EBioMedicine 13 (2016) 181–189

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Assessment of Human Tribbles Homolog 3 Genetic Variation (rs2295490) Effects on Type 2 Diabetes Patients with Glucose Control and Blood Pressure Lowering Treatment

Fazhong He [a,b], Mouze Liu [a,b], Zhangren Chen [a,b], Guojing Liu [a,b], Zhenmin Wang [a,b], Rong Liu [a,b], Jianquan Luo [b], Jie Tang [a,b], Xingyu Wang [c], Xin Liu [c], Honghao Zhou [a,b], Xiaoping Chen [a,b], Zhaoqian Liu [a,b], Wei Zhang [a,b,*]

[a] Department of Clinical Pharmacology, Xiangya Hospital, Central South University, Changsha 410008, China
[b] Hunan Key Laboratory of Pharmacogenetics, Institute of Clinical Pharmacology, Central South University, Changsha 410078, China
[c] Beijing Hypertension League Institute, 24 Shijingshan Road, Beijing 100043, China

ARTICLE INFO

Article history:
Received 1 September 2016
Received in revised form 8 October 2016
Accepted 18 October 2016
Available online 20 October 2016

Keywords:
TRIB3
Type 2 diabetes
Hypertension
Pharmacogenetics
Individualized drug therapy

ABSTRACT

Effects of human tribbles homolog 3 (TRIB3) genetic variation (c.251 A > G, Gln84Arg, rs2295490) on the clinical outcomes of vascular events has not been evaluated in patients with type 2 diabetes after blood pressure lowering and glucose controlling treatment. We did an analysis of a 2 × 2 factorial (glucose control axis and blood pressure lowering axis) randomized controlled clinical trial at 61 centers in China, with a follow-up period of 5 years. The major vascular endpoints were the composites of death from cardio-cerebral vascular diseases, non-fatal stroke and myocardial infraction, new or worsening renal and diabetic eye disease. A total of 1884 participants were included in our research with a 4.8 years median follow-up. For glucose lowering axis, patients with *TRIB3* (rs2295490) AA (n = 609) genotype exhibited significantly reduced risk of major vascular events compared with AG + GG (n = 335) genotype carriers (Hazard ratio 0.72, 95% CI 0.55–0.94, $p = 0.016$), Paradoxically, the risk of vascular events were significantly increased in patients with AA (n = 621) compared to AG + GG (n = 319) genotype for intensive glucose control (Hazard ratio 1.46, 95% CI, 1.06–2.17, 35 $p = 0.018$). For blood pressure lowering axis, marginally significant difference was found between *TRIB3* variant and coronary events. Our findings suggest that good glucose and blood pressure control exhibited greater benefits on vascular outcomes in patients with *TRIB3* (rs2295490) G allele.

1. Introduction

Recently, China faces an increasing burden of type 2 diabetes and its vascular complications with approximately 11.6% of overall prevalence in adults (Xu et al., 2013). People with diabetes impose about 3.38 times higher cost than those with normal glucose intolerance, while the cost will be 3.75 times higher for patients with longer duration of diabetes (≥10 years) compared with those with shorter duration (≤5 years) due to its complications (Yang et al., 2012). Therefore, it's important to conduct precise strategies to treat patients with diabetes effectively. Actually, clinical trials demonstrated that more intensive blood pressure lowering and glucose control yielded evident clinical benefits for diabetes patients with vascular endpoints (Zoungas et al., 2014).

On the other hand, inter-individual variation of drug response was observed in prophylactic medications for diabetes and its comorbidities (Tkac, 2015), which may be partially explained by accumulating the pharmacogenetics evidence. However, the influence of common genetic variations on the individual difference in drug response or clinical endpoints need to be confirmed by large and long-term follow-up studies, because of the progressive feature of type 2 diabetes mellitus (T2DM), and the mechanisms in molecular levels that related to clinical heterogeneity phenotype remains unclear.

Human tribbles homolog 3 (TRIB3, also called NIPK, SINK, TRB3, SKIP3) is a pseudokinase that can inhibit Akt by physically occupying its phosphorylation sites, which plays a pivotal role on subsequent insulin signaling (Du et al., 2003). Previous reports indicated that TRIB3 has a ubiquitous cellular functions by interacting with a host of molecules establishing a mechanic link between metabolic phenotypes and cardiovascular risk traits (Wang et al., 2012; Ti et al., 2011), renal diseases (Ding et al., 2014), tumor progression (Hua et al., 2015) in T2DM. Interestingly, in vitro study data showed *TRIB3* genetic variation (c.251 A > G, Gln84Arg, rs2295490) does not affect its protein level, but impacts on insulin-stimulated Akt (Thr308, Ser473) and eNOS (Ser1177) phosphorylation, which results in 2 to 3 times of decreased eNOS activity and nitric oxide production (Andreozzi et al., 2008). On another hand, valsartan (an antihypertensive drug), could

* Corresponding author at: Pharmacogenetics Research Institute, Institute of Clinical Pharmacology, Xiangya School of Medicine, Central South University, No.110, Xiangya Road, Changsha, Hunan 410078, China.
E-mail address: yjsd2003@163.com (W. Zhang).

http://dx.doi.org/10.1016/j.ebiom.2016.10.025

significantly down-regulate the expression of *TRIB3* mRNA levels and improve the cardiac functions in rats with diabetic cardiomyopathy (Zhang et al., 2006). Moreover, The presence of the gain-of-function mutation in *TIRB3*(rs2295490) is one of a leading cause of TRIB3 over-activity and subsequent Akt inhibition, which has linked to increased risk of cardiovascular diseases by metabolic syndrome, endothelial and/or cardiac dysfunction (Prudente et al., 2012). Therefore, we considered that TRIB3 may have an impartible role in mediating the development of the diabetic vascular complications by regulating insulin signal transduction pathway and (or) by regulating blood pressure homeostasis in patients with type 2 diabetes. And the relationship between *TRIB3* genetic variation and diabetes and its vascular complications merits further attention.

The achievement of therapeutic goals for plasma glucose, lipid and blood pressure levels is important to decrease the vascular diseases risk in patients with diabetes (Li et al., 2015). Although some evidence showed that a common *TRIB3* genetic variation (rs2295490) was a risk factor for diabetic nephropathy, atherosclerosis, and coronary artery disease (Prudente et al., 2005; De Cosmo et al., 2007; Formoso et al., 2011; Gong et al., 2009), some low-frequency genetic variants, which are detected by re-sequencing of *TRIB3*, can partially account for cardiovascular clinical outcomes in diabetes (Prudente et al., 2015). However, systematical study on the relationship between *TRIB3*genetic variant (rs2295490) and antidiabetic or antihypertensive drug response, vascular complications, especially in a large and long-term follow-up T2DM clinical trial cohort have not been reported. The aim of this study is to explore the effects of *TRIB3* genetic variation (rs2295490) on the clinical outcomes of vascular events after blood pressure lowering and glucose controlling treatment.

2. Methods

2.1. Patients

We conducted a retrospective study on the cohort from Action in Diabetes and Vascular disease: preterAx and diamicroN-MR Controlled Evaluation (ADVANCE) clinical trial at 61 centers in China, with a follow-up period of 5 years. Approval to conduct the trial was obtained from the ethics committee of each study center, and all participants provided written informed consent (registration number NCT00145925). Detailed rationale, design, follow-up schedule and clinical endpoints of ADVANCE trial have been described in previous studies (ADVANCE Management Committe, 2001a, 2001b). In brief, it was a 2 × 2 factorial randomized controlled trial. Patients with type 2 diabetes were randomly assigned (1:1) to receive perindopril-indapamide or matching placebo for blood pressure lowering, and modified-release gliclazide based intensive or local standard therapy for glycaemic control. For blood lowering cohort, participants were treated for 6 weeks as run-in period with combination of perindopril and indapamide, then randomly grouped into fixed combination regimen (perindopril/indapamide, initially 2.0/0.625 mg daily, increasing to 4.0/1.25 mg daily after 3 months) or matching placebo. For glucose controlling group, an open label, randomized protocol was implemented to an intensive glucose control or to local standard therapy based on local guidelines. The intensive glucose control was defined as the use of gliclazide modified release based regimen (30–120 mg daily) and other oral agents, then insulin aiming for a hemoglobin A1c (HbA1c) value of 6.5% or lower. The local standard treatment was defined as the patients who continue with their usual glucose control regimens, which may include any therapy except the use of gliclazide.

The major vascular endpoints include death from cardio-cerebral vascular diseases, nonfatal stroke or nonfatal myocardial infraction, and new or worsening renal or diabetic eye disease. Other vascular events such as cerebrovascular events (include death due to cerebrovascular disease, stroke, transient ischemic attack, and subarachnoid hemorrhage), coronary events (include myocardial infarction, angina pectoris, myocardial ischemia, and sudden death), heart disease (coronary heart disease, heart failure, atrial fibrillation), new or worsening microalbuminuria, and visual deterioration were also evaluated both jointly and separately. In our study, there was no other pre-specified criterion for the levels of blood pressure, HbA1c or other baseline clinical

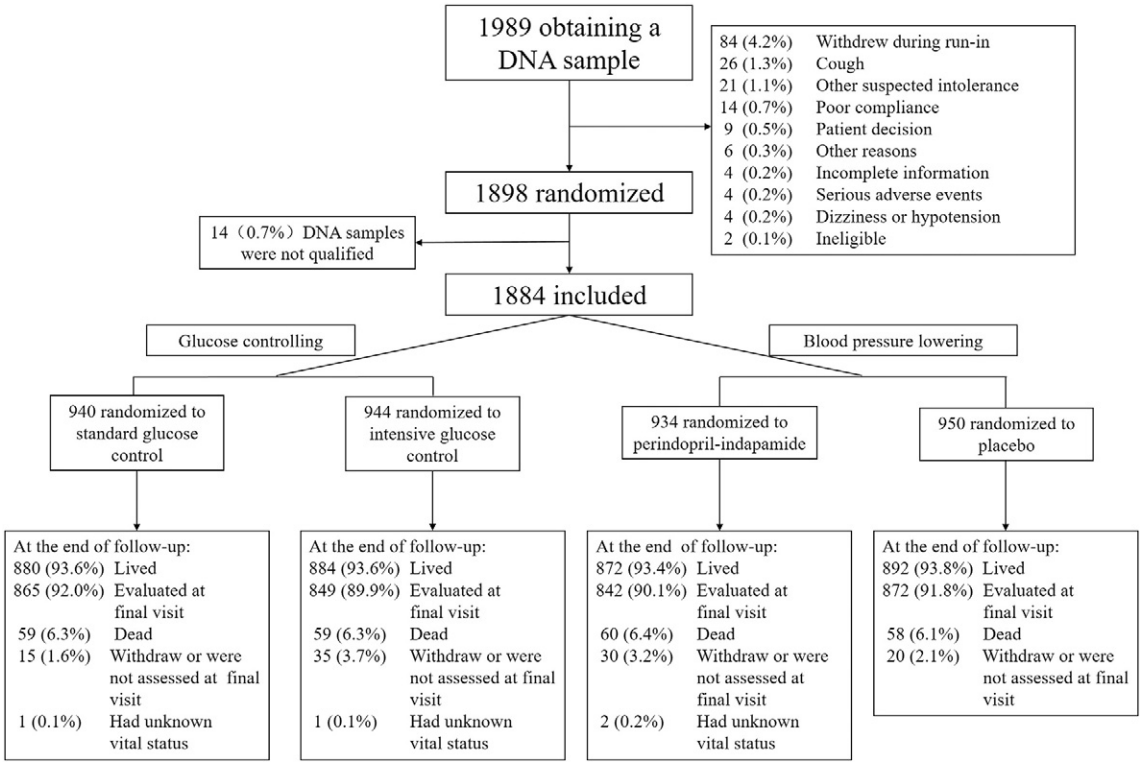

Fig. 1. Outline of DNA sample collection, randomization, and follow-up information of participants.

Table 1
Baseline characteristics of all participants in the subgroup according to *TRIB3* (251, A > G) genotype in the blood pressure lowering or glucose controlling groups.

Variable	Active drug (n = 934)		Placebo (n = 950)		Intensive control (n = 940)		Standard control (n = 944)	
	AA	AG/GG	AA	AG/GG	AA	AG/GG	AA	AG/GG
Age (years), mean (SD)	65(6)	65(5)	65(6)	64(6)	65(6)	65(6)	65(6)	65(6)
Male, n (%)	299(49)	165(51)	342(55)	163(50)	315(51)	152(48)	326(54)	176(53)
Duration of diabetes† (years), median (IQR)	7(3-11)	7(3–11)	7(3–11)	7(3−12)	7(3–12)	7(3–11)	7(3–11)	7(3–12)
Previous vascular disease								
History of major macrovascular disease, n (%)	109(18)	60(18)	120(19)	67(20)	117(19)	60(19)	112(18)	67(20)
History of myocardial infarction, n (%)	23(4)	9(3)	24(4)	13(4)	22(4)	9(3)	25(4)	13(4)
History of stroke, n (%)	90(15)	51(16)	103(17)	56(17)	102(16)	52(16)	91(15)	55(16)
History of major microvascular disease, n (%)	80(13)	52(16)	91(15)	39(12)	78(13)	46(14)	93(15)	45(13)
History of macroalbuminuria ‡, n (%)	46(8)	26(8)	52(8)	21(6)	44(7)	22(7)	54(9)	25(8)
History of microvascular eye disease§, n (%)	37(6)	30(9)	44(7)	21(6)	38(6)	27(9)	43(7)	24(7)
Blood pressure assessment								
Systolic blood pressure (mm Hg), mean (SD)	140(21)	139(22)	141(22)	138(19)	140(21)	137(21)	141(21)	140(21)
Diastolic blood pressure (mm Hg), mean (SD)	79(11)	78(11)	79(11)	78(10)	79(11)	78(11)	79(11)	77(11)
History of currently treated hypertension, n (%)	390(64)	216(66)	396(64)	211(64)	389(63)	212(67)	397(65)	215(64)
Blood glucose assessment								
Glycated hemoglobin (%), mean (SD)	7.7(1.8)	7.8(1.8)	7.7(1.7)	7.6(1.8)	7.7(1.8)	7.8(1.9)	7.7(1.8)	7.7(1.8)
Fasting plasma glucose (mmol/l), mean (SD)	8.7(3.0)	9.0(3.3)	8.5(2.8)	8.7(2.9)	8.7(3.0)	8.9(3.2)	8.5(2.8)	8.8(3.1)
Other major risk factors								
Current smokers, n (%)	139(23)	73(22)	158(25)	71(22)	150(24)	69(22)	147(24)	75(22)
Total cholesterol (mmol/L), mean (SD)	5.4(1.2)	5.4(1.2)	5.3(1.2)	5.3(1.3)	5.4(1.2)	5.4(1.3)	5.4(1.2)	5.2(1.2)
High-density lipoprotein (mmol/L), mean (SD)	1.3(0.5)	1.3(0.5)	1.3(0.5)	1.3(0.5)	1.3(0.5)	1.3(0.5)	1.3(0.5)	1.3(0.5)
Low-density lipoprotein (mmol/L), mean (SD)	3.3(1.0)	3.2(1.0)	3.2(1.0)	3.2(1.1)	3.2(1.1)	3.3(1.1)	3.2(1.0)	3.1(1.0)
Triglyceride (mmol/l), mean (SD)	2.0(1.6)	2.1(1.5)	2.0(1.5)	2.0(1.4)	1.9(1.4)	2.2(1.7)	2.1(1.7)	1.9(1.2)
Urinary albumin:creatinine ratio (mg/mmol), median (IQR)	2.4 (1.1–7.5)	2.3 (1.2–8.1)	2.5 (1.1–6.7)	2.4 (1.1–6.2)	2.4 (1.2–7.0)	2.6 (1.1–8.0)	2.4 (1.1–7.0)	2.1 (1.1–6.9)
Body-mass index (kg/m2), mean (SD)	25(3)	25(3)	25(3)	25(3)	25(3)	25(3)	25(3)	25(3)

† Durations of diabetes are shown as the age at first visit minus age when diabetes first diagnosed. ‡ Urinary albumin-creatinine ratio > 33.9 mg/mmol was defined as macroalbuminuria. § Proliferative diabetic retinopathy, retinal photocoagulation therapy, macular oedema, or blindness related to diabetes are defined as microvascular eye diseases.

characteristics at entry. A total of 1884 patients from 61 clinical centers were successfully genotyped in *TRIB3* (rs2295490) by Sanger sequencing.

2.2. Genotyping Procedure for TRIB3

Genotyping of *TRIB3* rs2295490 was determined by PCR-direct sequencing. The PCR primers for *TRIB3* (rs2295490) were as follows: the sense primer, 5′GTTGCCCCTGA-GCCCACCTACT3′; and the antisense primer, 5′TCCCTGGATGCTTCCCCACTAA3′, with a production length of 286 bp. The reaction mixture (25 μl) contained: 10× PCR buffer (2.5 μl), 10× dNTP (2.5 μl), 10 μM of each of the sense and antisense primers (0.5 μl), H_2O (16.8 μl), g - DNA (2 μl), Taq-ase (0.2 μl). Temperature cycling was proceeded as follows: initial denaturation for 5 min at 94 °C, followed by 36 cycles of 30 s at 94 °C, annealing at 57 °C for 30 s, and elongation at 72 °C for 30 s, and a terminal extension for 5 min. Genotypes were determined without knowledge of the status of patients and 10% blinded. Random DNA samples from the patients were

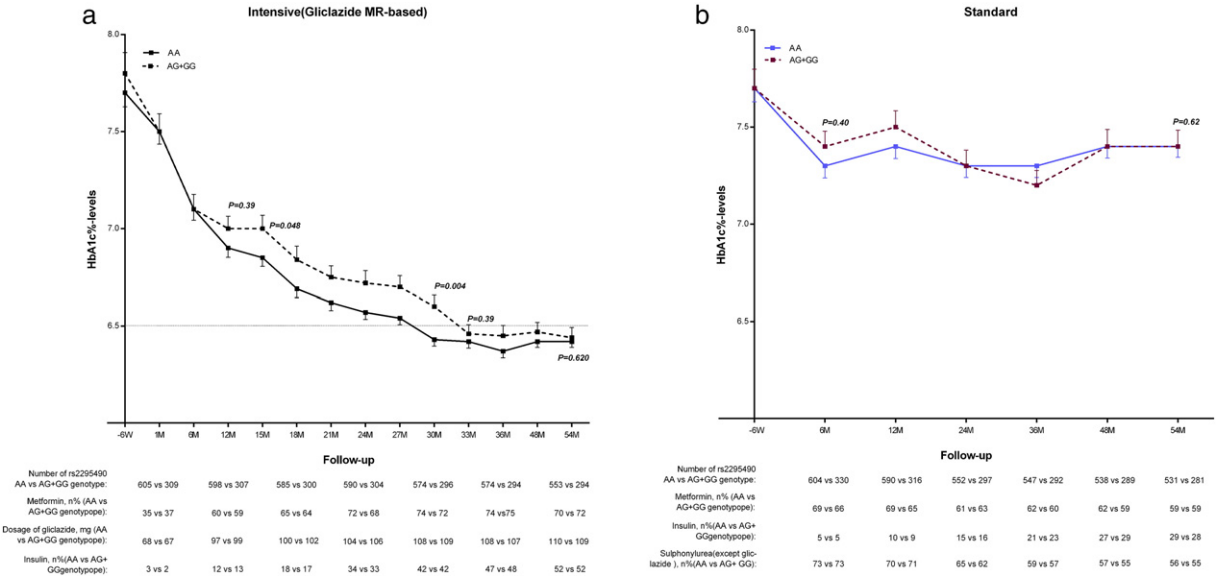

Fig. 2. Effects of *TRIB3* (rs2295490) genetic variation on HbA_{1c} (%) levels at baseline and during follow-ups between intensive and standard glucose control groups. Data are shown as mean ± SEM, *p*-values were estimated from stepwise linear regression model with adjustment for sex, age, duration of the disease, body mass index (BMI), combined medication and drug dosage between *TRIB3* (rs2295490) AA and AG + GG genotypes at the visit time.

genotyped twice with 100% concordance. Sequencing was assisted by Shanghai Majorbio Bio-pharmTechnology Company.

2.3. Statistical Analysis

All the analyses were performed with SPSS (version 20.0 for windows; Chicago, IL). Allele frequencies were determined by the genotypes of all the participants. Continuous data are presented as mean values ± SD, and frequencies or percentages are for categorical variables. The Hardy–Weinberg equilibrium analysis was carried out for the study participants using the χ^2-test. Differences in baseline characteristics among the phenotypes were assessed by independent-samples *t*-test or Wilcoxon rank sum test, appropriately. General linear model-multivariate ANOVA and linear regression analysis was performed in plasma glucose, lipid levels and blood pressure between *TRIB3* (rs2295490) AA, AG + GG genotype with adjustment for sex, age, duration of the disease, body mass index (BMI), combined medication and drug dosage. Cox backword (LR: entry $p = 0.05$ and removal $p = 0.10$) regression model adjustment by history of vascular disease, baseline of the clinical biochemical data, sex, age, duration of the disease, combined medication and drug dosage was used to investigate the relationship between genetic variation and the risk of clinical outcomes of vascular event endpoints. A two-tailed *P*-value < 0.05 was considered significant.

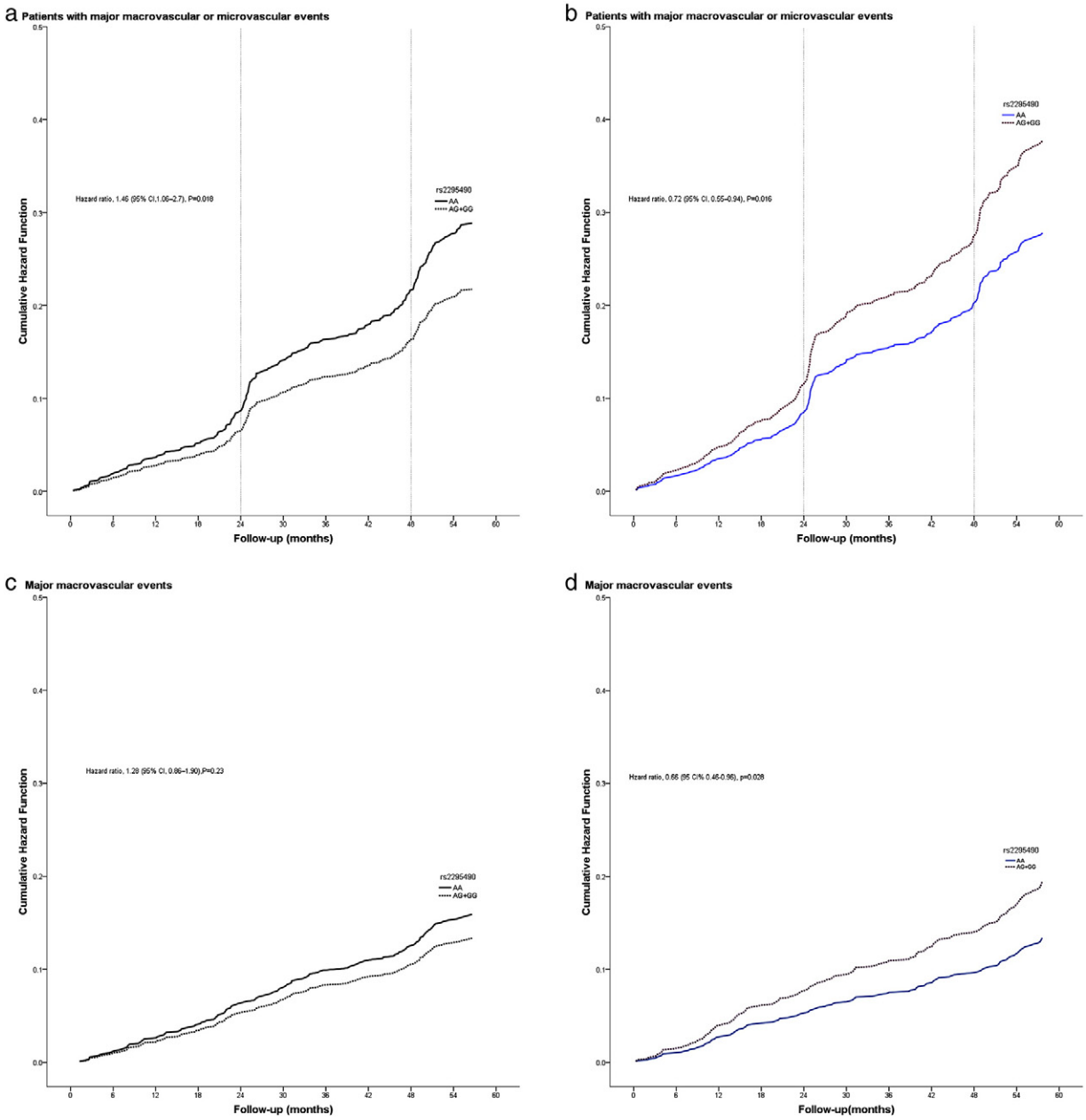

Fig. 3. Effects of *TRIB3* (rs2295490) genetic variation on the cumulative hazard function of clinical endpoints according to glucose control strategy. The major vascular endpoints include death from cardiovascular disease, stroke or myocardial infraction, and new or worsening renal or diabetic eye disease. Other vascular events such as cerebrovascular events (include death due to cerebrovascular disease, stroke, transient ischemic attack, and subarachnoid hemorrhage), coronary events (include myocardial infarction, angina pectoris, myocardial ischemia, and sudden death). Vertical broken lines indicate additional information on microvascular events (diabetes related nephropathy and retinopathy) were obtained at 24-month and 48-month visits. And the event times were recorded as the visit dates. The curves were truncated at Month 57, by which time 99% of events had occurred. The effects of treatment between *TRIB3* (rs2295490) AA and AG + GG genotypes (hazard ratios and *p*-values) were estimated from survival-cox regression models with backward LR adjustment for baseline available data.

3. Results

3.1. Genotyping and Characteristics of Patients

A total of 1898 Chinese participants were enrolled from May 2002 to December 2002 in ADVANCE trial (China centers) from 61 hospitals, with an median of 4.8 years follow-up duration in 1714/1884 (91.0%) participants who were successfully completed at scheduled end of follow-up (Fig. 1), and 1884/1898 (99.3%) of DNA samples from these patients passed quality control for genotyping of *TRIB3* (rs2295490). Genotyping data of *TRIB3* (rs2295490) in our study cohort showed a minor allele (G) frequency of 17.1%, the (AA, AG and GG) genotype frequencies were 1230/1884 (65.3%), 581/1884(30.8%) and 73 (3.9%), respectively, which were in Hardy-Weinberg equilibrium across the randomized groups (Table S1 in the Supplementary Appendix). No significant difference was observed across the baseline characteristics at entry between AA and AG + GG genotype of *TRIB3* (rs2295490) in blood pressure lowering or glucose controlling axis (Table 1).

3.2. Glucose Controlling Axis

For the intensive glucose controlling group, the mean glycosylated HbA_{1c} level was 6.8% throughout the follow-up period, and it was higher in patients with AG + GG genotypes (6.7%) compared to AA genotype (6.6%) after a follow-up of 6 months, while the difference observed was not significant until the period between 15 and 30 months (AA vs AG + GG, 6.6% vs 6.8%) (Fig. 2a). For the standard glucose control group, the mean glycosylated HbA_{1c} levels was 7·4% throughout the follow-up period, and it showed no significant difference between AA and AG + GG genotypes (AA vs AG + GG, 7.4% vs 7.4%) after randomized treatment (Fig. 2b). Additionally, in the intensive and standard glucose treatment groups, fast plasmid glucose and HbA1c levels between AA

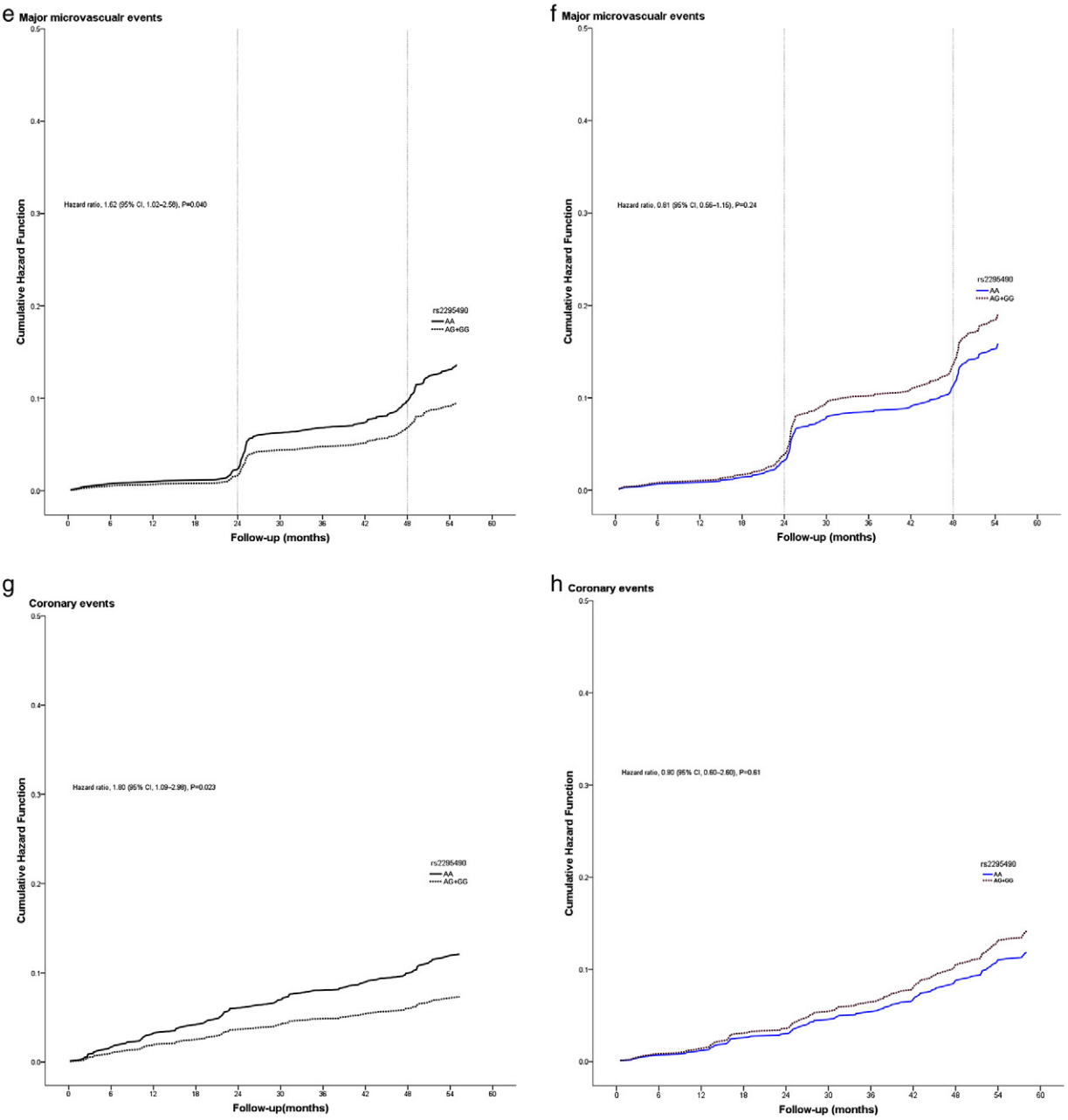

Fig. 3 (*continued*).

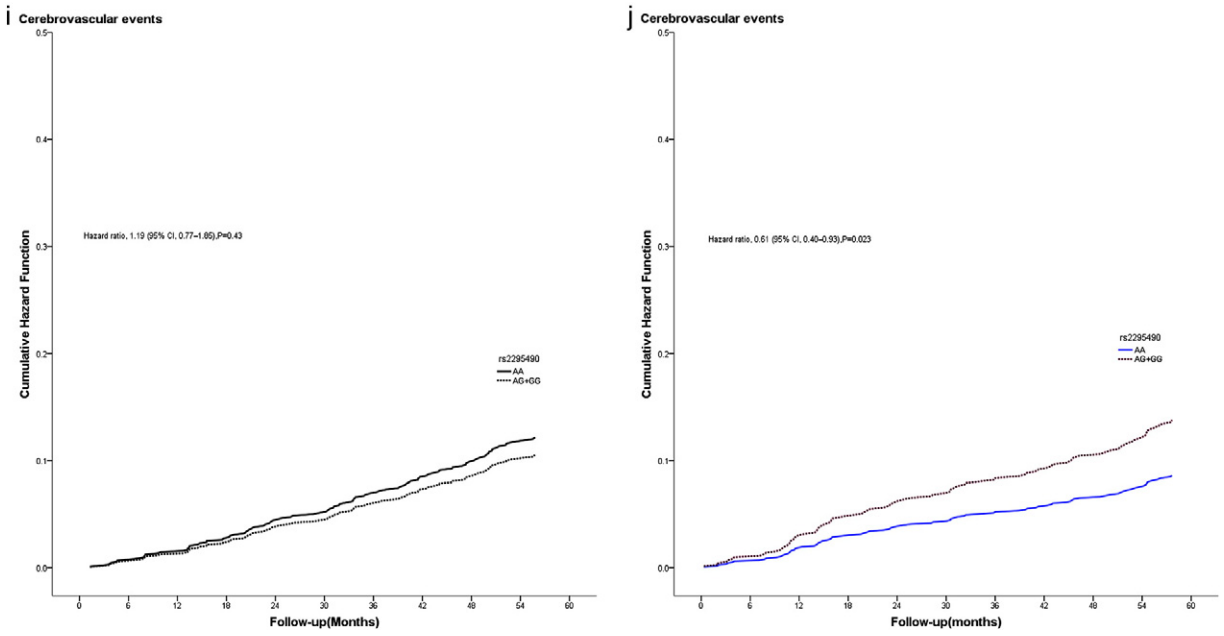

Fig. 3 (*continued*).

and AG + GG genotypes showed a similar trend, although not significant (data not shown).

A total of 458 patients had major macrovascular or microvascular events during the visits: 152/621 (24.5%) patients with AA genotype and 61/319 (19.1%) with AG + GG genotype (hazard ratio, 1.46; 95% CI, 1.06 to 2.17; $p = 0.018$) were observed in the intensive glucose control group (Fig. 3a); 143/609 (23.5%) patients with AA genotype and 102/335 (30.4%) with AG + GG genotype (hazard ratio, 0.72; 95% CI, 0.55 to 0.94; $p = 0.016$) were observed in the standard glucose control group (Fig. 3b). Additionally, *TRIB3* (rs2295490) genetic variation showed an inverse effect on the major macrovascular events (Fig. 3c and d) and microvascular events (Fig. 3e and f) when compared between the two groups, and the effects on the secondary clinical outcomes of coronary (Fig. 3g and h) and cerebrovascular events (Fig. 3i and j) are similar as well. There was no significant difference in other major and secondary macrovascular or microvascular events when analyzed jointly or separately, except for the secondary clinical endpoints of new or worsening microalbuminuria and death from any cause (Table 2).

3.3. Blood Pressure Controlling Axis

Over the duration of follow-ups, the mean systolic blood pressure (SBP) was 132.8 mm Hg and diastolic blood pressure (DBP) 74.6 mm Hg in perindopril–indapamide fix tablet treatment group; while the mean SBP was 137.8 mm Hg and DBP 76.9 mm Hg in placebo matching treatment group. In the randomized active blood lowering treatment group, compared with *TIRB3* (rs2295490) AA genotype carriers, patients with AG + GG genotype had a mean decrease of DBP of 0.8 mm Hg. Marginally significant difference ($p = 0.063$) between the two groups at the end of follow-up was observed (Fig. 4a). Conversely, in matched placebo treatment group, the mean DBP was increased by

Table 2
Effects of *TRIB3* (c.251, A > G) genetic variation on patients with major and secondary outcomes according to glucose-control strategy.

Subgroup	Intensive, n (%)		HR (95% CI), *P*-value	Standard n (%)		HR (95% CI), *P*-value
	AA	AG + GG		AA	AG + GG	
Major clinical outcomes						
Combined major macro- and micro-vascular events	152(24.5)	61(19.1)	1.46(1.06–2.17), 0.018	143(23.5)	102(30.4)	0.72(0.55–0.94), 0.016
Major macrovascular events	89(14.3)	39(12.2)	1.28(0.86–1.90), 0.22	73(12.0)	57(17.0)	0.66(0.46–0.96), 0.028
MI	14(2.3)	7(2.2)	0.91(0.34–2.46), 0.86	13(2.1)	13(3.9)	0.47(0.21–1.04), 0.061
Stroke	60(9.7)	28(8.8)	1.14(0.72–0.82), 0.57	55(9.0)	40(11.9)	0.70(0.45–1.06), 0.093
Death from Cardio-cerebral vascular cause	27(4.3)	8(2.5)	2.27(0.91–5.65), 0.078	21(3.4)	13(3.9)	0.78(0.33–1.84), 0.57
Major microvascular events	75(12.1)	28(8.8)	1.62(1.02–2.58), 0.040	86(14.1)	55(16.4)	0.81(0.56–1.15), 0.24
New or worsening nephropathy	28(4.5)	9(2.8)	1.57(0.70–3.54), 0.27	36(5.9)	25(7.5)	0.77(0.44–1.34), 0.35
New or worsening retinopathy	57(9.2)	21(6.6)	1.65(0.97–2.82), 0.065	57(9.4)	36(10.7)	0.924(0.60–1.43), 0.72
Secondary clinical outcomes						
Coronary events	69(11.1)	22(6.9)	1.80(1.09–2.98), 0.023	65(10.7)	42(12.5)	0.90 (0.60–2.60), 0.61
Heart disease	76(12.2)	29(9.1)	1.42(0.91–2.21), 0.12	80(13.1)	50(14.9)	0.91(0.64–1.29), 0.60
Cerebrovascular events	68(11.0)	31(9.7)	1.19(0.77–1.85), 0.43	48(7.9)	41(12.2)	0.61(0.40–0.93), 0.023
All macrovascular events	144(23.2)	63(19.7)	1.26(0.93–1.71), 0.14	128(21.0)	85(25.4)	0.82(0.61–1.09), 0.16
Visual deterioration	127(20.5)	56(17.6)	1.14(0.83–1.57), 0.42	87(14.3)	46(13.7)	1.03(0.71–1.49), 0.88
New or worsening microalbuminuria	23(3.7)	17(5.3)	0.60(0.32–1.15), 0.12	36(5.9)	11(3.3)	2.23(1.08–4.60), 0.030
All microvascular events	211(34.0)	90(28.2)	1.25(0.97–1.62), 0.079	188(30.9)	99(29.6)	1.06(0.82–1.36), 0.66
Death from any cause	45(7.2)	14(4.4)	1.96(1.02–3.75), 0.044	34(5.6)	22(6.6)	0.86(0.48–1.54), 0.62
Neuropathy	29(4.7)	11(3.4)	1.35(0.67–2.73), 0.41	24(3.9)	11(3.3)	1.28(0.60–2.70), 0.53

HR = Hazard Ratio, the effects of treatment between *TRIB3* (c. 251, A > G) AA and AG/GG genotypes (hazard ratios and p-values) were estimated from survival-cox regression models with backward LR adjustment for baseline available data.

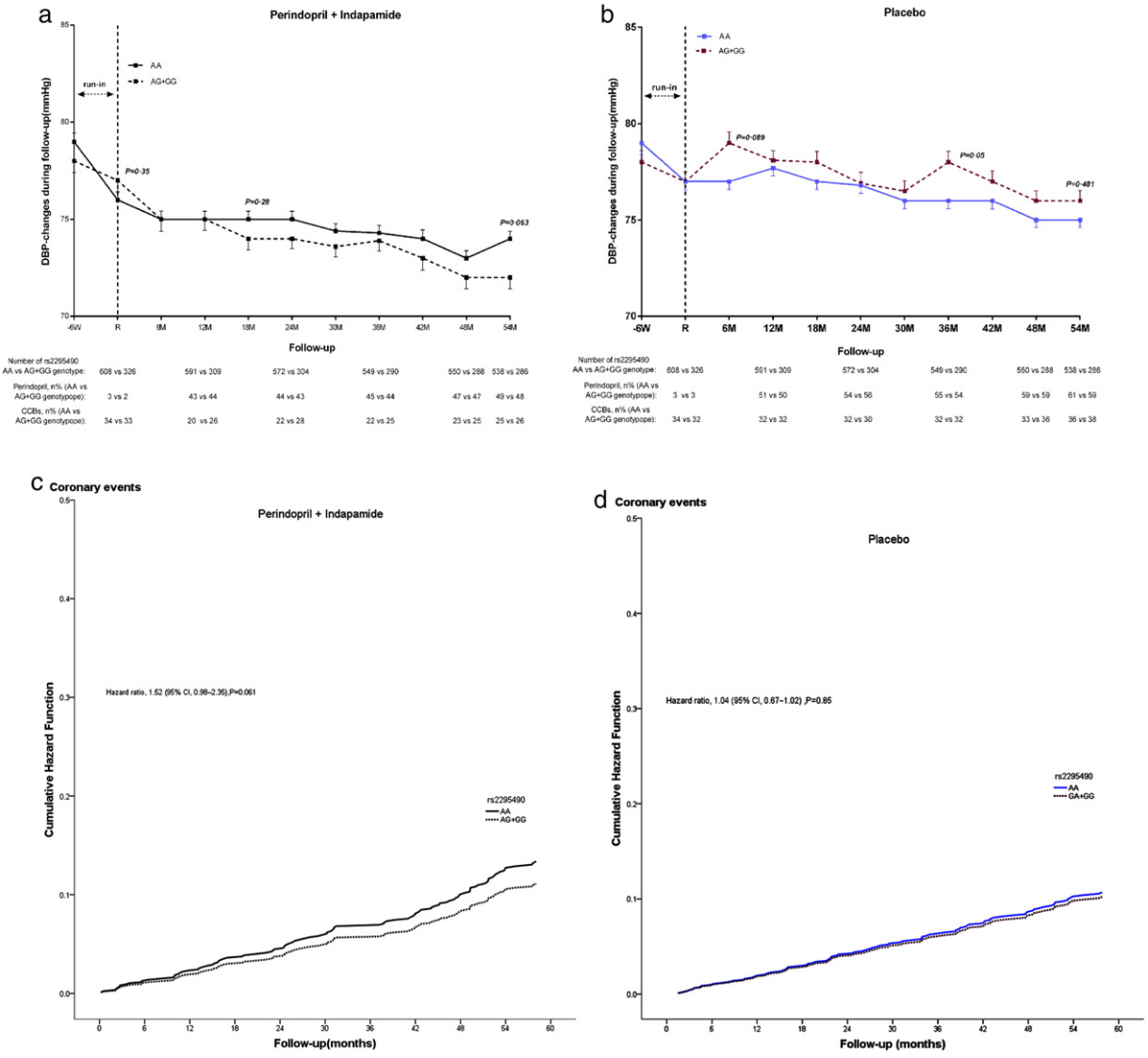

Fig. 4. Effects of *TRIB3* (rs2295490) genetic variation on diastolic blood pressure response and coronary clinical outcomes during follow-up between perindopril/indapamide and placebo treatment groups. Data of Panel a and b are shown as mean ± SEM, *p*-values were estimated from stepwise linear regression model with adjustment for sex, age, duration of the disease, body mass index (BMI), combined medication and drug dosage between *TRIB3* (rs2295490) AA and AG + GG genotypes at the visit time. Coronary events include myocardial infarction, angina pectoris, myocardial ischemia, and sudden death. The effects of treatment on coronary events between *TRIB3* (rs2295490) AA and AG + GG genotypes (hazard ratios and *p*-values) were estimated from survival-cox regression models with backward LR adjustment for baseline available data (panel c and d).

1.0 mm Hg respectively (Fig. 4b). However, SBP presented no remarkable difference between the AA and AG + GG groups (data not shown).

For blood pressure lowering axis, there was no significant effect of *TRIB3* (rs2295490) genetic variation on the risk of any major and secondary clinical outcomes between the active and placebo treatment groups (Table S2 in the Supplementary Appendix). However, it is worth noting that the genetic variation was weakly correlated to the risk of total coronary events in the active treatment group (hazard ratio, 1.52; 95% CI, 0.98 to 2.35; $p = 0.061$) (Fig. 4c), but not in the matched placebo treatment group (hazard ratio, 1.04, 95% CI, 0.67 to 1.02; $p = 0.85$) (Fig. 4d).

4. Discussion

For the intensive glucose control axis, as data shown in Fig. 2, *TRIB3* (rs2295490) genetic variation had a significant influence on patients that were assigned to the intensive glucose control group, but not on patients assigned to the standard glucose control group. For the intensive glucose control group, the gliclazide MR-based, along with other mainly prescribed drugs such as metformin, insulin preparations were required to make the glycated hemoglobin value target to 6.5% or lower. For the standard glucose control group, however, a routine administration of metformin and sulphonylureas (except gliclazide) glucose control strategies was recommended. The main difference in the clinical medication between the two groups was that the proportion of insulin combination treatment. Interestingly, TRIB3 is a pseudo-kinase that plays a pivotal role in the insulin signaling transduction pathway (Du et al., 2003), and *TRIB3* (rs2295490) G allele can lead to a gain-of-function amino acid substitution in the insulin target tissues (Liew et al., 2010). In addition, the genetic variation dose not directly affect fast blood glucose levels, but by altering the interplay between insulin sensitivity and secretion (Prudente et al., 2010), our results were in accordance with these findings.

Besides, our data showed that *TRIB3* (rs2295490) genetic variation had a significant clinical relevance to macrovascular or microvascular clinical outcomes. We were surprised to find that *TRIB3* (rs2295490) genetic variation played a reverse role between intensive and standard glucose control groups (Fig. 5). Subgroup analysis further confirmed that patients with G allele had greater benefits in the macrovascular and microvascular events from patients with intensive glucose

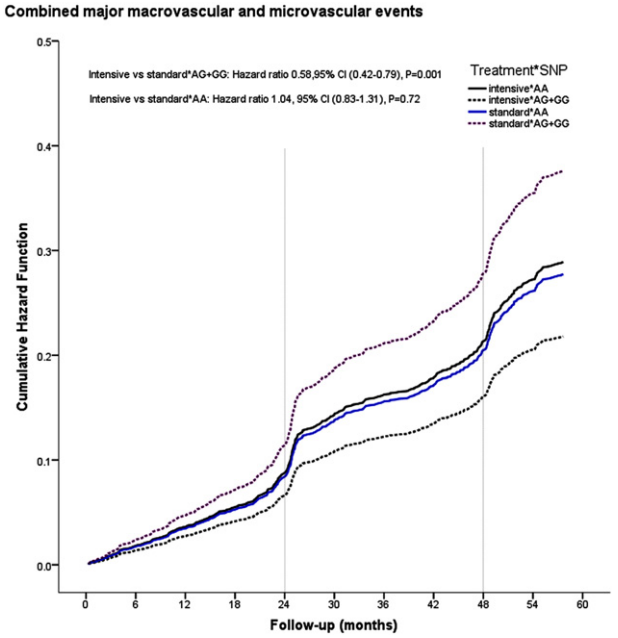

Fig. 5. Analysis of *TRIB3* (rs2295490) genetic variation and treatment-specific interaction in glycaemic control arm. Vertical broken lines indicate additional information on microvascular events (diabetes related nephropathy and retinopathy) were obtained at 24-month and 48-month visits. And the event times were recorded as the visit dates. The curves were truncated at Month 57, by which time 99% of events had occurred. The effects of *TRIB3* (rs2295490) genotypes and treatment-specific interaction in glycaemic control arm (hazard ratios and *p*-values) were estimated from survival-cox regression models with backward LR adjustment for baseline available data.

treatment, mainly as a consequence of a reduction in the major microvascular and all coronary clinical outcomes (Fig. 3e and g). For the patients in the standard glucose control group, however, the G allele was a risk factor of major macrovascular and cerebrovascular clinical outcomes (Fig. 3d and j). Our sub-center data illustrated that the incidence of major microvascular events was significantly reduced in the intensive glucose control when compared with the standard glucose control (hazard ratio, 0.72; 95% CI, 0.56 to 0.94; $p = 0.016$) (Fig. s1 in the Supplementary Appendix), and it was consistent with the results reported previously (Patel et al., 2008). Data from Zhang et al. have revealed that the induction of TRIB3 by high glucose was reversed to baseline by euglycemia achieved in vivo and in vitro (Zhang et al., 2015). It is suggested that the significant difference in blood glucose levels between intensive and standard glucose control groups may be a leading cause to the reverse role of *TRIB3* (rs2295490) genetic variation between the two groups, while the exact mechanism needs to be confirmed by further research.

For the blood pressure lowering axis, we found that *TRIB3* (rs2295490) genetic variation was not significantly associated with the blood pressure response after a fixed combination therapy of perindopril and indapamide. Interestingly, when taking *TRIB3* (rs2295490) AA genotype as the baseline, patients carrying *TRIB3* (rs2295490) AG + GG genotype had a flip effect on diastolic blood pressure response in a fixed combination of perindopril and indapamide treatment group compared with placebo matching treatment group (Fig. 4a and b). However, there was no significant difference between AA and AG + GG genotypes in the two groups on the incidence of major macrovascular, microvascular, coronary and cerebrovascular outcomes when analyzed jointly or separately. The more frequent use of perindopril, calcium channel blockers (CCBs) (57% and 33% at the end of follow-up, respectively) in the placebo group than the active treatment group (43% and 26% at the end of follow-up, respectively) might be relevant for such results. Details are shown in Fig. 4a and b.

However, in the continuous blood pressure lowering group, irrespective of initial blood pressure levels, *TRIB3* (rs2295490) G allele carriers in the active treatment group got more benefit on coronary events when compared with placebo group, although the difference was not significant. Limited data (Andreozzi et al., 2008; Zhang et al., 2006; Leiria et al., 2013; Chan et al., 2007) and our findings indicate that TRIB3 mediated signaling pathway may be related to blood pressure regulation and its genetic variation can affect the response of blood pressure lowering drugs, subsequently affect cardiovascular event outcomes. At this point, it is necessary to conduct further study.

Recently, investigation of common genetic variability in type 2 diabetes patients with cardiovascular complications from VNDS study showed that the genetic variations in gatekeepers along the insulin signaling pathway (*IRS1*, *ENPP1* and *TRIB3*) were associated with increased coronary heart disease (Trombetta et al., 2016). It is suggested that investigation of genetic variation in other new or key genes, which are related to insulin signaling pathway may have higher clinical value and significance (Menzaghi et al., 2014; Choi et al., 2016). However, up to now, the allele gene frequencies of the related genetic variations in *TRIB3* (except rs2295490 mutation) are rare in Chinese population (<1%). In addition, results from large-scale sequencing did not support any major role of lower-frequency variants in type 2 diabetes neither (Fuchsberger et al., 2016). Therefore, we appropriately selected rs2295490 as candidate single nucleotide polymorphism (SNP) for our study.

5. Conclusion

In this study, our findings include: 1) during follow-up period, a partially difference in glucose and blood pressure response were observed after the intensive and the active treatment between *TRIB3* (rs2295490) AA and AG + GGgenotype, it suggests this gene polymorphism may have a plausible effect on antidiabetic and antihypertensive drug response in patients with T2DM. 2) *TRIB3* (rs2295490) genetic variation was associated with the clinical outcomes of major macrovascular and microvascular complications in T2DM, while the effects of this genetic variation has a reverse role between the intensive and standard glucose control groups, which can be caused by the obvious differences in glucose levels between the two groups. 3) Our findings suggest that good glucose and blood pressure control exhibited greater benefits on vascular outcomes in patients with *TRIB3* (rs2295490) G allele.

Conflicts of Interest

We declare: no competing interests within the submitted manuscript; no other relationships or activities that could appear to have influenced the submitted work.

Author Contributions

FZH, WZ, XYW were responsible for the study design. FZH, ZRC, GJL, ZMW, JQL were responsible for the experiments: FZH, RL, ZRC did the data processing and statistical analysis. FZH, MZL and JT wrote and interpreted the paper. HHZ, XL, ZQL, XYW, XPC, WZ were responsible for data management and contributed reagents and materials.

Acknowledgements and Funding

We appreciate the work and help from the ADVANCE collaborative group. This research was supported by grants from the National Key Research and Development Program (No. 2016YFC0905000, 2016YFC0905001), National High Technology Research and Development Program of China, "863" Project (No. 2012AA02A518), National Natural Science Foundation of China (No. 81522048, 81573511, 81273595), Innovation Driven Project of Central South University (No. 2016CX024) and Central South University Innovation Foundation for

Postgraduate (2015zzts117). The sponsor of the study had no role in study design, data collection, data analysis, data interpretation or writing of the report. The corresponding author had full access to all data in the study.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2016.10.025.

References

ADVANCE Management Committe, 2001a. Rationale and design of the ADVANCE study: a randomised trial of blood pressure lowering and intensive glucose control in high-risk individuals with type 2 diabetes mellitus. Action in diabetes and vascular disease: PreterAx and DiamicroN modified-release controlled evaluation. J. Hypertens. Suppl. 19 (4), S21–S28.

ADVANCE Management Committe, 2001b. Study rationale and design of ADVANCE: action in diabetes and vascular disease—preterax and diamicron MR controlled evaluation. Diabetologia 44 (9), 1118–1120.

Andreozzi, F., Formoso, G., Prudente, S., Hribal, M.L., Pandolfi, A., Bellacchio, E., 2008. TRIB3 R84 variant is associated with impaired insulin-mediated nitric oxide production in human endothelial cells. Arterioscler. Thromb. Vasc. Biol. 28 (7), 1355–1360.

Chan, M.C., Nguyen, P.H., Davis, B.N., Ohoka, N., Hayashi, H., Du, K., Lagna, G., et al., 2007. A novel regulatory mechanism of the bone morphogenetic protein (BMP) signaling pathway involving the carboxyl-terminal tail domain of BMP type II receptor. Mol. Cell. Biol. 27 (16), 5776–5789.

Choi, E., Zhang, X., Xing, C., Yu, H., 2016. Mitotic checkpoint regulators control insulin signaling and metabolic homeostasis. Cell 166 (3), 567–581.

De Cosmo, S., Prudente, S., Andreozzi, F., Morini, E., Rauseo, A., Scarpelli, D., et al., 2007. Glutamine to arginine substitution at amino acid 84 of mammalian tribbles homolog TRIB3 and CKD in whites with type 2 diabetes. Am. J. Kidney Dis. 50 (4), 688–689.

Ding, W.Y., Li, W.B., Ti, Y., Bi, X.P., Sun, H., Wang, Z.H., et al., 2014. Protection from renal fibrosis, putative role of TRIB3 gene silencing. Exp. Mol. Pathol. 96 (1), 80–84.

Du, K., Herzig, S., Kulkarni, R.N., Montminy, M., 2003. TRB3: a tribbles homolog that inhibits Akt/PKB activation by insulin in liver. Science 300 (5625), 1574–1577.

Formoso, G., Di Tomo, P., Andreozzi, F., Succurro, E., Di Silvestre, S., Prudente, S., et al., 2011. The TRIB3 R84 variant is associated with increased carotid intima-media thickness in vivo and with enhanced MAPK signalling in human endothelial cells. Cardiovasc. Res. 89 (1), 184–192.

Fuchsberger, C., Flannick, J., Teslovich, T.M., Mahajan, A., Agarwala, V., Gaulton, K.J., et al., 2016. The genetic architecture of type 2 diabetes. Nature 536 (7614), 41–47.

Gong, H.P., Wang, Z.H., Jiang, H., Fang, N.N., Li, J.S., Shang, Y.Y., et al., 2009. TRIB3 functional Q84R polymorphism is a risk factor for metabolic syndrome and carotid atherosclerosis. Diabetes Care 32 (7), 1311–1313.

Hua, F., Li, K., Yu, J.J., Lv, X.X., Yan, J., Zhang, X.W., et al., 2015. TRB3 links insulin/IGF to tumour promotion by interacting with p62 and impeding autophagic/proteasomal degradations. Nat. Commun. 6 (7951).

Leiria, L.O., Sollon, C., Bau, F.R., Monica, F.Z., D'Ancona, C.L., De Nucci, G., et al., 2013. Insulin relaxes bladder via PI3K/AKT/eNOS pathway activation in mucosa: unfolded protein response-dependent insulin resistance as a cause of obesity-associated overactive bladder. J. Physiol. 591 (9), 2259–2273.

Li, P., Chen, K., Nie, Y., Guo, L.L., Wang, H.B., Wang, S.S., et al., 2015. Association of obesity with glucose, blood pressure, and lipid goals attainment in patients with concomitant diabetes and hypertension. Curr. Med. Res. Opin. 31 (9), 1623–1631.

Liew, C.W., Bochenski, J., Kawamori, D., Hu, J., Leech, C.A., Wanic, K., et al., 2010. The pseudokinase tribbles homolog 3 interacts with ATF4 to negatively regulate insulin exocytosis in human and mouse beta cells. J. Clin. Invest. 120 (8), 2876–2888.

Menzaghi, C., Fontana, A., Copetti, M., Rizza, S., Spoto, B., Buranasupkajorn, P., et al., 2014. Joint effect of insulin signaling genes on all-cause mortality. Atherosclerosis 237 (2), 639–644.

Patel, A., Macmahon, S., Chalmers, J., Neal, B., Billot, L., Woodward, M., et al., 2008. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N. Engl. J. Med. 358 (24), 2560–2572.

Prudente, S., Hribal, M.L., Flex, E., Turchi, F., Morini, E., De Cosmo, S., et al., 2005. The functional Q84R polymorphism of mammalian Tribbles homolog TRB3 is associated with insulin resistance and related cardiovascular risk in Caucasians from Italy. Diabetes 54 (9), 2807–2811.

Prudente, S., Baratta, R., Andreozzi, F., Morini, E., Farina, M.G., Nigro, A., et al., 2010. TRIB3 R84 variant affects glucose homeostasis by altering the interplay between insulin sensitivity and secretion. Diabetologia 53 (7), 1354–1361.

Prudente, S., Sesti, G., Pandolfi, A., Andreozzi, F., Consoli, A., Trischitta, V., 2012. The mammalian tribbles homolog TRIB3, glucose homeostasis, and cardiovascular diseases. Endocr. Rev. 33 (4), 526–546.

Prudente, S., Bailetti, D., Mendonca, C., Mannino, G.C., Fontana, A., Andreozzi, F., et al., 2015. Infrequent TRIB3 coding variants and coronary artery disease in type 2 diabetes. Atherosclerosis 242 (1), 334–339.

Ti, Y., Xie, G.L., Wang, Z.H., Bi, X.L., Ding, W.Y., Wang, J., et al., 2011. TRB3 gene silencing alleviates diabetic cardiomyopathy in a type 2 diabetic rat model. Diabetes 60 (11), 2963–2974.

Tkac, I., 2015. Genetics of drug response in type 2 diabetes. Curr. Diab. Rep. 15 (7), 43.

Trombetta, M., Dauriz, M., Bonetti, S., Travia, D., Boselli, L., Santi, L., et al., 2016. Is common genetic variation at IRS1, ENPP1 and TRIB3 loci associated with cardiometabolic phenotypes in type 2 diabetes? An exploratory analysis of the Verona newly diagnosed type 2 diabetes study (VNDS) 5. Nutr. Metab. Cardiovasc. Dis. 26 (3), 232–238.

Wang, Z.H., Shang, Y.Y., Zhang, S., Zhong, M., Wang, X.P., Deng, J.T., et al., 2012. Silence of TRIB3 suppresses atherosclerosis and stabilizes plaques in diabetic ApoE-/-/LDL receptor-/-mice. Diabetes 61 (2), 463–473.

Xu, Y., Wang, L., He, J., Bi, Y., Li, M., Wang, T., et al., 2013. Prevalence and control of diabetes in Chinese adults. JAMA 310 (9), 948–959.

Yang, W., Zhao, W., Xiao, J., Li, R., Zhang, P., Kissimova-Skarbek, K., et al., 2012. Medical care and payment for diabetes in China: enormous threat and great opportunity. PLoS One 7 (9), e39513.

Zhang, W., Zhong, M., Tang, M.X., Ma, X., Miao, Y., Sun, H., et al., 2006. Effect of valsartan on Tribble 3 gene expression in rats with experimental diabetic cardiomyopathy. Zhonghua Xin Xue Guan Bing Za Zhi 34 (3), 212–216.

Zhang, W., Yang, Z., Li, X., Wen, J., Zhang, H., Wang, S., et al., 2015. The functional Q84R polymorphism of TRIB3 gene is associated with diabetic nephropathy in Chinese type 2 diabetic patients. Gene 555 (2), 357–361.

Zoungas, S., Chalmers, J., Neal, B., Billot, L., Li, Q., Hirakawa, Y., et al., 2014. Follow-up of blood-pressure lowering and glucose control in type 2 diabetes. N. Engl. J. Med. 371 (15), 1392–1406.