Supplementary Online Content

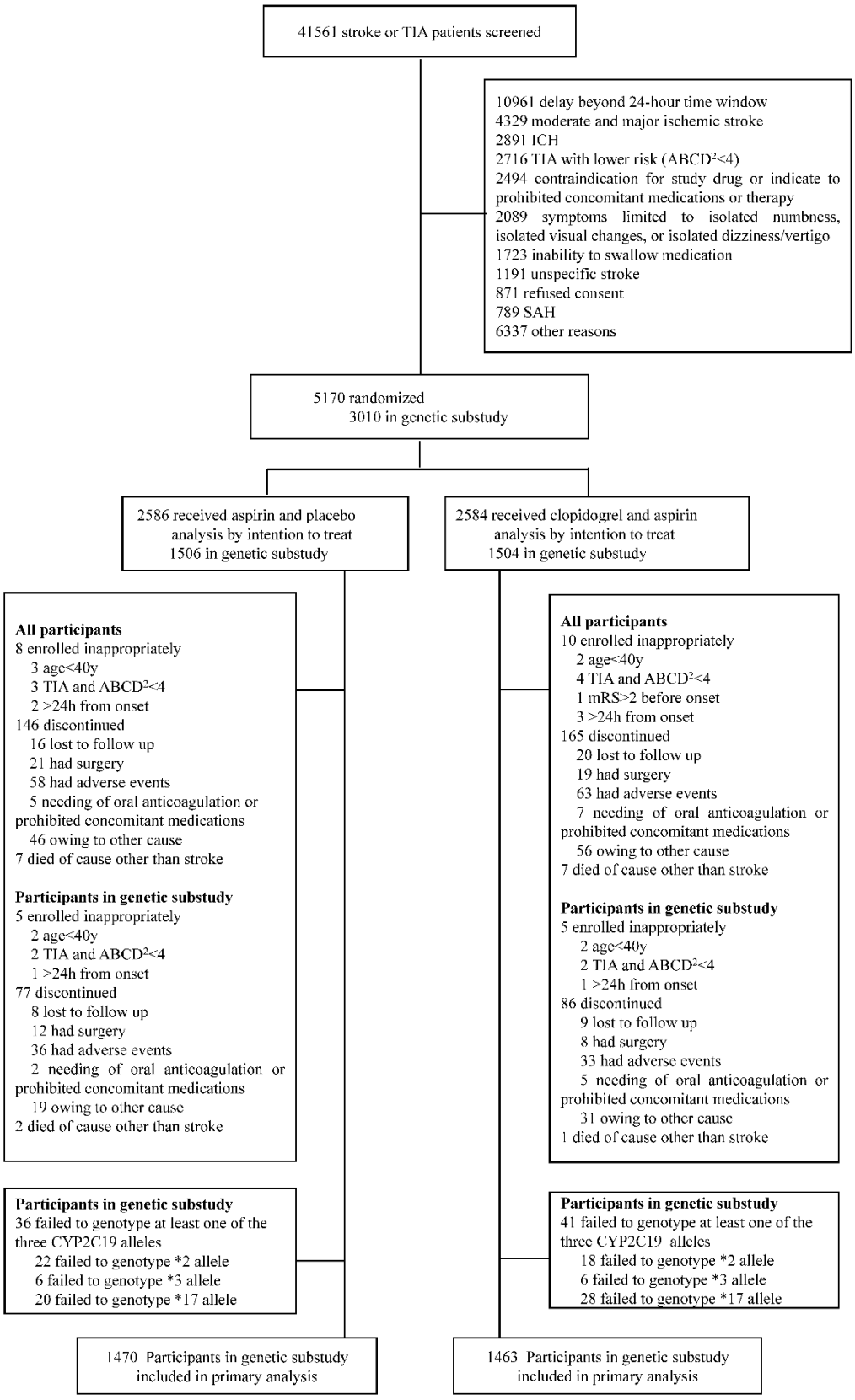
Wang Y, Zhao X, Lin J, et al. Association between *CYP2C19* loss-of-function allele status and efficacy of clopidogrel for risk reduction among patients with minor stroke or transient ischemic attack. *JAMA*. doi:10.1001/jama.2016.8662

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix. Details on genotyping technology

Three single-nucleotide polymorphisms (SNPs) for CYP2C19, including rs4244285 (*2), rs4986893 (*3), and rs12248560 (*17), were genotyped in 3010 participants. Genotyping of the three SNPs was performed using the Sequenom MassARRAY iPLEX platform (Sequenom, San Diego, CA). The locus–specific PCR primers were designed by the MassARRAY Assay Design software package (v4.0). The PCR products were dephosphorylated with Shrimp Alkaline Phosphatase enzymes before undertaking the iPLE X GOLD primer extension reactions. The desalted iPLEX reaction product was spotted onto a 384-format SpectroCHIP. Mass determination was discriminated by the MALDI-TOF mass spectrometer. Data acquisition was done by the MassARRAY Typer 4.0 software.

eFigure. Flow diagram of participants in the genetic substudy



eTable 1. Baseline differences between individuals with and without genetic data

Covariate	Without (n=2237)	With (n=2933)	P value
Age, year, median (IQR)	62.2 (54.5-71.3)	62.4 (54.8-71.2)	0.89
Female, No. (%)	1472 (65.8)	1948 (66.4)	0.64
BMI, median (IQR)	24.5 (22.9-26.4)	24.5 (22.7-26.6)	0.76
Medical history, No. (%)			
Ischemic stroke	480 (21.5)	553 (18.9)	0.02
TIA	84 (3.8)	90 (3.1)	0.18
Myocardial infarction	45 (2.0)	51 (1.7)	0.47
Congestive heart failure	31 (1.4)	49 (1.7)	0.41
Known atrial fibrillation or flutter	48 (2.1)	48 (1.6)	0.18
Valvular heart disease	6 (0.3)	8 (0.3)	0.98
Hypertension	1476 (66.0)	1923 (65.6)	0.75
Diabetes mellitus	513 (22.9)	580 (19.8)	0.006
Hypercholesterolemia	271 (12.1)	302 (10.3)	0.04
Current or previous smoker, No. (%)	977 (43.7)	1244 (42.4)	0.36
Index event			0.03
TIA	661 (29.5)	784 (26.7)	
Minor stroke	1576 (70.5)	2149 (73.3)	
Time from symptom onset to randomization, median (IQR)	12.0 (6.5-19.7)	12.0 (6.4-19.2)	0.34
Concomitant medication, No. (%)			
Proton pump inhibitors	26 (1.2)	20 (0.7)	0.07
Anti-hypertension agents	709 (31.7)	1105 (37.7)	<0.001
Anti-diabetes agents	293 (13.1)	363 (12.4)	0.44
Lipid-lowing agents	921 (41.2)	1250 (42.6)	0.30

Abbreviations: BMI, body mass index; IQR, interquartile range; TIA, transient ischemic attack

eTable 2. Event rate and effect of clopidogrel-aspirin *vs*. aspirin in patients with genotype data and in the parent trial

	Study Populaton (n=2933)				**Trial Population[a] (n=5710)**			
	Aspirin No. (%) (N=1470)	**Clopidogrel-aspirin No. (%) (N=1463)**	**HR (95%CI)**	**P value**	**Aspirin No. (%) (N=2586)**	**Clopidogrel-aspirin No. (%) (N=2584)**	**HR (95%CI)**	**P value**
Stroke	168 (11.4)	121 (8.3)	0.71 (0.56-0.90)	0.005	303 (11.7)	212 (8.2)	0.68 (0.57-0.81)	<0.001
Composite event [b]	170 (11.6)	121 (8.3)	0.70 (0.56-0.89)	0.003	307 (11.9)	216 (8.4)	0.69 (0.58-0.82)	<0.001
Any bleeding	22 (1.5)	35 (2.4)	1.57 (0.92-2.68)	0.098	41 (1.6)	60 (2.3)	1.41 (0.95-2.10)	0.09

Abbreviations: HR, Hazard ratio.

[a] Data were adapted from the parent trial Table 2. [1]

[b] Composite event was defined as a new clinical vascular event, including ischemic stroke, hemorrhagic stroke, myocardial infarction, or vascular death.

eTable 3. Event rates of composite event by genotype for each of the three *CYP2C19* SNPs

	Overall		**Aspirin**		**Clopidogrel-aspirin**	
	Frequency No. (%) (N=2933)	**Event rate No. (%)**	**Frequency No. (%) (N=1470)**	**Event rate No. (%)**	**Frequency No. (%) (N=1463)**	**Event rate No. (%)**
*CYP2C19**2 (681G>A)						
GG	1392 (47.5)	133 (9.6)	692 (47.1)	84 (12.1)	700 (47.9)	49 (7.0)
GA	1255 (42.8)	130 (10.4)	636 (43.3)	72 (11.3)	619 (42.3)	58 (9.4)
AA	286 (9.7)	28 (9.8)	142 (9.6)	14 (9.9)	144 (9.8)	14 (9.7)
*CYP2C19**3 (636G>A)						
GG	2669 (91.0)	267 (10.0)	1334 (90.7)	157 (11.8)	1335 (91.3)	110 (8.2)
GA	260 (8.9)	23 (8.8)	132 (9.0)	12 (9.1)	128 (8.7)	11 (8.6)
AA	4 (0.1)	1 (25)	4 (0.3)	1 (25.0)	0	
*CYP2C19**17 (-806C>T)						
CC	2875 (98.0)	289 (10.1)	1442 (98.1)	168 (11.7)	1443 (98.0)	121 (8.4)
CT	58 (2.0)	2 (3.4)	28 (1.9)	2 (7.1)	30 (2.0)	0 (0)

Composite event was defined as a new clinical vascular event, including ischemic stroke, hemorrhagic stroke, myocardial infarction, or vascular death.

eTable 4. Event rates of any bleeding by genotype for each of the three *CYP2C19* SNPs

	Overall		**Aspirin**		**Clopidogrel-aspirin**	
	Frequency No. (%) (N=2933)	**Event rate No. (%)**	**Frequency No. (%) (N=1470)**	**Event rate No. (%)**	**Frequency No. (%) (N=1463)**	**Event rate No. (%)**
*CYP2C19**2 (681G>A)						
GG	1392 (47.5)	28 (2.0)	692 (47.1)	11 (1.6)	700 (47.9)	17 (2.4)
GA	1255 (42.8)	23 (1.8)	636 (43.3)	7 (1.1)	619 (42.3)	16 (2.6)
AA	286 (9.7)	6 (2.1)	142 (9.6)	4 (2.8)	144 (9.8)	2 (1.4)
*CYP2C19**3 (636G>A)						
GG	2669 (91.0)	54 (2.0)	1334 (90.7)	21 (1.6)	1335 (91.3)	33 (2.5)
GA	260 (8.9)	3 (1.2)	132 (9.0)	1/ (0.8)	128 (8.7)	2 (1.6)
AA	4 (0.1)	2 (50.0)	4 (0.3)	0 (0)	0	NE
*CYP2C19**17 (-806C>T)						
CC	2875 (98.0)	56 (1.9)	1442 (98.1)	21 (1.5)	1443 (98.0)	35 (2.4)
CT	58 (2.0)	1 (1.7)	28 (1.9)	1 (3.6)	30 (2.0)	0 (0)

Abbreviations: NE, not estimable.

eTable 5. Effect for clopidogrel-aspirin as compared with aspirin on clinical outcome subtypes stratified by *CYP2C19* loss-of-function carrier status

Outcome	Carriers [a]			Hazard ratio (95%CI)	p value	Non-carriers [b]			Hazard ratio (95%CI)	p value	p value for interaction
	Total No.(%) (N=1726)	Aspirin No.(%) (N=872)	Clopidogrel-aspirin No.(%) (N=854)			Total No.(%) (N=1207)	Aspirin No.(%) (N=598)	Clopidogrel-aspirin No.(%) (N=609)			
Progressive ischemic stroke:	56 (3.2)	27 (3.1)	29 (3.4)	1.05 (0.62-1.77)	0.86	30 (2.5)	21 (3.5)	9 (1.5)	0.38 (0.17-0.84)	0.02	0.04
Recurrent ischemic stroke	115 (6.7)	66 (7.6)	49 (5.7)	0.75 (0.52-1.08)	0.12	83 (6.9)	53 (8.9)	30 (4.9)	0.53 (0.34-0.83)	<0.01	0.25
Etiology subtype											
Large-artery atherosclerosis	111 (6.4)	55 (6.3)	56 (6.6)	1.02 (0.70-1.48)	0.92	79 (6.5)	47 (7.9)	32 (5.3)	0.62 (0.39-0.97)	0.04	0.11
Small-artery occlusion	48 (2.8)	31 (3.6)	17 (2.0)	0.56 (0.31-1.01)	0.05	31 (2.6)	24 (4.0)	7 (1.1)	0.28 (0.12-0.65)	<0.01	0.17
Cardiogenic embolism	4 (0.2)	1 (0.1)	3 (0.4)	2.01 (0.11-35.35)	0.63	2 (0.2)	2 (0.3)	0 (0.0)	NE		
Other or undetermined	8 (0.5)	6 (0.7)	2 (0.2)	0.28 (0.05-1.44)	0.13	1 (0.1)	1 (0.2)	0 (0.0)	NE		
MI	1 (0.1)	1 (0.1)	0 (0.0)	NE		1 (0.1)	1 (0.2)	0 (0.0)	NE		

Abbreviation: CI, confidence interval; MI, myocardial infarction; NE, not estimable.

[a] Loss-of-function allele carriers were defined as patients with at least one *CYP2C19* loss-of-function allele (i.e., *2 or *3): *1/*2, *1/*3, *2/*2, *2/*3, *3/*3, *2/*17, or *3/*17;

[b] Loss-of-function non-carriers were defined as patients with no *CYP2C19* loss-of-function allele: *1/*1, *1/*17, or *17/*17.

eTable 6. Event rates and effect of clopidogrel-aspirin vs. aspirin in patients with and without proton pump inhibitors stratified by carrier status

Event	**PPI use**	**Carrier** [a]		**HR (95%CI)**	**Non-carrier** [b]		**HR (95%CI)**	**p for interaction**
		Aspirin no./N (%)	**Clopidogrel- aspirin no./N (%)**		**Aspirin no./N (%)**	**Clopidogrel- aspirin no./N (%)**		
Stroke								
	No	92/867 (10.6)	79/849 (9.3)	0.87 (0.64-1.18)	73/594 (12.3)	38/603 (6.3)	0.50 (0.34-0.74)	0.03
	Yes	2/5 (40.0)	1/5 (20.0)	NE	1/4 (25.0)	3/6 (50.0)	NE	NE
Composite event [c]								
	No	93/867 (10.7)	79/849 (9.3)	0.86 (0.64-1.16)	74/594 (12.5)	38/603 (6.3)	0.49 (0.33-0.73)	0.03
	Yes	2/5 (40.0)	1/5 (20.0)	0.39 (0.03-4.34)	1/4 (25.0)	3/6 (50.0)	2.80 (0.29-27.32)	0.26
Any bleeding								
	No	12/867 (1.4)	19/849 (2.2)	1.56 (0.76-3.22)	8/594 (1.3)	15/603 (2.5)	1.79 (0.76-4.23)	0.81
	Yes	0/5 (0.0)	1/5 (20.0)	NE	2/4 (50.0)	0/6 (0.0)	NE	NE

Abbreviations: PPI, proton pump inhibitors; HR, hazard ratio; CI, confidence interval; NE, not estimable.

[a] Loss-of-function allele carriers were defined as patients with at least one *CYP2C19* loss-of-function allele (ie, *2 or *3): *1/*2, *1/*3, *2/*2, *2/*3, *3/*3, *2/*17, or *3/*17;

[b] Loss-of-function non-carriers were defined as patients with no *CYP2C19* loss-of-function allele: *1/*1, *1/*17, or *17/*17;

[c] Composite event was defined as a new clinical vascular event, including ischemic stroke, hemorrhagic stroke, myocardial infarction, or vascular death.

eReferences

1. Wang Y, Wang Y, Zhao X, et al. Clopidogrel with aspirin in acute minor stroke or transient ischemic attack. *N Engl J Med*. 2013;369(1):11-19.