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Analytical validation of GMEX rapid point-of-care *CYP2C19* genotyping system for the CHANCE-2 trial

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ABSTRACT

Background and purpose Rapid genotyping is useful for guiding early antiplatelet therapy in patients with high-risk nondisabling ischaemic cerebrovascular events (HR-NICE). Conventional genetic testing methods used in *CYP2C19* genotype-guided antiplatelet therapy for patients with HR-NICE did not satisfy the needs of the Clopidogrel in High-Risk Patients with Acute Nondisabling Cerebrovascular Events (CHANCE)-2 trial. Therefore, we developed the rapid-genotyping GMEX (point-of-care) system to meet the needs of the CHANCE-2 trial.

Methods Healthy individuals and patients with history of cardiovascular diseases (n=408) were enrolled from six centres of the CHANCE-2 trial. We compared the laboratory-based genomic test results with Sanger sequencing test results for accuracy verification. Next, we demonstrated the accuracy, timeliness and clinical operability of the GMEX system compared with laboratory-based technology (YZY Kit) to verify whether the GMEX system satisfies the needs of the CHANCE-2 trial.

Results Genotypes reported by the GMEX system showed 100% agreement with those determined by using the YZY Kit and Sanger sequencing for all three *CYP2C19* alleles (*2, *3 and *17) tested. The average result's turnaround times for the GMEX and YZY Kit methods were 85.0 (IQR: 85.0–86.0) and 1630.0 (IQR: 354.0–7594.0) min ($p<0.001$), respectively.

Conclusions Our data suggest that the GMEX system is a reliable and feasible point-of-care system for rapid *CYP2C19* genotyping for the CHANCE-2 trial or related clinical and research applications.

INTRODUCTION

Ischaemic cerebrovascular events are one of the leading causes of death and disability in China.[1] Approximately 65% of ischaemic cerebrovascular events are nondisabling ischaemic cerebrovascular events (NICE), which include transient ischaemic attack (TIA) and minor stroke.[2] The rate of early strokes in these patients with NICE is between 10% and 20%,[3 4] which is much higher than previously reported.[5 6] In particular, patients with high-risk NICE (HR-NICE) require urgent antiplatelet intervention.[3 7] Our previously published results, from the Clopidogrel in High-Risk Patients with Acute Nondisabling Cerebrovascular Events (CHANCE) trial, showed for the first time that aspirin plus clopidogrel taken within 24 hours of symptom onset can significantly reduce the risk of subsequent stroke by 32% compared with aspirin alone in patients with HR-NICE, and that the risk of bleeding did not increase in the clopidogrel-aspirin group.[3] On the basis of the dramatic responses seen in our CHANCE trial, early (within 24 hours) and short-term dual antiplatelet therapy (DAPT) is recommended for patients with NICE.[8] This therapeutic strategy has been incorporated into stroke guidelines worldwide.[9–11]

However, clopidogrel is a prodrug whose bioactivation is primarily affected by hepatic cytochrome P450 family enzymes, with *CYP2C19* playing a predominant role.[12] Numerous genetic polymorphisms exist for *CYP2C19*. *CYP2C19**2 (49.3%) and *3 (13.2%) are the most prevalent loss-of-function (LOF) alleles in the Asian population and contribute to poor bioavailability of active clopidogrel resulting in nonresponsiveness to the antiplatelet therapy.[13 14] Interestingly, post hoc genetic tests of our CHANCE trial revealed that a large population (58.8%) of patients were *CYP2C19**2 and *3 LOF allele carriers and our subsequent analysis revealed that these patients did not significantly benefit from clopidogrel-aspirin treatment compared with aspirin-alone treatment. Meanwhile, patients without *CYP2C19**2 and *3 LOF alleles obtained a 17% increase in efficacy of clopidogrel-aspirin treatment compared with aspirin treatment alone.[15] Hence, personalisation of aspirin and clopidogrel DAPT based on *CYP2C19* genetic variation may be beneficial for patients receiving this therapeutic strategy.[8]

Genotype-guided antiplatelet therapy is effective and beneficial in the clinic.[16 17] However, this approach has not yet been clinically evaluated or used to treat cerebrovascular diseases. Therefore, we conceived the CHANCE-2 trial to evaluate the benefit and feasibility of incorporating rapid genotyping to guide early aspirin and clopidogrel DAPT (NCT04078737).[18] The CHANCE-2 trial is a multicenter, double-blinded, double-simulated, randomised, controlled clinical trial in which we plan to screen 10 878 patients by rapid genetic testing within 24 hours of symptom onset and compare the effect of DAPT using clopidogrel and aspirin with that of an alternative DAPT using ticagrelor (a non-*CYP2C19*-dependent $P2Y_{12}$ receptor antagonist) and aspirin.[19]

Most conventional genetic testing relies on the patient's peripheral blood as the source of genomic material. This requires extensive laboratory processing, including sample preparation, genotype detection reagent preparation and empirical interpretation of PCR amplification results.[20] These complicated procedures have dramatically increased the result turnaround time (TAT).[21] Additionally, there are many primary hospitals in the CHANCE-2 trial centres without professional PCR laboratories, and genetic testing cannot be performed in these hospitals. Moreover, prehospital delays are common in China.[22] Our previous CHANCE trial showed that the median time from symptom onset to the trial enrollment was 13 hours.[3] Therefore, our CHANCE-2 trial requires a rapid genetic testing strategy to enable *CYP2C19* genotype-guided DAPT.

We developed a novel GMEX (point-of-care) system for rapid genotyping, and we aimed to evaluate the accuracy and feasibility using the GMEX system for the CHANCE-2 trial or related clinical and research applications.

METHODS

Study design and ethics approval

We evaluated the performance of the GMEX point-of-care testing system against conventional clinical laboratory-based genomic testing for *CYP2C19* genotype detection. We conducted a multicentre study to assess the accuracy and result TAT between the two genotyping methods. Sanger sequencing is a gold standard test and was used to validate the genotyping results from two methods. The Ethics Committee of Beijing Tiantan Hospital Capital Medical University approved this study. All participants provided written informed consent.

Study subjects

Healthy individuals and patients with a clinical history of cardiovascular and cerebrovascular diseases were enrolled at six hospitals (Beijing Tiantan Hospital; Kaifeng Central Hospital; the Third People's Hospital of Tongzhong District, Nantong City; the People's Hospital of Wendeng District, Weihai City; Liaocheng people's Hospital and Yixing people's Hospital) between July and August in 2019. Inclusion criteria were healthy individuals by physical examination or patients with the ischaemic attack or other cardiovascular and cerebrovascular diseases. Individuals younger than 18 years of age and pregnant women were excluded.

GMEX point-of-care genotyping system

The GMEX point-of-care genotyping system was jointly developed by Chongqing Jingyin Bioscience and the China National Clinical Research Center for Neurological Diseases and was eventually transformed, produced and marketed by Chongqing Jingyin Bioscience. The GMEX system includes a portable DNA analyzer, genotyping reagents and a buccal sample collection kit. The system is user-friendly and can be easily operated, transmitted and analysed and presents an effective and simple approach to genotyping.

The operators associated with the GMEX system were doctors, nurses or clinical researchers. The GMEX system can be operated in the clinical department and bedside. Professional instructors trained all operators on-site and operators were assessed. Those that passed assessments obtained certificates. The training time for each hospital was about 2–4 hours.

The system uses noninvasive sampling by buccal swab at the bedside to improve patient compliance. It integrates automated steps of PCR-based amplification, fluorescent signal detection and genotype determination and is performed at the site of patient care by trained doctors, nurses or clinical researchers, dramatically improving the speed and user-friendliness of genotyping. The system has integrated controls to monitor the performance of a run and ensure ongoing quality of results. The innovative point-of-care test technology is in line with the affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end-users criteria proposed by the WHO.[23] It is user-friendly, rapid and robust, equipment-free and deliverable to those who need them.

Sample collection and processing

Three buccal swabs and 2 mL peripheral blood in EDTA were collected from healthy individuals and patients. The buccal swabs were either analysed immediately or stored under the manufacturer's recommended conditions prior to analysis by the GMEX system. Peripheral blood was subjected to analysis using the Human *CYP2C19* Gene Polymorphism Detection Kit (YZYMED, Wuhan, China, referred as YZY Kit), a clinically proven method approved by the National Medical Products Administration (NMPA), at the central laboratory of Beijing Tiantan Hospital Capital Medical University and Sanger sequencing at Beijing Genomics Institute (BGI, Beijing, China) for validation. Gender, age and process time were recorded in the data collection form for each subject . Process time of laboratory-based genotyping was only available for subjects whose blood sample was collected at the Beijing Tiantan Hospital, because the blood sample

Table 1 Primers and probes used for *GMEX system*

SNP	Primers*	Taqman MGB-probes†
CYP2C19*2	5'-CAGAGCTTGGCATATTGTATCTATA-3'	FAM-TTTCCCGGGAACC
	5'-CGAGGGTTGTTGATGTCCATC-3'	TexasRed-TTCCCAG +GAACCC
CYP2C19*3	5'-CAGCAATTTCTTAACTTGATGGA-3'	FAM-CCCCTGG +ATCCAG
	5'-CAATATAGAATTTTGGATTTCCCAG-3'	TexasRed-ACCCCCTG+AATCCA
CYP2C19*17	5'-TGAACAGGATGAATGTGGTATAT-3'	FAM-CAGAGATGCTTTG
	5'-GAGGTCTTCTGATGCCCA-3'	TexasRed-TCAGAGATACTTTG

*Upper line, forward primer; lower line, reverse primer;.
†'+' Locked nucleic acid.

would need to be transported from other centres to the central laboratory before testing.

Genotyping by the GMEX system

Buccal swabs were collected and analysed in accordance with the manufacturer's instructions. In brief, buccal swab samples taken from the healthy individuals or patients were directly inserted into separately packaged reaction tubes including 23 µL of reaction mixture. This allowed simultaneous mixing of the samples with reagents for detecting *CYP2C19 *2*, **3* or **17* alleles and sealing of the reaction tubes. The samples were then subjected to analysis by the GMEX DNA analyser using the Taqman assay (Applied Biosystems, Foster City, California, USA). Primers and probes used for three single nucleotide polymorphisms are listed in table 1.

The GMEX DNA analyser is a portable fluorescent PCR machine with 12 reaction wells. Wells cannot be run independently and the temperature increase or decrease and fluorescence excitation or collection are performed at the same time for 12 reaction wells. The GMEX DNA analyser should not be paused while it is running and samples or reagents cannot be added midway.

The PCR conditions used were 95°C for 5 min, followed by 50 cycles of 95°C for 8 s and 55°C for 35 s. The genotyping results were interpreted with the accompanying software. The GMEX system was operated by nonlaboratory trained healthcare personnel.

Clinical laboratory-based genotyping

Genomic DNA from peripheral blood was extracted using the MagNA Pure 96 System (Roche, Basel, Switzerland) and subsequently analysed using the YZY Kit according to the manufacturer's instructions. In brief, 2 µL (10–30 ng) of genomic DNA was added to a 23 µL reaction tube and subjected to quantitative PCR analysis using the LightCycler 480 PCR system (Roche, Switzerland). According to the criteria set by the YZY Kit manufacturer, the genotype was determined by professional laboratory personnel.

Validation of CYP2C19 genotypes

The genotypes of study participants were validated at the BGI Laboratory using Sanger sequencing. PCR primers (*2 forward: 5′-CAGAGCTTGGCATATTGTATC-3′ and *2 reverse: 5′-GTAAACACAAAACTAGTCAATG-3′, *3 forward: 5′-TGTGCTCCCTGCAATGTGAT-3′ and *3 reverse: 5′-TTTGGGGCTGTCACCAAAGT-3′,*17 forward: 5′-GCCCTTAGCACCAAATTCTC-3′ and *17 reverse: 5′-ATTTAACCCCCTAAAAAAACACG-3′) were designed to amplify *CYP2C19**2, *3, and *17 genomic fragments. The PCR conditions were 96°C for 5 min, followed by 10 cycles of 96°C for 20 s, 62°C–52°C touchdown for 20 s and 72°C for 30 s and 35 cycles of 96°C for 20 s, 55°C for 20 s and 72°C for 30s. The final extension was performed at 72°C for 5 min. Peripheral blood samples were processed and subsequently analysed using a 3730XL DNA sequencer (Applied Biosystems, Massachusetts, USA).

Statistical analysis

Patient characteristics were described as medians with IQRs for continuous variables and frequencies and percentages for categorical variables. Kappa statistics were used for the assessment of diagnostic value agreement among the three platforms. The genotype was tested by Hardy-Weinberg equilibrium. All analyses were performed with SAS software V.9.4 (SAS Institute, Cary, North Carolina, USA).

RESULTS

On-site performance of the GMEX system

We compared the TAT and accuracy of our GMEX system to that of the YZY Kit for detecting *CYP2C19 *2*, **3* and **17* alleles, Sanger sequencing was the gold standard test. To accurately reflect the recruitment process during the CHANCE-2 trial, buccal swabs and peripheral blood samples from 408 subjects (270 men and 138 women) with a mean age of 60.8 (IQR: 53.3–67.1) years (age range 22–90 years) were tested on-site and sent to the clinical laboratory for further processing.

The *CYP2C19 *2/*3/*17* genotyping results are shown in table 2, and genotypes reported by the GMEX system showed 100% agreement with those determined by both laboratory-based genotyping and Sanger sequencing for all three *CYP2C19* alleles tested. Hardy-Weinberg equilibrium revealed that the three genotypes were all consistent with the equilibrium, suggesting that the subjects in this study had no significant natural selection or migration and that they were representative of the population ($p>0.05$, table 3).

Table 2 Comparison of *CYP2C19*2*, **3* and **17* genotype results obtained with the three methods

	Genotype results	GMEX system	Laboratory-based genotyping	Sanger sequencing	Kappa statistic
	GG	191 (46.81%)	191 (46.81%)	191 (46.81%)	1.000*
*CYP2C19*2*	GA	176 (43.14%)	176 (43.14%)	176 (43.14%)	
	AA	41 (10.05%)	41 (10.05%)	41 (10.05%)	
	GG	363 (88.97%)	363 (88.97%)	363 (88.97%)	
*CYP2C19*3*	GA	45 (11.03%)	45 (11.03%)	45 (11.03%)	
	AA	0	0	0	
	CC	399 (97.79%)	399 (97.79%)	399 (97.79%)	
*CYP2C19*17*	CT	9 (2.21%)	9 (2.21%)	9 (2.21%)	
	TT	0	0	0	

Sanger sequencing and laboratory-based genotyping versus Sanger sequencing.
*GMEX *system vs laboratory-based genotyping and GMEX system*.

Result TAT of the GMEX system and conventional laboratory genetic testing

The rapid genotyping approach to clinical *CYP2C19* testing is important for acute antiplatelet therapy prescribed after obtaining genotyping results. Our results from a total of 408 patients showed that the average length of workflow time for GMEX system and laboratory-based genotyping were 85.0 (IQR: 85.0–86.0) and 1630.0 (IQR: 354.0–7594.0) min ($p<0.001$), respectively. The times of sample-to-start, start-to-end and end-to-reports for the GMEX system were 6.0 (IQR: 5.0–6.0), 62.0 (IQR: 61.5–62.0) and 18.0 (IQR: 18.0–18.0) min, respectively. The GMEX system genotyping results were available about 1.5 hour after sample collection and were substantially faster than those produced by laboratory-based genotyping (2–3) days.

DISCUSSION

In this study, we applied the GMEX (point-of-care) system, a novel point-of-care genetic testing technology in clinical practice, for the first time. Our results show that this system has demonstrated advantages in clinical practice and satisfies the requirements for the CHANCE-2 trial.

The point-of-care test technology has the advantages of providing fast results, being user-friendly, and having flexible application scenarios. The GMEX (point-of-care) system is widely used in emergency, outpatient and rapid clinical diagnosis. At present, the point-of-care technologies in the field of molecular diagnostics mainly include microfluidic lab-on-a-chip, isothermal amplification and extraction-free direct amplification technologies. The microfluidic lab-on-a-chip technology integrates sample lysis, nucleic acid purification, amplification and detection. Lab-on-a-chip technology has been used to directly analyse saliva samples and has been successfully applied to pathogen detection.[24] Isothermal temperature amplification technology uses a constant and moderate temperature, which does not require a large temperature control device, and it has also been used for pathogen detection.[25] Extraction-free direct amplification technology relies on a strong anti-inhibition PCR reagent and innovative molecular diagnostic equipment and using this approach oral cells can be directly analysed. Extraction-free direct amplification technology has been approved by The Food and Drug Administration for *CYP2C19* genotyping (Spartan RX *CYP2C19* Test System).[26 27] However, owing to the high testing cost, difficult technical operation and failure to obtain registration approval in the NMPA, none of the products described above is marketable molecular diagnostic point-of-care products in China, with the exception of the GMEX system.

Table 3 HWE analysis

	HWE χ^2	P value
*CYP2C19*2*	0.0024	0.9611
*CYP2C19*3*	1.3899	0.2384
*CYP2C19*17*	0.0507	0.8218

HWE, Hardy-Weinberg equilibrium.

In our study, compared with the laboratory-based genotyping test, the GMEX system can shorten the average TAT time by approximately 20-fold. Our results show the successful application of the GMEX system as a point-of-care model. It not only has the characteristics of rapid detection but also the characteristics of high detection accuracy. The accuracy of the GMEX (point-of-care) system was been verified against that of the laboratory-based testing and Sanger sequencing methods. It is worth noting that the accuracy and first-run success rates of GMEX® in Beijing Tiantan Hospital and five primary hospitals were both 100%. None of the patients were retested or excluded owing to incorrect operation or first-run test failure in the GMEX genetic testing group. These data indicate that the accuracy and operability of GMEX would not be affected by hospital rank, operator or geographic regions. The correct operation and positive and daily negative quality control testing before sample testing can better guarantee the validity of this method when it is performed as a point-of-care model.

There were several limitations of our study. First, we only included subjects 408, and this small sample size may lead to statistical bias. Second, this matching should not be paused, and samples or reagents cannot be added part way through the process.

In this study, 59.31% (242/408) of patients carried *CYP2C19* LOF alleles, and 35.5% of these patients were taking or intended to take, clopidogrel, which may have caused serious adverse outcomes.**15 28 29** Despite increasing recognition of *CYP2C19* genetic testing among physicians, there remain a lack of facilities for genetic testing in a large number of grassroots hospitals in China. The GMEX (point-of-care) system can provide a good solution for these hospitals because it is both user-friendly and portable.

In conclusion, the GMEX (point-of-care) system resolved the problems of accurate genotyping before the start of antiplatelet therapy and the cost-effectiveness of pharmacogenetic guidance. Our data suggest that the GMEX (point-of-care) system meets the requirement of rapid and accurate genotyping and is a reliable and feasible point-of-care system for rapid *CYP2C19* genotyping for the CHANCE-2 trial. Further prospective studies are needed to ascertain whether the rapid genotyping system can improve treatment outcomes.

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Patient consent for publication Not required.

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