Stroke

ORIGINAL CONTRIBUTION

CYP2C19 Genotype and Efficacy of Clopidogrel Initiated Between 24 to 72 Hours for Ischemic Stroke

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BACKGROUND: The aim of this study was to investigate the clinical outcomes of clopidogrel-aspirin therapy initiated between 24 and 72 hours from the symptom onset among patients with minor stroke or transient ischemic attack stratified by *CYP2C19* loss-of-function allele status.

METHODS: This was a prespecified secondary analysis of the INSPIRES trial (Intensive Statin and Antiplatelet Therapy for Acute High-Risk Intracranial or Extracranial Atherosclerosis), which was a randomized clinical trial conducted across 222 centers in China from September 2018 to October 2022. Two loss-of-function alleles (*CYP2C19*2*, *CYP2C19*3*) and 1 gain-of-function allele (*CYP2C19*17*) were genotyped in the INSPIRES study. Patients with *CYP2C19* loss-of-function allele carriers were patients with either *CYP2C19*2* or *CYP2C19*3*. All participants were randomized to receive clopidogrel-aspirin or aspirin treatment, and those started treatment between 24 and 72 hours from the symptom onset were included in this study. The primary efficacy outcome was new stroke within 90 days. The primary safety outcome was moderate-to-severe bleeding. Cox proportional hazards models were performed to estimate the interaction between treatment assignment and *CYP2C19* loss-of-function allele status for the primary outcomes.

RESULTS: Among 5003 patients, 2911 (58.2%) patients were loss-of-function carriers, and 2092 (41.8%) patients were noncarriers. Relative to aspirin alone, clopidogrel-aspirin reduced the rate of new stroke in the noncarriers (hazard ratio, 0.67 [95% CI, 0.49–0.91]; *P*=0.01) but not in the carriers (hazard ratio, 0.96 [95% CI, 0.73–1.25], *P*=0.74; *P*=0.09 for interaction). For the safety outcome, moderate-to-severe bleeding did not vary significantly between the carriers (hazard ratio, 1.83 [95% CI, 0.68–4.95]; *P*=0.23) and noncarriers (hazard ratio, 2.07 [95% CI, 0.62–6.88], *P*=0.23; *P*=0.88 for interaction).

CONCLUSIONS: Clopidogrel-aspirin treatment presented a priority to aspirin in reducing the risk of new stroke in *CYP2C19* loss-of-function noncarriers when administered between 24 and 72 hours after stroke onset. These findings supported the necessity of *CYP2C19* genotyping in the choice of antiplatelet therapy with an extended treatment window to 72 hours.

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GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: alleles ■ clopidogrel ■ humans ■ stroke ■ transient ischemic stroke

Acute mild ischemic stroke or transient ischemic attack (TIA) is followed by a high rate of ≈5% to 10% risk of subsequent stroke during the first 3 months from the symptom onset.[1] Dual-antiplatelet therapy of clopidogrel with aspirin has become a recommended treatment for patients with acute minor stroke and TIA, based

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Nonstandard Abbreviations and Acronyms

ATAMIS	Antiplatelet Therapy in Acute Mild to Moderate Ischemic Stroke
CHANCE	Clopidogrel in High-Risk Patients With Acute Nondisabling Cerebrovascular Events
CONSORT	Consolidated Standards of Reporting Trials
HR	hazard ratio
INSPIRES	Intensive Statin and Antiplatelet Therapy for Acute High-Risk Intracranial or Extracranial Atherosclerosis
NIHSS	National Institutes of Health Stroke Scale
SNP	single-nucleotide polymorphism
TIA	transient ischemic stroke

on the evidence provided by the CHANCE trial (Clopidogrel in High-Risk Patients With Acute Nondisabling Cerebrovascular Events), in which a reduced risk of new stroke has been reported in patients (with a National Institutes of Health Stroke Scale [NIHSS] score of ≤3) randomized to the clopidogrel-aspirin assignment.[2] For better understanding of who may benefit the most from dual-antiplatelet therapy, *CYP2C19* genetic status helps stratify the efficacy and safety outcomes of clopidogrel treatment.[3,4] Patients without *CYP2C19* loss-of-function alleles have presented a reduced risk of new stroke,[5] whereas those carrying *CYP2C19* loss-of-function alleles may benefit more from ticagrelor-aspirin therapy within 24 hours from the symptom onset.[6–8]

As treatment window is essential in the secondary stroke prevention,[9] the INSPIRES trial (Intensive Statin and Antiplatelet Therapy for Acute High-Risk Intracranial or Extracranial Atherosclerosis) has extended the treatment window of dual-antiplatelet therapy to 72 hours (patients with an NIHSS score of ≤5). The subgroup analysis showed clopidogrel-aspirin therapy initiated within 24 to 48 hours after the stroke onset was of a 15% relative risk reduction in new stroke and a 133% relative risk increase in moderate-to-severe bleeding, compared with aspirin alone. When initiated within 48 to 72 hours, the clopidogrel-aspirin therapy demonstrated a 30% relative risk reduction in new stroke and a 125% relative risk increase in bleeding risk.[10] In the ATAMIS study (Antiplatelet Therapy in Acute Mild to Moderate Ischemic Stroke), patients (NIHSS score of 4–10) who received clopidogrel with aspirin within 24 hours had a reduced risk of neurological deterioration compared with patients who received assignment between 24 and 48 hours (P=0.01 for interaction).[11] The evidence indicates that clopidogrel efficacy varies with the time course of randomization, while limited studies have been provided.

To determine whether the *CYP2C19* loss-of-function status influences antiplatelet choice >24 hours, we aimed to evaluate the interaction of *CYP2C19* loss-of-function status on the treatment efficacy and safety (clopidogrel-aspirin versus aspirin alone) between 24 and 72 hours from the symptom onset among the patients with minor ischemic stroke or TIA.

METHODS

Data Availability

Data are available to researchers upon request by contacting the corresponding author.

Study Design and Participants

The INSPIRES study was a multicenter, double-blind, placebo-controlled, randomized, 2-by-2 factorial trial at 222 sites in China from September 17, 2018 to October 15, 2022. The protocol and data collection were approved by ethics committees of Beijing Tiantan Hospital and all other study centers. All participants or their representatives provided written informed consent before being entered into the study. The study protocol has been published previously,[12,13] and the trial protocol was placed in Supplemental Material 3. Our study was a prespecified analysis of the INSPIRES study (detailed in Supplemental Material 3). In brief, patients with acute TIA or minor ischemic stroke within 72 hours from the symptom onset were assigned in a 1:1 ratio to receive clopidogrel (n=3050, loading dose of 300 mg followed by 75 mg daily for days 2–90) plus aspirin (loading dose of 100–300 mg followed by 100 mg daily for days 2–21) or aspirin alone (n=3050, loading dose of 100–300 mg followed by 100 mg daily for days 2–90), and immediate intensive statin therapy or 3-day delayed intensive statin therapy. The aspirin dosage on day 1 was determined by the physician in both groups. No interaction was observed between the antiplatelet treatment and statin treatment in the factorial trial. Here, we focused on the effect of clopidogrel-aspirin and aspirin therapy. The study followed the CONSORT (Consolidated Standards of Reporting Trials) reporting guideline[14] (Supplemental Material).

CYP2C19 Genotyping

CYP2C19 loss-of-function carriers had a reduced ability for clopidogrel metabolism.[4] *CYP2C19*2* and *CYP2C19*3* were identified as the most prevalent loss-of-function variants of *CYP2C19* gene, whereas *CYP2C19*17* was the most common *CYP2C19* gain-of-function allele.[15] In this study, these 3 single-nucleotide polymorphisms (SNPs) for *CYP2C19*, including rs4244285 (*CYP2C19*2*, 681G>A), rs4986893 (*CYP2C19*3*, 636G>A), and rs12248560 (*CYP2C19*17*, -806C>T), were genotyped. Complete *CYP2C19* SNP genotyping data were acquired from 5742 patients. Individuals with complete information for each of these 3 *CYP2C19* SNPs were included in the current analyses. In the present study, 5003 patients were included as they started clopidogrel-aspirin or aspirin alone from symptom onset >24 to 72 hours.

Genotyping of the 3 SNPs was performed using the Matrix-Assisted Laser Desorption/Ionization Time-of-Flight

Mass Spectrometry (MALDI-TOF MS) technique based on the MassARRAY system (Agena Bioscience, San Diego, CA). The procedure was described as follows: first, the DNA samples from the patient samples were subjected to multiplexed polymerase chain reaction (PCR) amplification following the system: 5 μL reaction volume for each sample, which contained a mixture of 1× PCR buffer, 2 mmol/L $MgCl_2$, 500 μM deoxyribonucleoside triphosphates (dNTP), 0.1 pmol/μL, 0.5 U of HotStarTaq enzyme, and 1 μL DNA template. An initial denaturation stage for 2 minutes at 94 ℃ was followed by 45 cycles of denaturation for 20 seconds at 94 ℃, annealing for 30 seconds at 56 ℃, extension for 1 minute at 72 ℃, and a final extension at 72 ℃ for 3 minutes. Second, 0.5 U of shrimp alkaline phosphatase enzyme was added to the PCR subjects to neutralize the unincorporated dNTPs at 37 ℃ for 40 minutes. Then, the extension reaction was performed using the iPLEX pro-extension reaction system, and the iPLEX reaction products were desalted to optimize the spectral signals. Fourth, samples were placed onto the MassARRAY SpectroCHIP, which was put into the MassARRAY Analyzer Compact for detection and analysis.

Patients were categorized by *CYP2C19* loss-of-function status based on**2*, *3* and**17* genotypes. Those with at least 1 loss-of-function allele (**2* or**3*) were classified as loss-of-function allele carriers (**1/*2*, *1/*3*, *2/*2*, *2/*3*, *3/*3*, *2/*17* or **3/*17*) and those with no loss-of-function allele were classified as *CYP2C19* loss-of-function noncarriers (**1/*1*, *1/*17* or**17/*17*).[8,16]

For better understanding of the effect of *CYP2C19* genetic status on clopidogrel usage, patients were further categorized by *CYP2C19* metabolizer status. In the *CYP2C19* loss-of-function carriers, patients with**2* or**3* loss-of-function allele homozygotes (**2/*2*, *2/*3*, or**3/*3*) were classified as poor metabolizers, and those with**2* or**3* loss-of-function allele heterozygotes (**1/*2*, *1/*3*, *2/*17*, or**3/*17*) were classified as intermediate metabolizers. In the *CYP2C19* loss-of-function noncarriers, patients with**17* allele (**1/*17* or**17/*17*) were classified as ultra metabolizers, and those without**2*, *3* or**17* allele (**1/*1*) were classified as extensive metabolizers.

Clinical Outcomes

The primary efficacy outcome was any new stroke (ischemic or hemorrhagic) within 90 days. The secondary efficacy outcomes included the composite events (stroke, myocardial infraction, or death from cardiovascular causes), ischemic stroke, TIA, hemorrhagic stroke, poor functional outcome (modified Rankin Scale score of 2 to 6) , and a 6-level scale classifying the new stroke or TIA within 90 days.[17]

The primary safety outcome was moderate-to-severe bleeding.[18] Other safety outcomes included death from any cause, intracranial hemorrhage and any bleeding within 90 days.

Statistical Analysis

The baseline characteristics were compared between treatment groups, including the total patients included in this subanalysis and the subgroups stratified by *CYP2C19* loss-of-function allele carrier status. Proportions were used for categorical variables, and medians with interquartile ranges were used for continuous variables. The nonparametric Kruskal-Wallis test was used to compare group differences for nominal variables, and χ^2 tests for dichotomous variables. Similar approaches were performed in *CYP2C19* genotypes stratified by homozygotes or heterozygotes.

Efficacy and safety analyses were performed in accordance with the intention-to-treat principle. Differences in the rate of stroke (ischemic or hemorrhagic), the composite outcome, TIA, 6-level scale on stroke or TIA, moderate-to-severe bleeding, death from any cause, and any bleeding (intracranial or mild bleeding) during the 90-day follow-up period were assessed using Cox proportional hazards regression with adjustment for pooled trial centers (those with ≥20 patients were enrolled). Hazard ratios (HR) with 95% CIs were reported. For poor functional outcome, differences between assignment groups and assignment groups within *CYP2C19* loss-of-function status were calculated using generalized linear models, and relative risks with 95% CIs were reported. For the 6-level scale of stroke or TIA outcome between 2 groups were calculated by ordinal logistic regression. At the 90-day follow-up, 2 patients were lost to follow-up and 4 patients had missing data. Following the intention-to-treat principle, these patients were included in the other outcome analyses to maintain trial validity. For evaluating the interaction between treatment assignments and *CYP2C19* loss-of-function allele status, we incorporated terms of treatment assignment and *CYP2C19* loss-of-function status in the Cox proportional hazards model (generalized linear model for poor functional outcomes and the model of ordinal logistic regression for 6-level scale of stroke or TIA outcome). The models included 1 interaction term (treatment assignment×*CYP2C19* status), which contributed to 1 degree of freedom. We conducted cumulative event curves for the new stroke and moderate-to-severe bleeding by Kaplan-Meier analyses, and the differences between groups were tested by the log-rank test. The log-rank test compared the differences of the multiple cumulative event curves among the 4 groups.

All tests were 2-sided, and a *P* value of 0.05 was defined to indicate statistical significance. All statistical analyses were performed with SAS software (SAS Institute), version 9.4.

RESULTS

Study Patients

A total of 6100 patients with acute mild ischemic stroke and high-risk TIA enrolled in the INSPIRES study,[12] among which 5317 patients were randomized between 24 and 72 hours from the stroke onset. *CYP2C19* genotype data of *CYP2C19*2*, *CYP2C19*3* and *CYP2C19*17* were acquired from 5003 patients, and 314 patients were excluded for no blood samples or failed to genotype at least one of the 3 *CYP2C19* alleles. The baseline characteristics of the patients included in this study and those excluded were provided in Table S1. At the 90-day follow-up, 4 patients had missing data and 2 patients were lost to follow-up. The data of patients who were lost to follow-up were treated as censored. A patient flow diagram was shown in Figure 1.

In this genetic subgroup, 2517 patients received aspirin therapy, and 2486 patients received clopidogrel-aspirin therapy. The baseline characteristics of patients classified by randomization were similar to that in the parent study,[12] and the results were shown in

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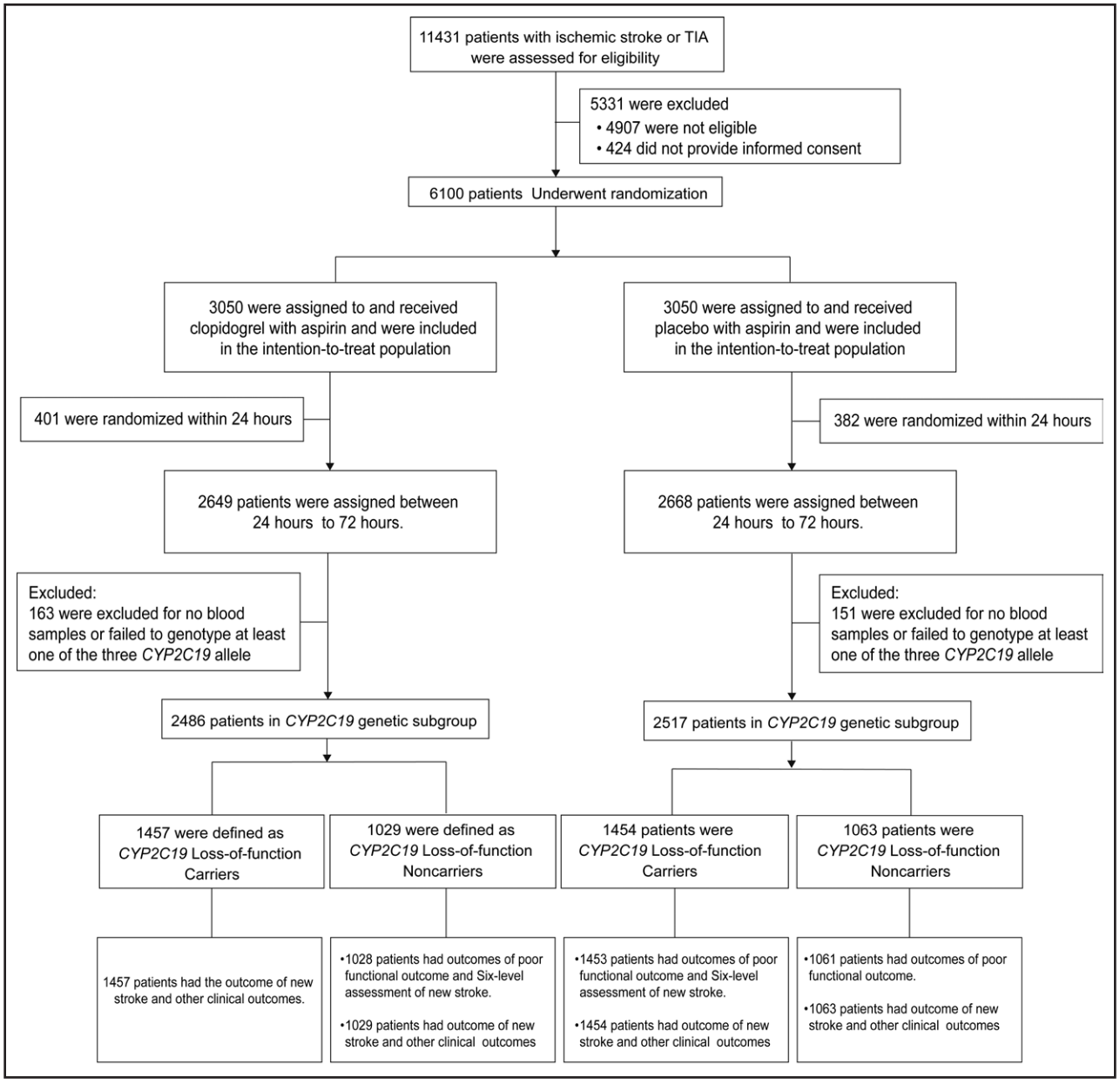

Figure 1. Study flowchat.
TIA indicates transient ischemic stroke.

Table S2. Among the 5003 patients, 2911 (58.2%) patients were defined as *CYP2C19* loss-of-function carriers, and 2092 (41.8%) patients were defined as loss-of-function noncarriers. Baseline demographics, clinical condition and medical history of patients stratified by loss-of-function carriers and noncarriers were detailed in Table 1, and the patient characteristics were similar between *CYP2C19* loss-of-function carriers and noncarriers.

Efficacy Outcomes

In the genetic subgroup population, new stroke occurred within 90 days was 8.2% in 2517 patients receiving aspirin alone (206 of 2517) versus 6.8% in 2486 patients receiving clopidogrel-aspirin (168 of 2486, HR, 0.82 [95% CI, 0.67–1.00]; *P*=0.054). The primary efficacy, the composite event and the individual vascular events were similar to that in the parent population and time dependent course of treatment assignment,[10,12] detailed in Table S3.

The frequency distribution and rate of new stroke for each genotype of the 3 *CYP2C19* SNPs and the total population before subgrouping by genotyping were shown in Table 2. Carriers of the *CYP2C19*2* accounted for 42.6% (2133 of 5003) for *GA* genotype and 9.0% (450 of 5003) for *AA* genotype. *CYP2C19*3* carriers accounted for 9.6% (480 of 5003) of the study population, including 9.3% (464 of 5003) for *GA* genotype and 0.3% (14 of 5003) for *AA* genotype. Frequency

Table 1. Baseline Characteristics Between Carriers and Noncarriers of *CYP2C19* Loss-of-Function Alleles Stratified by Treatment Allocation

Covariate	Carriers*			Noncarriers†		
	Total (N=2911)	Aspirin (n=1454)	Clopidogrel-aspirin (n=1457)	Total (N=2092)	Aspirin (n=1063)	Clopidogrel-aspirin (n=1029)
Age, y; median (IQR)	65.0 (57.0–71.0)	66.0 (57.0–71.0)	65.0 (57.0–71.0)	65.0 (57.0–71.0)	65.0 (57.0–71.0)	64.0 (57.0–71.0)
Male, n (%)	1847 (63.4)	910 (62.6)	937 (64.3)	1350 (64.5)	664 (62.5)	686 (66.7)
BMI, median (IQR)	24.5 (22.6–26.6)	24.5 (22.7–26.7)	24.4 (22.5–26.4)	24.5 (22.7–26.7)	24.5 (22.7–26.7)	24.4 (22.8–26.6)
Medical story, n (%)						
Hypertension	1964 (67.5)	972 (66.9)	992 (68.1)	1409 (67.4)	712 (67.0)	697 (67.7)
Diabetes	809 (27.8)	401 (27.6)	408 (28.0)	568 (27.2)	296 (27.8)	272 (26.4)
Dyslipidemia	108 (3.7)	61 (4.2)	47 (3.2)	73 (3.5)	40 (3.8)	33 (3.2)
Previous ischemic stroke	843 (29.0)	425 (29.2)	418 (28.7)	644 (30.8)	330 (31.0)	314 (30.5)
Current or previous smoker	824 (28.3)	391 (26.9)	433 (29.7)	619 (29.6)	326 (30.7)	293 (28.5)
Use of agents before qualifying event, n (%)						
Aspirin	371 (12.7)	193 (13.3)	178 (12.2)	270 (12.9)	145 (13.6)	125 (12.1)
Clopidogrel	19 (0.7)	8 (0.6)	11 (0.8)	8 (0.4)	3 (0.3)	5 (0.5)
Lipid lowering agent	264 (9.1)	137 (9.4)	127 (8.7)	205 (9.8)	105 (9.9)	100 (9.7)
Qualifying event, n (%)						
TIA	346 (11.9)	173 (11.9)	173 (11.9)	271 (13.0)	141 (13.3)	130 (12.6)
Acute single infarction	545 (18.7)	279 (19.2)	266 (18.3)	433 (20.7)	221 (20.8)	212 (20.6)
Acute multiple infractions	2020 (69.4)	1002 (68.9)	1018 (69.9)	1388 (66.3)	701 (65.9)	687 (66.8)
≥50% symptomatic stenosis, n (%)						
Yes	2331 (81.8)	1163 (81.8)	1168 (81.8)	1694 (82.4)	860 (82.5)	834 (82.2)
No	518 (18.2)	258 (18.2)	260 (18.2)	362 (17.6)	182 (17.5)	180 (17.8)
NIHSS score in qualifying ischemic stroke, n (%)‡						
≤ 3	1978 (77.1)	991 (77.4)	987 (76.9)	1431 (78.6)	719 (78.0)	712 (79.2)
> 3	587 (22.9)	290 (22.6)	297 (23.1)	390 (21.4)	203 (22.0)	187 (20.8)
ABCD2 score in qualifying TIA, n (%)§						
4 or 5	274 (79.2)	131 (75.7)	143 (82.7)	214 (79.0)	108 (76.6)	106 (81.5)
>5	72 (20.8)	42 (24.3)	30 (17.3)	57 (21.0)	33 (23.4)	24 (18.5)
Statin therapy, n (%)						
Immediate intensive statin	1443 (49.6)	739 (50.8)	704 (48.3)	1049 (50.1)	522 (49.1)	527 (51.2)
Delayed intensive statin	1468 (50.4)	715 (49.2)	753 (51.7)	1043 (49.9)	541 (50.9)	502 (48.8)

ABCD2 indicates age, blood pressure, clinical features, duration of symptoms, and diabetes; BMI, body mass index; IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; and TIA, transient ischemic stroke.

*Carriers are defined as those with at least one *CYP2C19* loss-of-function allele, including**1/*2,*1/*3,*2/*2,*2/*3,*3/*3,*2/*17*, or **3/*17*.

†Noncarriers are defined as those with no *CYP2C19* loss-of-function allele, including**1/*1,*1/*17*, or **17/*17*.

‡NIHSS score ranges from 0 to 42 for evaluating conditions of patients with ischemic stroke, and higher scores suggest more severe stroke.

§ABCD2 score ranges from 0 to 7, and higher scores suggest higher risk of stroke.

of *CYP2C19*17* of *CT* genotype was 2.0% (104 of 5003) in this subanalysis. The events rate for the composite vascular event of *CYP2C19*2*, *CYP2C19*3* and *CYP2C19*17* genotype were shown in Table S4.

In the population of *CYP2C19* loss-of-function noncarriers, the treatment effect of clopidogrel-aspirin compared with aspirin in reducing the occurrence of new stroke was significant (HR, 0.67 [95% CI, 0.49–0.91]), while in the population of carriers, the effect was not significant (HR, 0.96 [95% CI, 0.73–1.25]; *P*=0.09 for treatment×*CYP2C19* loss-of-function carrier status interaction effect). The cumulative risk of new stroke among patients with the status of *CYP2C19* loss-of-function carrier or noncarrier by treatment assignment was shown in Figure 2A. Similar tendencies of the effect between the clopidogrel-aspirin and aspirin alone group were observed in the secondary efficacy outcomes of the composite vascular events, ischemic stroke, and TIA, detailed in Table 3.

Safety Outcomes

Among the 5003 patients, moderate-to-severe bleeding occurred within 90 days was 0.4% in 2517 patients receiving aspirin alone (10 of 2517) versus 0.8% in 2486 patients receiving clopidogrel-aspirin (19 of 2486,

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Table 2. Distribution and Event Rate of New Stroke by Genotype for Each of the 3 *CYP2C19* Single-Nucleotide Polymorphisms and in the Total Population Before the Subgrouping by Genotype

	Overall, n (%)	Event rate	Aspirin, n (%)	Event rate	Clopidogrel-aspirin, n (%)	Event rate
Total population	5003	374 (7.5)	2517 (50.3)	206 (8.2)	2486 (49.7)	168 (6.8)
*CYP2C19*2 (681G>A)*						
GG	2420 (48.4)	186 (7.7)	1220 (48.5)	109 (8.9)	1200 (48.3)	77 (6.4)
GA	2133 (42.6)	146 (6.8)	1085 (43.1)	79 (7.3)	1048 (42.2)	67 (6.4)
AA	450 (9.0)	42 (9.3)	212 (8.4)	18 (8.5)	238 (9.6)	24 (10.1)
*CYP2C19*3 (636G>A)*						
GG	4525 (90.4)	347 (7.7)	2280 (90.6)	193 (8.5)	2245 (90.3)	154 (6.9)
GA	464 (9.3)	27 (5.8)	229 (9.1)	13 (5.7)	235 (9.5)	14 (6.0)
AA	14 (0.3)	0 (0.0)	8 (0.3)	0 (0.0)	6 (0.2)	0 (0.0)
*CYP2C19*17 (-806C>T)*						
CC	4902 (98.0)	367 (7.5)	2464 (97.9)	200 (8.1)	2438 (98.1)	167 (6.8)
CT	101 (2.0)	7 (6.9)	53 (2.1)	6 (11.3)	48 (1.9)	1 (2.1)

HR, 1.93 [95% CI, 0.90–4.15]; *P*=0.09). The event rates of the moderate-to-severe bleeding for *CYP2C19*2*, *CYP2C19*3* and *CYP2C19*17* were shown in Table S5.

Combined with the *CYP2C19* genetic status, the risk for moderate-to-severe bleeding between the aspirin group and clopidogrel with aspirin group were not significant in *CYP2C19* loss-of-function noncarriers (HR, 2.07 [95% CI, 0.62–6.88]) or carriers (HR, 1.83 [95% CI, 0.68–4.95]) and no difference was observed according to the *CYP2C19* genetic status (*P*=0.88 for interaction). The secondary safety outcomes including death from any cause and any bleeding were following the similar tendency between the clopidogrel-aspirin and aspirin group stratified with the *CYP2C19* genetic status. The cumulative risk of moderate-to-severe bleeding among patients with the status of *CYP2C19* loss-of-function carrier or noncarrier by treatment assignment was shown in Figure 2B.

Clinical Outcomes Stratified by *CYP2C19* Metabolizer Status

The association between the poor metabolizers and intermediate metabolizers with the clinical outcomes was tested in the *CYP2C19* loss-of-function carriers. Among the 2911 loss-of-function carriers, 2297 (78.9%) patients were intermediate metabolizers. As shown in Table S6, the relative risk reduction for new stroke with aspirin versus clopidogrel-aspirin was not significant among the intermediate metabolizers (HR, 0.90 [95% CI, 0.66–1.22]) and the poor metabolizers (HR, 1.18 [95% CI, 0.67–2.10]).

Among the 2092 *CYP2C19* loss-of-function noncarriers, 2021 (96.6%) patients were extensive metabolizers, and 71 patients were ultra metabolizers. The association between the extensive metabolizers and the ultra metabolizers with the clinical outcome was shown in Table S7. The relative risk reduction for new stroke with aspirin versus clopidogrel-aspirin was significant among the extensive metabolizers (HR, 0.70 [95% CI, 0.51–0.95]). The moderate-to-severe bleeding rate was not significant in the extensive metabolizers with aspirin versus clopidogrel-aspirin therapy (HR, 2.06 [95% CI, 0.62–6.86]). Due to the limited number of **17* carriers, no stroke events were observed in the 33 ultra metabolizers who received clopidogrel-aspirin therapy, and the risk ratio was not estimated.

DISCUSSION

In this genetic study, we focused on the therapeutic effect stratified by *CYP2C19* loss-of-function allele status between 24 and 72 hours from the symptom onset in patients with acute minor stroke or TIA. Clopidogrel-aspirin treatment was superior to aspirin alone in noncarriers of the *CYP2C19* loss-of-function allele, while those *CYP2C19* loss-of-function allele carriers had no reduced risk of new stroke in the clopidogrel-aspirin group. In the aspect of individual stroke events, there was a reduced risk of ischemic stroke and TIA within 90 days in the noncarriers in the clopidogrel-aspirin group, but not in the carriers. No difference in bleeding was found between carriers and noncarriers between the clopidogrel-aspirin and aspirin alone groups. Our findings provide evidence for identifying that acute minor stroke or TIA patients without *CYP2C19* loss-of-function allele can benefit from clopidogrel-aspirin therapy with an extended treatment time window from within 24 to 72 hours after symptom onset.

In our total genetic population without *CYP2C19* stratification, a tendency towards reduced risk of new stroke between the clopidogrel with aspirin and aspirin alone (*P*=0.054) in patients (NIHSS score of ≤5) started treatment between 24 and 72 hours. In the subgroup of the INSPIRES study on randomization time, reduced risk of new stroke was the most obvious in >48 within 72 hours between the clopidogrel with aspirin and aspirin

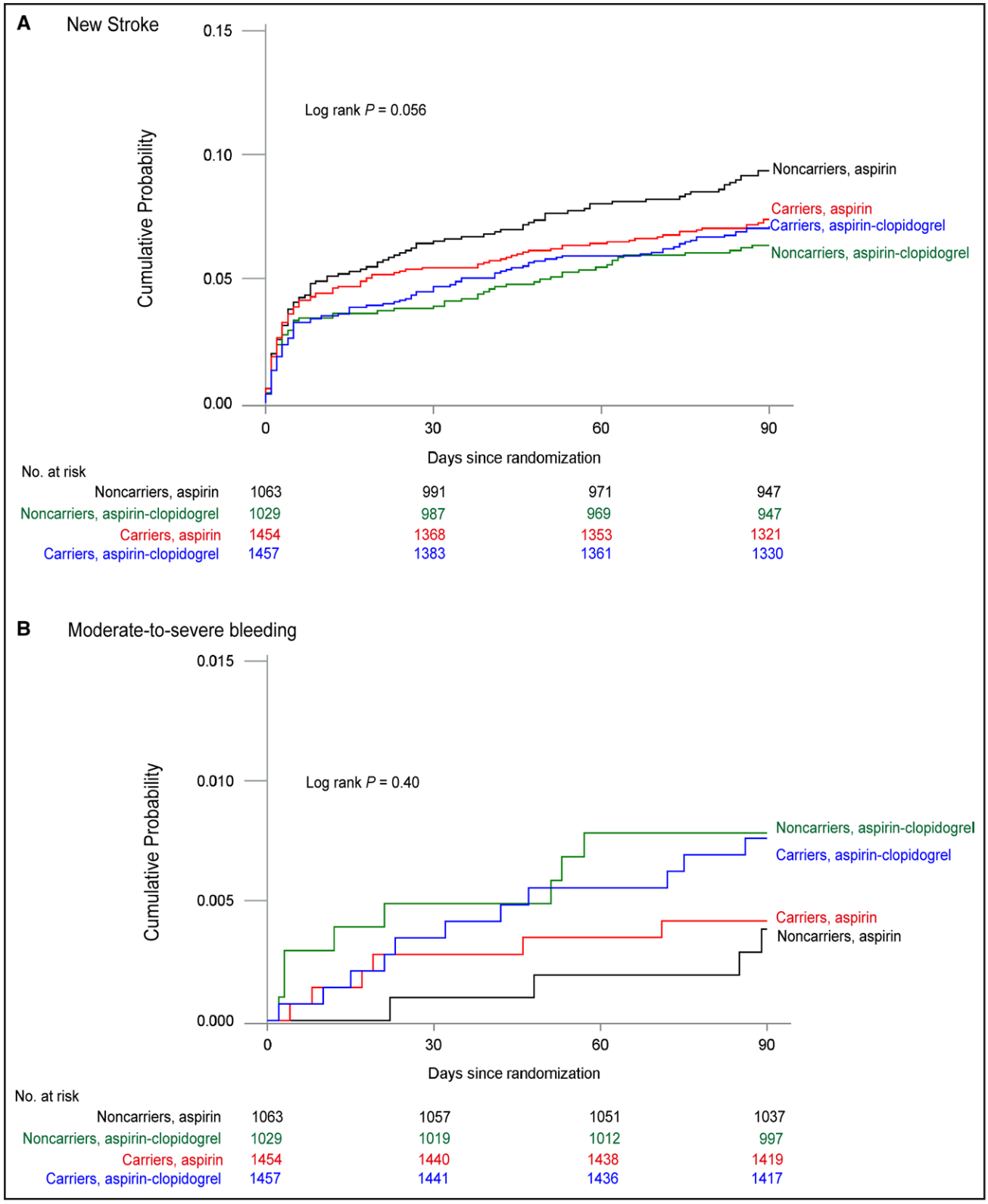

Figure 2. Cumulative probability of new stroke and moderate-to-severe bleeding according to the loss-of-function allele carrier status.

Carriers are defined as those with at least one *CYP2C19* loss-of-function allele, including* *1/*2,*1/*3,*2/*2,*2/*3,*3/*3,*2/*17,* or *3/*17.* Noncarriers are defined as those with no *CYP2C19* loss-of-function allele, including *1/*1,*1/*17,* or *17/*17.* Log-rank test was performed to compare the difference of new stroke and moderate-to-severe bleeding rate among the 4 groups.

Table 3. Effect of Clopidogrel-Aspirin Compared With Aspirin on Clinical Outcomes Stratified by *CYP2C19* Loss-of-Function Carrier Status

Outcome	Carriers* Total (N=2911)	n (%) Aspirin (n=1454)	Clopidogrel -aspirin (n=1457)	HR or RR or cOR† (95% CI)	*P* value	Noncarriers‡ Total (N=2092)	n (%) Aspirin (n=1063)	Clopidogrel-aspirin (n=1029)	HR or RR or cOR† (95% CI)	*P* value	*P* value for interaction
Primary efficacy outcome											
Stroke, including ischemic and hemorrhagic stroke	210 (7.2)	107 (7.4)	103 (7.1)	0.96 (0.73–1.25)	0.74	164 (7.8)	99 (9.3)	65 (6.3)	0.67 (0.49–0.91)	0.01	0.09
Secondary efficacy outcome											
Composite events§	213 (7.3)	109 (7.5)	104 (7.1)	0.95 (0.72–1.24)	0.69	167 (8.0)	100 (9.4)	67 (6.5)	0.68 (0.50–0.93)	0.02	0.12
Ischemic stroke	204 (7.0)	106 (7.3)	98 (6.7)	0.92 (0.70–1.21)	0.54	156 (7.5)	96 (9.0)	60 (5.8)	0.64 (0.46–0.88)	0.006	0.09
Recurrent stroke	147 (5.0)	72 (5.0)	75 (5.1)	1.03 (0.75–1.43)	0.84	126 (6.0)	76 (7.1)	50 (4.9)	0.67 (0.47–0.96)	0.03	0.08
TIA with infraction	8 (0.3)	5 (0.3)	3 (0.2)	0.60 (0.14–2.49)	0.48	7 (0.3)	6 (0.6)	1 (0.1)	0.17 (0.02–1.40)	0.10	0.33
Progressive stroke	49 (1.7)	29 (2.0)	20 (1.4)	0.69 (0.39–1.21)	0.19	23 (1.1)	14 (1.3)	9 (0.9)	0.66 (0.29–1.52)	0.33	0.94
Hemorrhagic stroke	7 (0.2)	1 (0.1)	6 (0.4)	6.00 (0.72–49.84)	0.10	8 (0.4)	3 (0.3)	5 (0.5)	1.73 (0.41–7.22)	0.46	0.34
TIA	29 (1.0)	18 (1.2)	11 (0.8)	0.61 (0.29–1.29)	0.19	21 (1.0)	16 (1.5)	5 (0.5)	0.32 (0.12–0.88)	0.03	0.32
Poor functional outcome¶	302 (10.4)	165 (11.4)	137 (9.4)	0.83 (0.66–1.04)	0.10	197 (9.4)	113(10.7)	84 (8.2)	0.77 (0.60–0.99)	0.04	0.67
Six-level assessment of new stroke, n/total N				0.93 (0.71–1.21)	0.58				0.61 (0.44–0.83)	0.002	0.04
5: Fatal stroke	10 (0.3)	3 (0.2)	7 (0.5)			13 (0.6)	4 (0.4)	9 (0.9)			
4: Severe stroke	23 (0.8)	10 (0.7)	13 (0.9)			15 (0.7)	9 (0.8)	6 (0.6)			
3: Moderate stroke	72 (2.5)	44 (3.0)	28 (1.9)			52 (2.5)	34 (3.2)	18 (1.8)			
2: Mild stroke	104 (3.6)	49 (3.4)	55 (3.8)			83 (4.0)	52 (4.9)	31 (3.0)			
1: TIA	27 (0.9)	16 (1.1)	11 (0.8)			20 (1.0)	15 (1.4)	5 (0.5)			
0: No stroke or TIA	2674 (91.9)	1331 (91.6)	1343 (92.2)			1908 (91.2)	949 (89.3)	959 (93.3)			
Primary safety outcome											
Moderate-to-severe bleeding#	17 (0.6)	6 (0.4)	11 (0.8)	1.83 (0.68–4.95)	0.23	12 (0.6)	4 (0.4)	8 (0.8)	2.07 (0.62–6.88)	0.23	0.88
Secondary safety outcome											
Death from any cause	28 (1.0)	13 (0.9)	15 (1.0)	1.15 (0.55–2.42)	0.71	22 (1.1)	9 (0.8)	13 (1.3)	1.50 (0.64–3.50)	0.35	0.65
Any bleeding #	69 (2.4)	28 (1.9)	41 (2.8)	1.47 (0.91–2.37)	0.12	49 (2.3)	22 (2.1)	27 (2.6)	1.27 (0.72–2.23)	0.41	0.71
Intracranial hemorrhage	10 (0.3)	3 (0.2)	7 (0.5)	2.33 (0.60–9.01)	0.22	8 (0.4)	3 (0.3)	5 (0.5)	1.73 (0.41–7.22)	0.46	0.76
Mild bleeding	54 (1.9)	23 (1.6)	31 (2.1)	1.35 (0.79–2.31)	0.28	38 (1.8)	18 (1.7)	20 (1.9)	1.15 (0.61–2.17)	0.67	0.71

cOR indicates common odds ratio; HR, hazard ratio; RR, relative risk; and TIA, transient ischemic stroke.

Carriers are defined as those with at least one CYP2C19 loss-of-function allele, including *1/*2,*1/*3,*2/*2,*2/*3,*3/*3,*2/*17*, or *3/*17*.

†Relative risks were shown for poor functional outcomes. cORs were shown for 6-level assessment of new stroke. Hazards ratios were shown for other outcomes.

‡Noncarriers are defined as those with no CYP2C19 loss-of-function allele, including *1/*1,*1/*17*, or *17/*17*.

§Composite event includes stroke, myocardial infarction, or death from cardiovascular causes.

¶Poor functional outcome includes modified Rankin Scale scores of 2 to 6. The modified Rankin Scale scores range from 0 to 6, with higher scores indicating more disability. The modified Rankin Scale at 90 d was missing for 3 patients in the *CYP2C19* loss-of-function noncarriers and for 1 patient in *CYP2C19* loss-of-function carriers.

#Bleeding events were defined in accordance with the Global Utilization of Streptokinase and tissue-type plasminogen activator for Occluded Coronary Arteries criteria.

alone (5.8% versus 8.2%; *P*<0.02), and a reduced incidence of new stroke was also observed (7.6% versus 8.9%; *P*=0.25) but not statistically significant in patients who were randomized between 24 and 48 hours from the symptom onset.[10] Our study results were similar to those in the previous study, which also indicated that the efficacy of clopidogrel was associated with the randomization time. For a more accurate therapy, a priority of clopidogrel with aspirin treatment stands out combined with the *CYP2C19* genetic stratification (*P*<0.01 in the *CYP2C19* loss-of-function noncarriers). A nonsignificant trend toward an interaction (*P*=0.09) of the efficacy outcome between clopidogrel-aspirin and aspirin alone stratified by *CYP2C19* loss-of-function carriers or noncarriers was observed between 24 and 72 hours from the symptom onset. In the previous genetic subgroup of the CHANCE study, a significant interaction was observed between the carriers and noncarriers within 24 hours (*P*=0.02). The incidence of new stroke in that study of the clopidogrel with aspirin therapy was 9.4% in the carriers and 6.7% in the noncarriers,[5] while in our study the incidence was 7.4% in the carriers and 6.3% in the noncarriers. In addition, compared with the genetic subgroup of the CHANCE study, patients were with a NIHSS score of ≤3, while the patients included in the INSPIRES study had a NIHSS score of ≤5, in which patients were with a

severe stroke. A different inclusion of patients and the low overall risk of new stroke incidence may explain for a the nonsignificant trend toward an interaction between the carriers and noncarriers. As the ability of clopidogrel metabolism is still important in the application of clopidogrel between 24 and 72 hours from the symptom onset, the *CYP2C19* genotyping is still recommended in the early stroke onset extended to 72 hours.

Patients with reduced ability of clopidogrel metabolism were reported accounting for 50% to 60% in the Asian population,[19,20] and an ≈30% of whom were found as *CYP2C19* loss-of-function carriers in non-Asians.[21,22] In this study, 58.2% patients were defined as carriers (51.6% of the patients are *CYP2C19*2* carriers and 9.6% are *CYP2C19*3* carriers in this study), which was similar to the previous results.[23,24] Given the high prevalence of *CYP2C19* loss-of-function allele, genetic testing before the clopidogrel with aspirin therapy is required. This study provides evidence that noncarriers of *CYP2C19* loss-of-function status are recommended for dual-antiplatelet therapy of clopidogrel with aspirin administration between 24 and 72 hours from the symptom onset in patients with acute minor stroke or TIA. Whereas the carriers of *CYP2C19* loss-of-function will not benefit from the clopidogrel with aspirin treatment in patients who were randomized between 24 and 72 hours, compared with aspirin alone. Considering that >50% of patients are with reduced metabolism ability of clopidogrel in the Asian population, more studies are required to personalize the treatment. Beyond its application in stroke therapy, clopidogrel remains a major therapeutic choice in secondary prevention of atherothrombotic events in atherosclerotic diseases, including coronary artery disease and peripheral arterial disease.[25] Clinical evidence supports *CYP2C19* genotyping (**2* and**3* alleles) to guide clopidogrel therapy in patients with coronary artery disease, where those patients with *CYP2C19* loss-of-function alleles are recommended to an alternative therapy of ticagrelor or prasugrel for their reduced drug efficacy and higher thrombotic risk.[26–28] In one randomized clinical trial with critical limb ischemic patients, *CYP2C19*-based intermediate and poor metabolizers taking clopidogrel therapy presented a higher risk of amputation and mortality.[29] Collectively, these findings underscore the clinical value of genotype-guided strategies across cardiocerebrovascular diseases.

Our study has several limitations. First, this study evaluated the efficacy and safety outcomes between *CYP2C19* loss-of-function carriers and noncarriers. While there is a gap between clopidogrel metabolism and *CYP2C19* genotype,[30] clopidogrel response was not determined, so we didn't compare the clopidogrel responsiveness with the efficacy of clopidogrel with aspirin therapy. In this study, *CYP2C19* genetic status was determined by genotyping 3 SNPs of *CYP2C19*, while more *CYP2C19* genetic SNPs should be included in determining the *CYP2C19* genetic status.[31] Up to date, 39 SNPs of *CYP2C19* are found associated with clopidogrel metabolism (https://www.pharmvar.org/gene/CYP2C19). Second, all patients in our study were Chinese, and the frequencies of *CYP2C19* genotypes differ in populations, so these findings may not be applicable in other populations. Third, no significant difference in bleeding risk between *CYP2C19* loss-of-function noncarriers received clopidogrel-aspirin therapy or aspirin alone was observed, while a higher risk of moderate-to-severe bleeding was observed in the parent study of the INSPIRES trial (P=0.03),[12] so the statistical power was limited, which might be due to a lower number of bleeding events. The POINT study (Platelet-Oriented Inhibition in New TIA and Minor Ischemic Stroke) and a previous meta-analysis reported a higher risk of hemorrhagic stroke in clopidogrel-treated patients,[32] so more attention should be placed on the bleeding events during clopidogrel usage. Fourth, the INSPIRES trial especially included patients who were with stroke attributed to major intracranial or extracranial artery atherosclerosis, so our results are not applicable to those patients with presumed cardioembolic stroke, small vessel disease, and cryptogenic stroke.

In summary, among the patients with acute minor stroke or TIA who received clopidogrel-aspirin assignment between 24 and 72 hours have a reduced risk for new stroke who are *CYP2C19* loss-of-function noncarriers. These findings support the role of *CYP2C19* genotyping in the efficacy of clopidogrel-aspirin treatment with an extended treatment window up to 72 hours.

ARTICLE INFORMATION

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Author Contributions

Drs Yun Chen, Jiang, Gao, Weiqi Chen, Pan, and Yilong Wang contributed to the conception and design of the study. Drs Yun Chen, Jiang, Gao, Weiqi Chen, Yan, Tingting Wang, Yang, Yao, Liu, Pan, and Yilong Wang contributed to the acquisition and analysis of data. Drs Yun Chen, Jiang, Gao, Weiqi Chen, Pan, and Yilong Wang contributed to drafting the text or preparing the figures. Drs Pan and Yilong Wang contributed to the study supervision.

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Disclosures

None.

Supplemental Material

Tables S1–S7
CONSORT Checklist
Trial Protocol

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