, mean arterial pressure.