Ticagrelor plus aspirin versus clopidogrel plus aspirin for platelet reactivity in patients with minor stroke or transient ischaemic attack: open label, blinded endpoint, randomised controlled phase II trial

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ABSTRACT

OBJECTIVE

To test the hypothesis that ticagrelor plus aspirin is safe and superior to clopidogrel plus aspirin for reducing high platelet reactivity at 90 days and stroke recurrence in patients with minor stroke or transient ischaemic attack, particularly in carriers of the CYP2C19 loss-of-function allele and patients with large artery atherosclerosis.

DESIGN

Open label, blinded endpoint, randomised controlled phase II trial.

SETTING

Prospective studies conducted at 26 centres in China, August 2015 to March 2017.

PARTICIPANTS

675 patients with acute minor stroke or transient ischaemic attack.

INTERVENTION

Ticagrelor (180 mg loading dose, 90 mg twice daily thereafter) or clopidogrel (300 mg loading dose, 75 mg daily thereafter) on a background of aspirin (100 mg daily for the first 21 days) within 24 hours of symptom onset.

MAIN OUTCOME MEASURES

Primary outcome was the proportion of patients with high platelet reactivity at 90 days. High platelet reactivity was defined as P2Y12 reaction units of more than 208. Secondary outcomes included high platelet reactivity at 90 days (7 days either way) in patients carrying genetic variants that would affect clopidogrel metabolism, and any stroke (ischaemic or haemorrhagic) recurrence at 90 days (7 days either way), six months, and one year.

RESULTS

At 90 days, high platelet reactivity occurred in 35 (12.5%) of 280 patients in the ticagrelor/aspirin group and 86 (29.7%) of 290 patients in the clopidogrel/aspirin group (risk ratio 0.40; 95% confidence interval 0.28 to 0.56; P<0.001), and in 10.8% versus 35.4% (0.31; 0.18 to 0.49; P<0.001) of patients carrying CYP2C19 loss-of-function alleles. Stroke occurred in 21 (6.3%) of 336 patients in the ticagrelor/aspirin group and 30 (8.8%) of 339 patients in the clopidogrel/ aspirin group (hazard ratio 0.70; 95% confidence interval 0.40 to 1.22; P=0.20). Patients with large artery atherosclerosis in the ticagrelor/aspirin group had a lower stroke recurrence at 90 days than those in the clopidogrel/aspirin group (6.0% *v* 13.1%; hazard ratio 0.45, 95% confidence interval 0.20 to 0.98; P=0.04). No difference was seen in the rates of major or minor haemorrhagic events between the ticagrelor/aspirin and clopidogrel/aspirin groups (4.8% *v* 3.5%; P=0.42).

CONCLUSION

Patients with minor stroke or transient ischaemic attack who are treated with ticagrelor plus aspirin have a lower proportion of high platelet reactivity than those who are treated with clopidogrel plus aspirin, particularly for those who are carriers of the CYP2C19 loss-of-function allele. The results of this study should be evaluated further in large scale, phase III trials and in different populations.

TRIAL REGISTRATION

Clinicaltrials.gov NCT02506140.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Studies have shown that patients who are carriers of the cytochrome P450 (CYP) 2C19*2 and *3 loss-of-function alleles do not benefit from dual antiplatelet therapy (aspirin combined with clopidogrel), compared with aspirin alone

Ticagrelor combined with aspirin has been shown to be more efficacious than clopidogrel combined with aspirin for acute coronary syndromes, regardless of CYP2C19 genotypes

However, the safety and efficacy of ticagrelor/aspirin versus clopidogrel/aspirin has not been evaluated in patients with minor stroke or transient ischaemic attack

WHAT THIS STUDY ADDS

This study suggests the efficacy of ticagrelor/aspirin in reducing high platelet reactivity compared with clopidogrel/aspirin, especially in patients with CYP2C19 loss-of-function alleles at 90 days after symptoms onset

The rate of major or minor haemorrhagic events did not differ between the two groups

As a phase II trial, these results would need to be replicated and investigated further in larger studies and in different populations in the future

Introduction

Patients with acute minor ischaemic stroke and transient ischaemic attack are at high risk of recurrent stroke and cardiovascular events.[1] The Clopidogrel in High-risk patients with Acute Non-disabling Cerebrovascular Events (CHANCE) trial indicated

that combined clopidogrel and aspirin treatment is superior to aspirin alone in reducing the risk of stroke,[2] but could increase the risk of non-intracranial haemorrhage.[1 3] Additionally, about 50% patients with acute ischaemic stroke had a risk of intracranial large artery atherosclerosis (LAA) in Asia, and patients with intracranial arterial stenosis and minor stroke (or a high risk of transient ischaemic attack) had a higher rate of recurrent stroke than those without.[4 5] The CHANCE genetic substudy showed that patients who were carriers of the cytochrome P450 (CYP) 2C19*2 and *3 loss-of-function alleles benefitted more from using aspirin alone than from using dual antiplatelet therapy.[6]

The metabolism of ticagrelor is primarily via the CYP3A4 enzyme and does not involve CYP2C19, unlike clopidogrel.[7] A genetic substudy of the Platelet Inhibition and Patient Outcomes (PLATO) trial indicated that ticagrelor is more efficacious than clopidogrel for acute coronary syndromes, regardless of CYP2C19 genotype, but was associated with an increased risk of haemorrhage in patients with a history of stroke.[8] The Acute Stroke or Transient Ischaemic Attack Treated With Aspirin or Ticagrelor and Patient Outcomes (SOCRATES) trial revealed a trend towards better efficacy in reducing the risk of vascular events in the ticagrelor treated group than in the aspirin group in an Asian subpopulation. However, limited data are available on the safety and efficacy of ticagrelor for the treatment of stroke, compared with data for clopidogrel on a background of aspirin in patients with acute stroke.[4 9 10]

High platelet reactivity is defined as resistance or non-responsiveness to antiplatelet agents and is a known marker for recurrent ischaemic events in patients with acute coronary syndrome or those patients with percutaneous coronary intervention.[11 12] Several studies have shown the predictive value of high platelet reactivity for ischaemic and bleeding events after percutaneous coronary intervention or in patients with acute coronary syndrome. Multiple factors can contribute to the variability in platelet function testing results, thus defining the high platelet reactivity status. High platelet reactivity is associated with poor cerebrovascular outcomes, and might be of clinical value for the evaluation of recurrent events in patients with stroke.[13-16]

We conducted the Platelet Reactivity in Acute Stroke or Transient Ischaemic Attack (PRINCE) trial as a phase II study to compare the efficacy of ticagrelor plus aspirin with clopidogrel plus aspirin in reducing high platelet reactivity at 90 days in patients with minor stroke or transient ischaemic attack.[17] We also compared the clinical outcomes in terms of efficacy and safety before a large scale, phase III study.

Methods

The PRINCE study protocol (NCT02506140) and data collection were approved by the ethics committee of Beijing Tiantan Hospital (ethical approval number KY2014-048-03) and all of the study centres, and conducted in accordance with the Declaration of Helsinki.[17] The trial was a prospective, multicentre, randomised, open label, active controlled, blinded endpoint trial. All participants or their representatives provided written consent before study enrolment.

Study design and participants

From August 2015 to March 2017 in 26 study centres in China, the PRINCE trial enrolled patients aged 40-80 years who had had an acute minor ischaemic stroke (National Institutes of Health Stroke Scale score of ≤3 at the time of randomisation) or those with a moderate to high risk of transient ischaemic attack (ABCD[2] stroke risk score of ≥4 at the time of randomisation or ≥50% stenosis of cervical or intracranial vessels that could account for the presentation) who could be treated with the study drug within 24 hours of symptom onset. Patients were excluded from the trial if they had a diagnosis intracranial haemorrhage, acute coronary syndrome, or other pathology that could account for the neurological symptoms; had a modified Rankin scale score of more than 2 at randomisation; or had a contraindication to ticagrelor, clopidogrel, or aspirin.

The trial included six visits: randomisation (baseline), seven to nine days, 21 days (two days either way), 90 days (seven days either way), six months (14 days either way), and one year (14 days either way). Additional visits at two and 24 hours after the first dose was administered were optional. All visits involved face-to-face interviews, with the exception of the six month follow-up, which was conducted by telephone, with data collected on electronic case report forms. The general schedule of the trial and the collection times for blood and urine samples are listed in the supplementary appendix (blood and urine samples collecting schedule).

Randomisation and procedures

Immediately after signing the written informed consent form, eligible patients were assigned to receive the following within one hour of randomisation, in a 1:1 ratio:

- Intervention (ticagrelor/aspirin): aspirin (a loading dose of 100-300 mg given as one to three 100 mg tablets on day 1, followed by 100 mg once daily until day 21) combined with ticagrelor (180 mg loading dose given as two 90 mg tablets on day 1, followed by 90 mg twice daily until day 90)
- Control (clopidogrel/aspirin): aspirin (a loading dose of 100-300 mg given as one to three 100 mg tablets on day 1, followed by 100 mg once daily until day 21) combined with clopidogrel (300 mg loading dose given as four 75 mg tablets on day 1, followed by 75 mg once daily until day 90).

Patients were allocated via a block randomisation process by investigators at the clinical centres. The block randomisation sequence was provided by an independent statistician using computer generated random numbers with a block size of four. The block size was not listed in the Chinese version of the protocol

(which was provided to the investigators) in order to prevent the investigators from speculating about the group assignment. The loading and maintenance doses of ticagrelor were administered as in the SOCRATES and PLATO studies.[10 18]

VerifyNow testing was conducted in each study centre by qualified personnel who were blinded to the treatment allocation. The platelet reaction units were measured in each study centre by specially trained and qualified personnel according to a standardised procedure manual. To ensure the validity and reproducibility of the assay, we held two separate training course for all the testing personnel from each centre. Both the investigators and the patients were aware of the study drug assignment, but were blinded to the platelet reactivity data until the end of the trial.

Outcomes

The primary outcome of the PRINCE trial was the proportion of patients with high platelet reactivity at 90 days. High platelet reactivity was defined as a P2Y12 reaction unit of more than 208 measured using the VerifyNow P2Y12 assay. Prespecified secondary outcomes included high platelet reactivity at 90 days (seven days either way) in patients carrying genetic variants that would affect clopidogrel metabolism; any stroke (ischaemic or haemorrhagic); and composite clinical vascular events (ischaemic/haemorrhagic stroke, transient ischaemic attack, myocardial infarction, or vascular death) at 90 days (seven days either way), six months, and one year. Each reported composite clinical vascular event and safety outcome was independently adjudicated by two members (KD and Jimei Li) of the clinical event adjudication committee, who were blinded to the treatment group assignments. All discrepancies were reviewed by all five members of the committee and resolved by consensus.

The primary safety outcome was major bleeding, which was defined as that in the PLATO study classification of haemorrhagic events: fatal or life threatening bleed, major bleed, and other (supplementary appendix, PLATO bleeding classification). Secondary safety outcomes included the incidence of intracranial bleeding; dyspnoea events; and mortality at 90 days (seven days either way), six months, and one year.

Genotyping

The prespecified analysis included the single nucleotide polymorphisms (SNPs) CYP2C19*2 (681G >A, rs4244285), CYP2C19*3 (636G >A, rs4986893), and CYP2C19*17 (−806C >T, rs12248560), which were genotyped in all participants with adequate blood samples. Most genotyping of the three SNPs was performed by the Sequenom MassARRAY iPLEX platform (Sequenom, San Diego, CA, USA). We used Sanger sequencing (ABI 3500 Genetic Analyzer, Applied Biosystems) if the results were otherwise inconclusive.

We used star allele nomenclature to categorise patients by the CYP2C19 metaboliser status, based on *2, *3, and *17 genotypes.[19] Patients with at least one gain-of-function allele (*17) were classified as "gain-of-function allele carriers," and those with at least one loss-of-function allele (*2 or *3) were classified as "loss-of-function allele carriers."[20] Patients who carried at least one *17 allele (*1/*17 or *17/*17) were classified as "ultra-metabolisers," those without any *2, *3, or *17 allele (*1/*1) were classified as "extensive metabolisers," those with one *2 or *3 allele (*1/*2 or *1/*3) were classified as "intermediate metabolisers," and those with at least two *2 or *3 alleles (*2/*2, *2/*3, or *3/*3) were classified as "poor metabolisers."[20] Patients with one *17 and a loss-of-function allele (*2/*17 or *3/*17) were classified as "unknown metabolisers," because the clinical effect of these alleles is uncertain.[21 22]

Based on our preplanned aims, we compared the recurrence of stroke in patients whose stroke subtype was intracranial large artery atherosclerosis (LAA) with those whose stroke subtype was non-LAA based on the stroke subtype of SSS-TOAST (Stop Stroke Study Trial of Org 10172 in Acute Stroke Treatment stroke aetiology classification; supplementary appendix, SSS-TOAST classification criteria). Non-LAA included cardioaortic embolism, small artery occlusion, other causes, and undetermined causes.

Statistical analysis

An interim analysis was preplanned in the published protocol.[17] We calculated that 952 patients (estimated 10% dropout rate) would be required to achieve 90% power with a two sided $\alpha=0.05$ to detect a relative reduction of 24% in the proportion of the primary outcome in the ticagrelor/aspirin group compared with that in the clopidogrel/aspirin group. The data safety monitoring board opted to terminate the study after the interim analysis based on 476 patients (50% of the projected necessary sample size) who completed 90 days of follow-up, based on achieving a prespecified threshold for efficacy (P<0.005). At the time of this decision, an additional 199 patients had already been recruited and randomised into the trial, and these patients were also followed up to study completion. Therefore, a total of 675 patients were included in the intention-to-treat analyses.

Proportions were presented for categorical variables, and medians with interquartile ranges or means (standard deviation) were presented for continuous variables. We compared the proportion of high platelet reactivity at the 90 day follow-up (the primary outcome) between the two study groups using genmod models adjusted by the high platelet reactivity status at baseline, reported as a risk ratio with 95% confidence intervals. To evaluate the influence of missing data for the primary outcome, we did sensitivity analyses assuming that all the missing data were high platelet reactivity or not.

The differences in the rates of stroke, composite outcome, death, and bleeding events during the 90 day follow-up were assessed by use of Cox proportional hazards regression, and were reported as hazard ratios with 95% confidence intervals. The proportional hazard assumption for the Cox models

was examined by including a time dependent covariate with interaction of treatment group, and a logarithmic function of survival time in the model.

We assessed whether the treatment effect differed in certain genotype categories by testing the treatment-by-genotype interaction effect in genmod models for the primary outcome and Cox models for other outcomes, as described earlier. We also tested whether the treatment effect in reducing stroke recurrence differed between patients with LAA and those without LAA by testing the treatment-by-stroke subtype interaction effect in a genmod model. The models also included the main effects of treatment group and genotype or stroke subtype. All statistical analyses were two sided, and differences with a P value of less than 0.05 were considered to be statistically significant. Analyses were

Table 1 | Baseline characteristics of patients in the PRINCE trial

Characteristic	Trial group	
	Ticagrelor/aspirin (n=336)	Clopidogrel/aspirin (n=339)
Age (years)		
Mean (standard deviation)	61.1 (8.5)	60.5 (9.0)
Median (interquartile range)	62.0 (55.0-67.0)	61.0 (54.0-67.0)
Female sex (No (%))	91 (27.1)	90 (26.5)
Systolic blood pressure (mm Hg)		
Mean (standard deviation)	152.3 (22.5)	154.9 (21.2)
Median (interquartile range)	150.0 (137.5-168.0)	154.0 (140.0-170.0)
Diastolic blood pressure (mm Hg)		
Mean (standard deviation)	87.7 (13.0)	89.4 (12.8)
Median (interquartile range)	87.5 (80.0-96.0)	88.0 (80.0-97.0)
Body mass index*		
Mean (standard deviation)	25.0 (3.8)	25.0 (3.8)
Median (interquartile range)	24.6 (22.6-27.0)	24.8 (22.7-27.3)
Pulse rate (beat/min; mean (SD))	75.1 (10.1)	76.3 (11.5)
Medical history (No (%))		
Hypertension	203 (60.4)	208 (61.4)
Dyslipidaemia	20 (6.0)	21 (6.2)
Diabetes mellitus	79 (23.5)	85 (25.1)
Ischaemic stroke	59 (17.6)	62 (18.3)
Transient ischaemic attack	8 (2.4)	10 (2.9)
Coronary artery disease	26 (7.7)	25 (7.4)
Known atrial fibrillation	0 (0.0)	4 (1.2)
Flutter valvular heart disease	1 (0.3)	0 (0.0)
Pulmonary embolism	0 (0.0)	0 (0.0)
Smoking status (No (%))		
Non-smoker	150 (44.6)	155 (45.7)
Current smoker	160 (47.6)	159 (46.9)
Ex-smoker	26 (7.7)	25 (7.4)
Drug use before randomisation (No (%))		
Proton pump inhibitor	2 (0.6)	3 (0.9)
Statin	36 (10.7)	30 (8.8)
Aspirin	77 (22.9)	69 (20.4)
Clopidogrel	5 (1.5)	10 (2.9)
Ticagrelor	0 (0.0)	0 (0.0)
Time to randomisation after onset of symptoms (h; mean (range))	14.0 (8.3-20.6)	13.8 (8.0-20.8)
Time to randomisation after onset of symptoms (No (%))		
<12 h	139 (41.4)	144 (42.5)
≥12 h	197 (58.6)	195 (57.5)
Qualifying event (No (%))		
Minor stroke	275 (81.8)	289 (85.3)
Transient ischaemic attack	61 (18.2)	50 (14.7)
Baseline ABCD[2] score among patients with transient ischaemic attack as the qualifying event (median (interquartile range))†	5.0 (4.0-5.0)	4.5 (4.0-5.0)
SSS-TOAST stroke subtype (No (%))‡		
Large artery atherosclerosis	151 (54.9)	153 (52.9)
Cardioaortic embolism	8 (2.9)	5 (1.7)
Small artery occlusion	104 (37.8)	109 (37.7)
Other causes	7 (2.5)	9 (3.1)
Undetermined causes	5 (1.8)	13 (4.5)
Unknown	2 (0.7)	7 (2.4)
Unclassified	3 (1.1)	6 (2.1)

*Body mass index is the weight in kilograms divided by the square of the height in metres.
†ABCD[2] stroke risk scores range from 0 to 7, with higher scores indicating higher risk; data provided in the table are only for the group of 111 patients whose qualifying event was transient ischaemic attack for inclusion in the trial.
‡SSS-TOAST stroke subtype=Stop Stroke Study Trial of Org 10172 in Acute Stroke Treatment stroke aetiology classification (supplementary appendix, SSS-TOAST classification criteria); data provided in the table are only for the group of 564 patients whose qualifying event was minor stroke for inclusion in the trial.

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performed by use of SAS software, version 9.4 (SAS Institute, Cary, NC, USA).

Patient and public involvement

The design, outcome measurement, recruiting plans, or implementation of the study were independent of any patient. The gene and platelet reactivity testing results of every patient will be delivered to the patient himself or his appointed relatives after the primary results of the results published through email or telephone. The results of the research will be broadcasted to all the participants and general public through internet news, popular science articles, newspapers, and social media.

Results

Between August 2015 and March 2017, 5644 patients with stroke or transient ischaemic attack were screened at 26 hospitals, and 675 patients (mean age
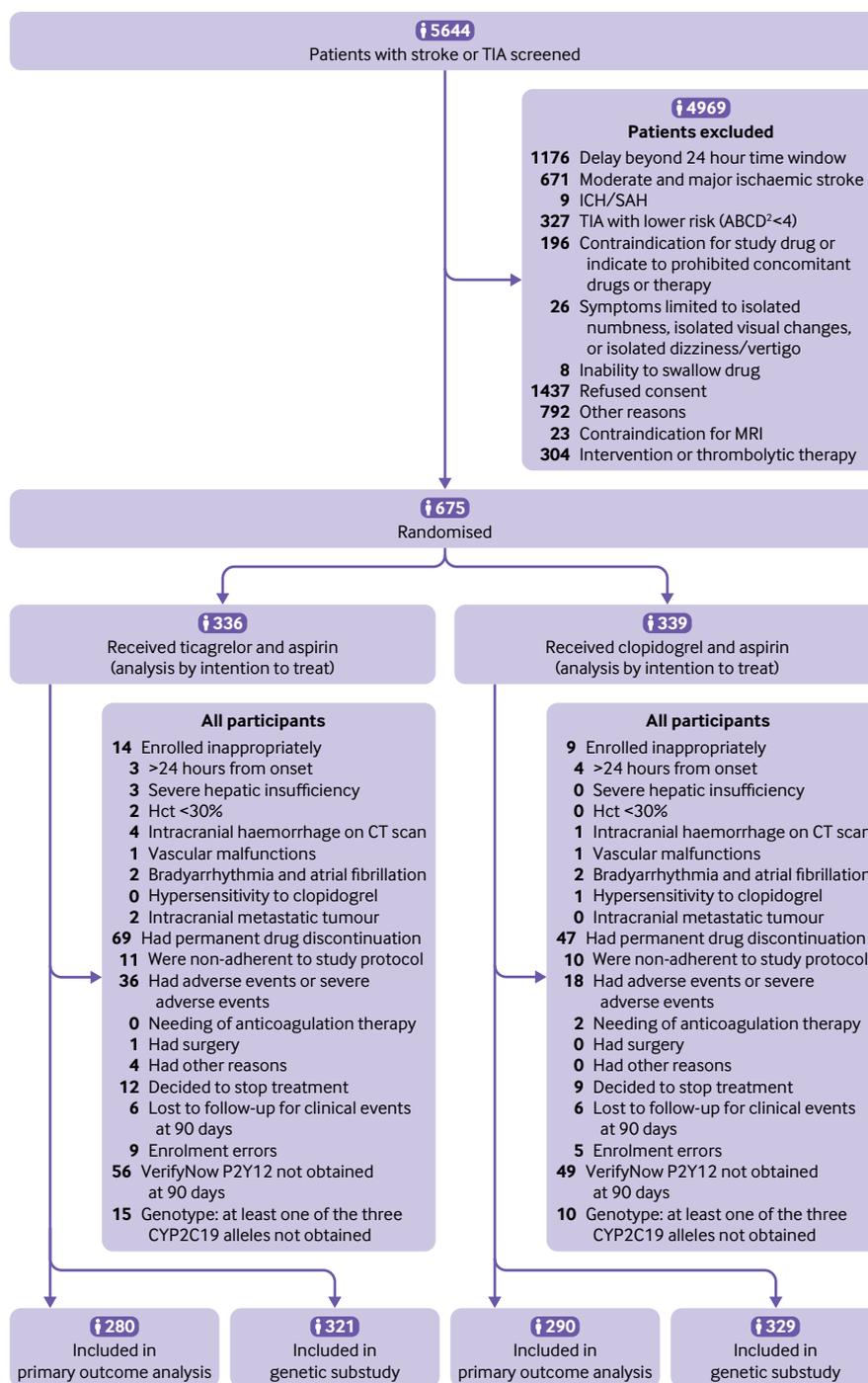
Fig 1 | Trial profile. TIA=transient ischaemic attack; MRI=magnetic resonance imaging; ICH/SAH=intracerebral haemorrhage/subarachnoid haemorrhage; Hct=haematocrit; CT=computed tomography; VerifyNow P2Y12=VerifyNow P2Y12 platelet reactivity assay
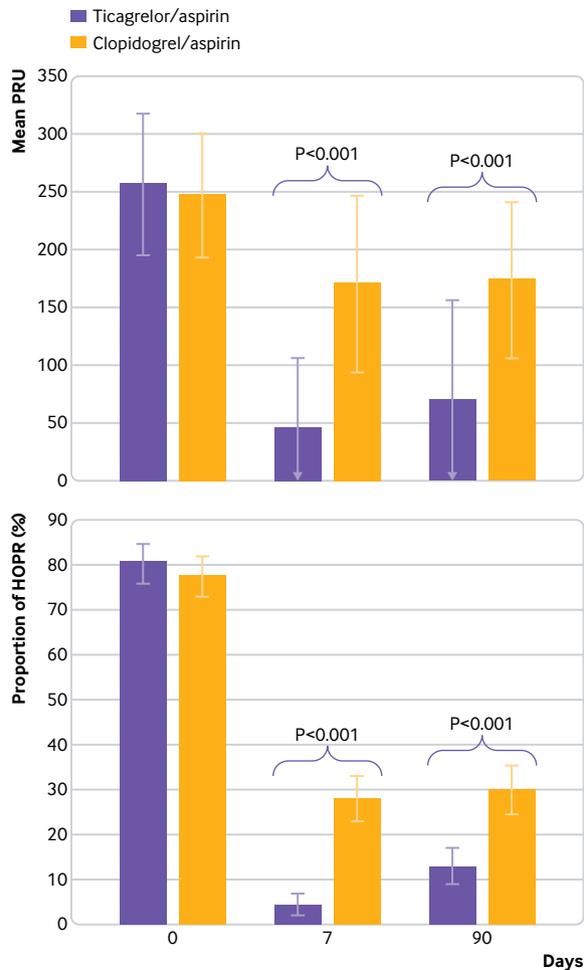
Fig 2 | Platelet reactivity in trial groups at baseline and follow-up, showing P2Y12 reaction units (PRU; mean (standard deviation)) and proportion (%; 95% confidence intervals) of patients with high platelet reactivity (HOPR; PRU >208 as measured by the VerifyNow P2Y12 assay). A total of 333, 306, and 280 patients in the ticagrelor/aspirin group and 336, 321, and 290 patients in the clopidogrel/aspirin group were included in the 0, 7, and 90 day analyses, respectively

(supplementary tables B, C, and D show details of the baseline characteristics of patients enrolled in the genetic substudy). Of the 650 participants, 374 (57.5%) were classified as CYP2C19 loss-of-function carriers.

We obtained valid measurements in 627 (92.9%) and 570 (84.4%) patients for the VerifyNow P2Y12 assay at the seven day and 90 day follow-up periods, respectively. The P2Y12 reaction units before receiving the study drugs were similar in the ticagrelor/aspirin and clopidogrel/aspirin groups (mean 256.4 (standard deviation 61.3) v 246.9 (53.7), P=0.13). The ticagrelor/aspirin group had significantly fewer P2Y12 reaction units than the clopidogrel/aspirin group (69.3 (87.0) v 173.5 (67.6); P<0.001) at 90 day follow-up (fig 2). The primary outcome (high platelet reactivity) was observed in 35 of 280 patients (12.5%) in the ticagrelor/aspirin group, and in 86 of the 290 patients (29.7%) in the clopidogrel/aspirin group at 90 days (risk ratio 0.40; 95% confidence interval 0.28 to 0.56; P<0.001; fig 2 and table 2). Similar results were observed for the primary outcome in sensitivity analyses assuming that all missing data for the primary outcome showed high platelet reactivity or not (supplementary table E).

Stroke, the main secondary outcome, occurred in 21 (6.3%) of the 336 patients in the ticagrelor/aspirin group and 30 (8.8%) of the 339 patients in the clopidogrel/aspirin group (hazard ratio 0.70; 95% confidence interval 0.40 to 1.22; P=0.20). Composite clinical vascular events occurred in 22 patients (6.5%) in the ticagrelor/aspirin group and 32 patients (9.4%) in the clopidogrel/aspirin group (0.68; 0.40 to 1.18; P=0.17; table 2). All of the proportional hazard assumptions were met (P=0.89 for stroke and P=0.85 for composite events).

Among the CYP2C19 loss-of-function carriers, high platelet reactivity at 90 days was markedly less frequent in the ticagrelor/aspirin group than in the clopidogrel/aspirin group (10.8% v 35.4%; risk ratio 0.31, 95% confidence interval 0.18 to 0.49; P<0.001; supplementary table F). The event rates of stroke, composite events, and haemorrhagic events varied by treatment assignment and phenotype (fig 3). Other primary and secondary outcomes in patients with available genotype data are listed in supplementary table G.

Among the patients with LAA, stroke recurrence at 90 days was markedly lower in the ticagrelor/aspirin group than in the clopidogrel/aspirin group (6.0% v 13.1%; hazard ratio 0.45, 95% confidence interval 0.20 to 0.98; P=0.04; table 3 and fig 4). However, among patients with non-LAA, the risk of stroke recurrence was similar in the ticagrelor/aspirin group and clopidogrel/aspirin groups (8.1% v 7.4%; 1.1, 0.46 to 2.63; P=0.84; table 3 and fig 4). The proportional hazard assumption was met (P=0.93).

The primary safety outcome (PLATO major hae-morrhagic event) occurred in five patients (1.5%) in the ticagrelor/aspirin group and four (1.2%) in the clopidogrel/aspirin group (hazard ratio 1.27; 95% confidence interval 0.34 to 4.72; table 2). Three patients (0.9%) in the ticagrelor/aspirin group and two (0.6%)

61.1, standard deviation 8.5) were enrolled before the data safety monitoring board opted to terminate the trial after reviewing the results of the interim analysis. Thus, 336 patients were randomly assigned to the ticagrelor/aspirin group and 339 patients were assigned to the clopidogrel/aspirin group (table 1). Patients were excluded from the trial mainly because of a delay beyond the 24 hour time window and because they refused consent (fig 1). The average age was 60.8 (standard deviation 8.7), and 26.8% (n=181) of the patients were women. At 90 days, 12 patients were lost to follow-up owing to clinical events, and the VerifyNow P2Y12 assay data were not obtained from 105 patients (supplementary table A shows details of the baseline characteristics of patients with and without platelet function data).

Among the 675 patients enrolled, 650 (mean age 60.8 (standard deviation 8.7)) had complete genotype data for all three SNPs, of whom 321 were randomly assigned to the ticagrelor/aspirin group and 329 were assigned to the clopidogrel/aspirin group

Table 2 | Effect of ticagrelor/aspirin versus clopidogrel/aspirin on efficacy and safety outcomes in PRINCE trial

Outcomes	Trial participants (No with event/total No (%))		Hazard ratio or risk ratio (95% CI)*	P
	Ticagrelor/aspirin	Clopidogrel/aspirin		
Primary efficacy outcomes†				
Baseline	268/333 (80.5)	260/336 (77.4)	1.04 (0.96 to 1.13)	0.33
7+2 days	12/306 (3.9)	89/321 (27.7)	0.14 (0.07 to 0.23)	<0.001
90±7 days	35/280 (12.5)	86/290 (29.7)	0.40 (0.28 to 0.56)	<0.001
Secondary efficacy outcomes				
Stroke	21/336 (6.3)	30/339 (8.8)	0.70 (0.40 to 1.22)	0.20
Composite events‡	22/336 (6.5)	32/339 (9.4)	0.68 (0.40 to 1.18)	0.17
Ischaemic stroke	18/336 (5.4)	28/339 (8.3)	0.64 (0.35 to 1.16)	0.14
Haemorrhagic stroke	3/336 (0.9)	2/339 (0.6)	1.52 (0.25 to 9.08)	0.65
Myocardial infarction	0/336 (0.0)	1/339 (0.3)	—	—
Death from cardiovascular causes	1/336 (0.3)	2/339 (0.6)	0.50 (0.05 to 5.55)	0.58
Death from any cause	3/336 (0.9)	2/339 (0.6)	1.50 (0.25 to 9.00)	0.65
Transient ischaemic attack	1/336 (0.3)	2/339 (0.6)	0.50 (0.05 to 5.53)	0.57
Primary safety outcomes§				
Major bleeding	5/336 (1.5)	4/339 (1.2)	1.27 (0.34 to 4.72)	0.72
Major, fatal, life threatening bleeding	4/336 (1.2)	3/339 (0.9)	1.35 (0.30 to 6.03)	0.69
Fatal bleeding	1/336 (0.3)	1/339 (0.3)	1.01 (0.06 to 16.13)	1.00
Intracranial haemorrhage	3/336 (0.9)	2/339 (0.6)	1.27 (0.34 to 4.72)	0.72
Major, other	1/336 (0.3)	1/339 (0.3)	1.01 (0.06 to 16.18)	0.99
Minor bleeding	11/336 (3.3)	8/339 (2.4)	1.40 (0.56 to 3.47)	0.47
Major or minor bleeding	16/336 (4.8)	12/339 (3.5)	1.36 (0.64 to 2.88)	0.42
Minimal bleeding	64/336 (19.0)	36/339 (10.6)	1.86 (1.24 to 2.80)	0.003
Any bleeding	75/336 (22.3)	48/339 (14.2)	1.65 (1.15 to 2.37)	0.007
Other safety outcomes				
Respiratory, thoracic, and mediastinal disorders	22/336 (6.5)	0/339 (0.0)	—	<0.001
Dyspnoea	14/336 (4.2)	0/339 (0.0)	—	<0.001
Epistaxis	6/336 (1.8)	0/339 (0.0)	—	0.04

*Risk ratios used for the primary efficacy outcome and hazard ratios used for secondary efficacy outcome.
†Primary outcome indicates high platelet reactivity, which was defined as a P2Y12 reaction unit of more than 208, as measured by a VerifyNow P2Y12 assay.
‡A composite event was defined as a new clinical vascular event, including stroke, transient ischaemic attack, myocardial infarction, or death from cardiovascular causes.
§Primary safety outcomes were defined according to the PLATO criteria (supplementary appendix, PLATO bleeding classification). All 675 patients were included in the analysis of safety outcomes. Other safety outcomes included those leading to permanent drug discontinuation.

in the clopidogrel/aspirin group had intracranial haemorrhage. However, the rate of any haemorrhagic events occurring was higher in the ticagrelor/aspirin group (22.3%) than in the clopidogrel/aspirin group (14.2%; 1.65, 1.15 to 2.37; table 2). All of the proportional hazard assumptions were met (P=0.99 for major haemorrhagic event and P=0.82 for any haemorrhagic events). The rate of major bleeding did not vary significantly between the ticagrelor/aspirin and clopidogrel/aspirin groups amongst the carriers of the CYP2C19 loss-of-function allele (rate 0.0% v 1.6%; hazard ratio 0.35, 95% confidence interval 0.04 to 3.33; P=0.43) and non-carriers (0.0% v 0.7%; supplementary table G).

Dyspnoea was more common in the ticagrelor/aspirin group (n=54, 16.1%) than in the clopidogrel/aspirin group (n=11, 3.2%; supplementary table H). A total of 69 (20.5%) patients in the ticagrelor/aspirin group and 47 (13.9%) in the clopidogrel/aspirin group stopped receiving the study drug before 90 days (fig 1); the most common reasons were dyspnoea and epistaxis. The rate of permanent discontinuation caused by dyspnoea was 4.2% (14/336) in the ticagrelor/aspirin group and 0.0% in the clopidogrel/aspirin group, and the rate of permanent discontinuation caused by epistaxis was 1.8% (n=6) in the ticagrelor/aspirin group and 0.0% in the clopidogrel/aspirin group (supplementary table

1). Serious adverse events and adverse events leading to permanent drug discontinuation within 90 days are listed in supplementary table 1.

Discussion

Principal findings

This PRINCE trial results indicated that the proportion of high platelet reactivity at 90 days reduced with ticagrelor compared with clopidogrel, in patients with acute minor stroke and those at moderate-to-high risk of transient ischaemic attack treated with aspirin. This trial was not powered to study clinical events. However, we observed fewer strokes and composite outcomes at 90 days in patients who were treated with dual antiplatelet therapy using ticagrelor/aspirin compared with clopidogrel/aspirin within 24 hours of the onset of minor stroke or transient ischaemic attack, without increasing the risk of major, minor, or intracranial haemorrhage. Treatment discontinuation was, however, higher with ticagrelor due to an increased rate of dyspnoea and minimal haemorrhagic events (that is, epistaxis).

Comparison with other studies

The major haemorrhage rate in the present study was lower than that found in the PLATO study (ticagrelor/aspirin group 1.5% v 11.6%; clopidogrel/aspirin group
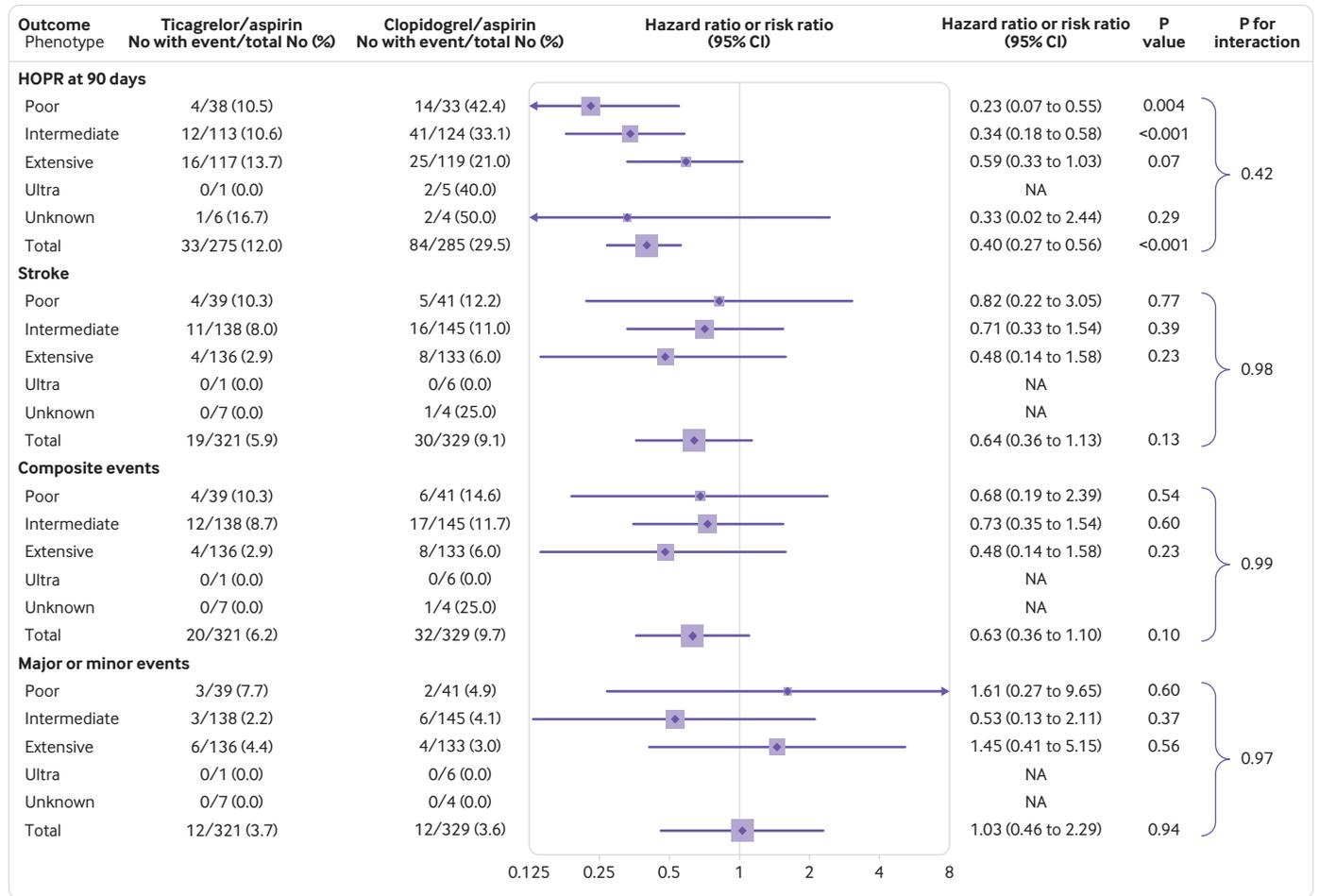
Outcome Phenotype	Ticagrelor/aspirin No with event/total No (%)	Clopidogrel/aspirin No with event/total No (%)	Hazard ratio or risk ratio (95% CI)	Hazard ratio or risk ratio (95% CI)	P value	P for interaction
HOPR at 90 days						
Poor	4/38 (10.5)	14/33 (42.4)		0.23 (0.07 to 0.55)	0.004	
Intermediate	12/113 (10.6)	41/124 (33.1)		0.34 (0.18 to 0.58)	<0.001	
Extensive	16/117 (13.7)	25/119 (21.0)		0.59 (0.33 to 1.03)	0.07	
Ultra	0/1 (0.0)	2/5 (40.0)		NA		0.42
Unknown	1/6 (16.7)	2/4 (50.0)		0.33 (0.02 to 2.44)	0.29	
Total	33/275 (12.0)	84/285 (29.5)		0.40 (0.27 to 0.56)	<0.001	
Stroke						
Poor	4/39 (10.3)	5/41 (12.2)		0.82 (0.22 to 3.05)	0.77	
Intermediate	11/138 (8.0)	16/145 (11.0)		0.71 (0.33 to 1.54)	0.39	
Extensive	4/136 (2.9)	8/133 (6.0)		0.48 (0.14 to 1.58)	0.23	
Ultra	0/1 (0.0)	0/6 (0.0)		NA		0.98
Unknown	0/7 (0.0)	1/4 (25.0)		NA		
Total	19/321 (5.9)	30/329 (9.1)		0.64 (0.36 to 1.13)	0.13	
Composite events						
Poor	4/39 (10.3)	6/41 (14.6)		0.68 (0.19 to 2.39)	0.54	
Intermediate	12/138 (8.7)	17/145 (11.7)		0.73 (0.35 to 1.54)	0.60	
Extensive	4/136 (2.9)	8/133 (6.0)		0.48 (0.14 to 1.58)	0.23	
Ultra	0/1 (0.0)	0/6 (0.0)		NA		0.99
Unknown	0/7 (0.0)	1/4 (25.0)		NA		
Total	20/321 (6.2)	32/329 (9.7)		0.63 (0.36 to 1.10)	0.10	
Major or minor events						
Poor	3/39 (7.7)	2/41 (4.9)		1.61 (0.27 to 9.65)	0.60	
Intermediate	3/138 (2.2)	6/145 (4.1)		0.53 (0.13 to 2.11)	0.37	
Extensive	6/136 (4.4)	4/133 (3.0)		1.45 (0.41 to 5.15)	0.56	
Ultra	0/1 (0.0)	0/6 (0.0)		NA		0.97
Unknown	0/7 (0.0)	0/4 (0.0)		NA		
Total	12/321 (3.7)	12/329 (3.6)		1.03 (0.46 to 2.29)	0.94	

Axis scale: 0.125 0.25 0.5 1 2 4 8

Fig 3 | Effect of ticagrelor/aspirin versus clopidogrel/aspirin on high platelet reactivity and clinical outcome in PRINCE trial participants at 90 days, stratified by metaboliser status. A total of 321 patients in the ticagrelor/aspirin group and 329 patients in the clopidogrel/aspirin group were included in the genetic analysis. Patients with two *2 or *3 alleles (*2/*2, *2/*3, or *3/*3) were classified as having a poor metaboliser phenotype, those with one *2 or *3 allele (*1/*2 or *1/*3) were classified as having an intermediate metaboliser phenotype, those without a *2, *3, or *17 allele (*1/*1) were classified as having an extensive metaboliser phenotype, and those with a single *17 allele (*1/*17) and *17 homozygotes were classified as having an ultra-metaboliser phenotype. HOPR=P2Y12 reaction units of more than 208, as measured the VerifyNow P2Y12 assay; composite event=a new clinical vascular event, including stroke, transient ischaemic attack, myocardial infarction, or death from cardiovascular causes; NA=not applicable

1.2% v 11.2%).[18] This difference could be partly due to the short term use of dual antiplatelet therapy in our study compared with the PLATO study (21 v 277 days). By contrast, a slightly higher major haemorrhagic rate was found in PRINCE than in the Asian subgroup in the SOCRATES trial (1.5% in the ticagrelor/aspirin group in PRINCE v 0.6% in the ticagrelor used as monotherapy in SOCRATES).[4] This difference could be related to the combined use of two drugs in our study. Similar to that reported in the PLATO and SOCRATES trials, dyspnoea occurred more commonly with ticagrelor than with clopidogrel treatment.[10 18]

Safety of study drug
The premature discontinuation of the study drug was more frequent in the ticagrelor/aspirin group than in the clopidogrel/aspirin group, although the rate of serious adverse events did not differ between the two groups. The treating physician or patient had the option of temporarily or permanently discontinuing

Table 3 | Stroke recurrence at 90 days, by cause

	Trial participants (No with event/total No (%))		Hazard ratio (95% CI)*	P	P for interaction
Cause of stroke*	Ticagrelor/aspirin (n=336)	Clopidogrel/aspirin (n=339)			
Large artery atherosclerosis	9/151 (6.0)	20/153 (13.1)	0.45 (0.20 to 0.98)	0.04	0.13
Non-large artery atherosclerosis	10/124 (8.1)	10/136 (7.4)	1.10 (0.46 to 2.63)	0.84	—

*Cause of stroke classified by the SSS-TOAST stroke subtype (SSS-TOAST=Stop Stroke Study Trial of Org 10172 in Acute Stroke Treatment stroke aetiology classification (supplementary appendix, SSS-TOAST classification criteria)). Non-large artery atherosclerosis included patients with cardioaortic embolism, small artery occlusion, other causes, and undetermined causes.
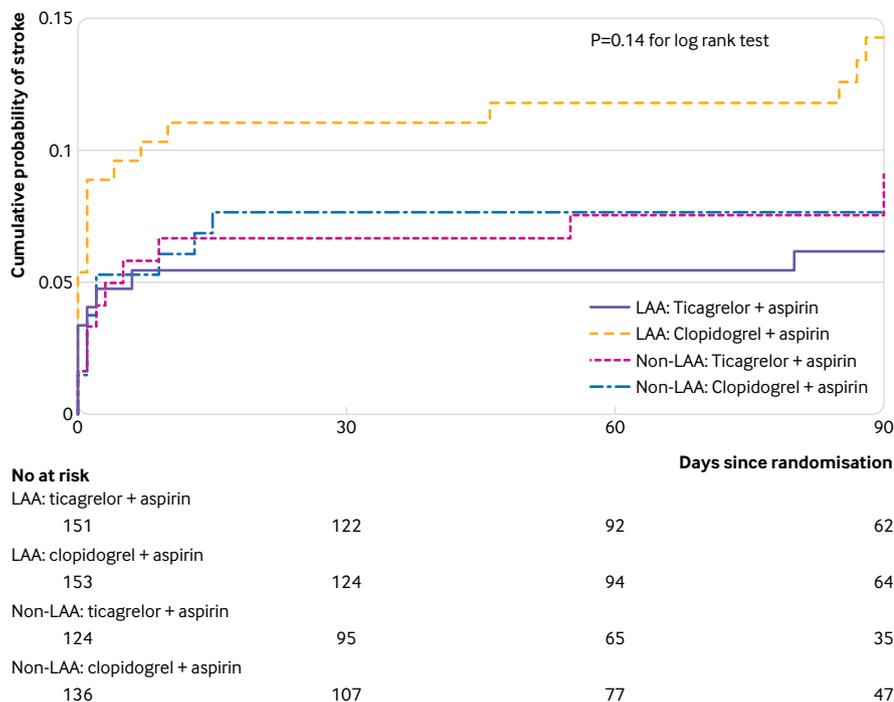
Fig 4 | Stroke recurrence risk with ticagrelor/aspirin versus clopidogrel/aspirin in PRINCE trial participants at 90 days, based on cause of stroke. LAA=large-artery atherosclerosis; Non-LAA=non-large-artery atherosclerosis (including cardioaortic embolism, small artery occlusion, other causes, and undetermined causes)

the drug in case of an adverse event, and could have taken this opportunity more frequently because of the open label design, when using an unapproved drug (that is, ticagrelor) in this indication. Furthermore, because early discontinuation and non-adherence are likely to contribute to the increased platelet reactivity observed at 90 days (compared with that observed at seven days), the efficacy of ticagrelor on high platelet reactivity could have been even higher in patients in the case of a blinded study design.

Clinical efficacy
Although we tested the clinical efficacy (recurrent stroke) between the two groups, the sample size was small and this phase II trial was not powered to find a clinical effect. Our finding of fewer recurrent stroke and composite events in patients treated with ticagrelor/aspirin than in those treated with clopidogrel/aspirin would need to be replicated, because the current study provided only a 25.5% power to show a 30% relative risk difference with a two sided test at a 5% significance level. Given a 90% power and a significance level of 5% (two sided), a total of 4690 patients would need to be included in a phase III trial to detect the relative risk difference between the ticagrelor/aspirin and clopidogrel/aspirin groups, based on the event rates in our study.

The stroke recurrence rate in LAA group was higher than that in the non-LAA group in our study. Patients with minor stroke or transient ischaemic attack with LAA might benefit more from ticagrelor plus aspirin than from clopidogrel plus aspirin treatment. Considering the large numbers required for the stroke

subtyping analyses, our results were exploratory and hypothesis generating, so future studies are needed. We also found a relatively high (7.7%) stroke recurrence rate in patients with non-LAA (table 3), compared with studies conducted in other developed countries.[9] This might be relevant with the overall recurrent stroke rate after minor stroke or transient ischaemic attack also being relatively high, because the recurrent stroke included a new stroke and also rapid worsening (National Institutes of Health Stroke Score ≥4) of an existing focal neurological deficit in our study.

Among patients randomised to clopidogrel, we saw a dose-response association between the number of CYP2C19 loss-of-function alleles and the proportion of high platelet reactivity, with patients carrying more loss-of-function alleles having a higher proportion of platelet aggregation. A larger benefit with ticagrelor compared with clopidogrel was observed in the proportion of high platelet reactivity in patients carrying more loss-of-function alleles. While a similar trend in clinical outcomes was seen among patients randomly allocated to clopidogrel, those allocated to ticagrelor also had higher rates of clinical events if they carried a greater number of loss-of-function allele. However, these results should be interpreted with caution, owing to the very low number of patients and events.

Limitations
This study had several limitations. Firstly, the primary outcome used high platelet reactivity as a marker of risk for events but not yet proved as a causal risk

factor of thrombotic events. Further studies are needed to evaluate the clinical efficacy of dual antiplatelet therapy as the primary endpoint in this target population. Secondly, about 15% of patients were lost to follow-up for the evaluation of high platelet reactivity at 90 days. However, similar results were observed after assuming all the missing data were high platelet reactivity or not. Thirdly, potential selection bias could exist because we enrolled patients from sites that were mostly urban hospitals and that had more experts and medical resources. Fourthly, the cause of stroke and the genetic differences in the CYP2C19 gene differ between Chinese patients with stroke and European patients with stroke. The results of our study should be evaluated in different populations in the future. Finally, the open label design could have led to a placebo effect,[23] which might have caused potential bias in adverse events assessment, drug continuation, and even the physicians' or patients' decisions.

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