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1 **The Impact of CYP2C19 Genotype on The Platelet Reactivity Index (PRI) Among chronic**
2 **coronary syndromes (CCS) Patients Undergoing Percutaneous Coronary Intervention**
3 **(PCI): Affectability of Rapid Genetic Testing**

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20 **Abstract**

21 **Background:** In the Asian population, the presence of the CYP2C19 loss-of-function (LOF) allele
22 is a known genetic variation. This allele is associated with a reduced capacity to metabolize
23 clopidogrel into its active forms through the CYP2C19 enzyme, resulting in diminished platelet
24 inhibition and an elevated risk of recurrent cardiovascular events. Regulatory authorities have
25 recommended an alternative P2Y12 inhibitor, ticagrelor, for individuals carrying the LOF allele.
26 Consequently, this study seeks to assess the impact of the CYP2C19 genotype on the Platelet
27 reactivity index (PRI) using a rapid genetic testing approach in Asian patients with chronic
28 coronary syndromes (CCS) who undergo percutaneous coronary intervention (PCI).

29 **Methods:** This prospective study employed a parallel design, single-center design, and
30 randomized approach. Genotyping for the CYP2C19*2 and *3 polymorphisms was conducted
31 using the Nested Allele-Specific Multiplex PCR (NASM-PCR) technique. Patients meeting the
32 inclusion criteria underwent genotyping for CYP2C19 polymorphisms. Following PCI, patients
33 were randomly assigned to receive either ticagrelor or clopidogrel. PRI assessments were
34 performed four hours after loading dose administration. The trial was registered with
35 ClinicalTrials.gov under the identifier NCT05516784.

36 **Results:** Among the 94 patients recruited for the study, 40 (42.55%) were identified as carriers of
37 the LOF allele for CYP2C19*2 and *3 (*1/*2, *2/*2, *1/*3). Out of the 84 patients evaluated for
38 PRI (44 receiving clopidogrel and 40 receiving ticagrelor), 21 (47.7%) of the clopidogrel group
39 and 39 (97.5%) of the ticagrelor group exhibited a favorable response to antiplatelet therapy
40 (PRI<50). Patients treated with ticagrelor demonstrated superior antiplatelet responses compared
41 to those receiving clopidogrel, regardless of LOF carrier status (P = 0.005 and <0.001 for non-
42 LOF and LOF carriers, respectively).

43 **Conclusion:** NASM-PCR as a rapid genetic test holds promise for personalizing antiplatelet
44 therapy in Asian CCS patients.

45 **Keywords:** Clopidogrel, Ticagrelor, CYP2C19, Platelet reactivity index, and chronic coronary
46 syndromes

47 **Introduction**

48 The sustained efficacy of dual antiplatelet therapy (DAPT) following percutaneous coronary
49 intervention (PCI), irrespective of clinical symptoms, is well-established. Current guidelines
50 advocate the 12-month use of aspirin with a P2Y12 inhibitor [1, 2]. Platelet aggregation plays a
51 pivotal role in arterial thrombosis and stent-related complications. To mitigate platelet activation
52 and aggregation, DAPT must effectively inhibit the platelet aggregation pathway [3].
53 Nevertheless, persistent thrombotic events can occur despite DAPT, possibly attributable to
54 substantial interindividual genetic variations affecting the clinical response to antiplatelet drugs
55 [4-6].

56 DAPT has traditionally been the gold standard for managing chronic coronary syndromes (CCS).
57 Clopidogrel has long been the preferred therapeutic option, with ticagrelor emerging as a relatively
58 newer alternative [7, 8]. The PLATO study in acute coronary syndrome (ACS) patients
59 demonstrated superior effectiveness of ticagrelor over clopidogrel [7, 9]. Recent investigations
60 have suggested substituting ticagrelor for clopidogrel may mitigate resistance and enhance clinical
61 outcomes in specific patient populations [4, 10-12]. Despite Clopidogrel being the most commonly
62 prescribed P2Y12 inhibitor, the clinical response to clopidogrel is often unpredictable. Certain
63 patients receiving clopidogrel face an elevated risk of thrombosis due to high platelet reactivity
64 (HPR) [13-15].

65 Genetic polymorphisms within the CYP2C19 gene have been categorized into distinct groups,
66 commonly referred to as alleles. These preliminary allele classifications encompass a total of 36
67 distinct alleles, denoted as CYP2C19 *1, *2, *3, *4, *5, *6, *7, *8, and so forth. Among these
68 alleles, the most clinically significant in terms of their impact on the metabolism of clopidogrel
69 are the *2/*3 mutations, which result in reduced metabolic activity, and the *17 mutations,

70 associated with enhanced metabolic activity. It is noteworthy that the prevalence of other allelic
71 variations in the majority of population groups is relatively low [16]. Genetic polymorphisms
72 affecting the primary enzyme responsible for clopidogrel metabolism, cytochrome CYP2C19,
73 have been identified as the underlying cause of this variability in platelet reactivity [4, 17-20].
74 These genetic polymorphisms in the CYP2C19 gene, collectively called loss-of-function (LOF)
75 alleles, result in diminished CYP2C19 enzyme activity, thereby reducing the conversion of
76 clopidogrel into active metabolites. This compromise in clopidogrel's efficacy for platelet
77 inhibition elevates the risk of thrombotic events and adverse cardiovascular outcomes, particularly
78 in patients treated with clopidogrel post-PCI [19, 21-24]. These LOF alleles are prevalent among
79 individuals of East Asian descent [25-27]. Despite the widespread use of clopidogrel in this
80 population, approximately 60% carry CYP2C19 LOF alleles, leading to a substantial incidence of
81 HPR and major adverse cardiovascular events [28-30]. Notable LOF alleles of the CYP2C19 gene
82 encompass CYP2C19*2 and *3. While the CYP2C191 allele, known as the wild-type allele, is
83 associated with normal drug metabolism [31]. the CYP2C19*3 allele harbors a 636 G>A point
84 mutation in exon 4, resulting in an early stop codon. The CYP2C19*2 allele is marked by a 681
85 G>A substitution in exon 5, leading to a defective splice site [32, 33].
86 In response, the U.S. Food and Drug Administration (FDA) has recommended alternative P2Y12-
87 blocking therapies, such as ticagrelor, for individuals with LOF alleles [34, 35]. Nevertheless,
88 clinical practice selection integration for oral P2Y12 inhibitors remains limited [36-38]. Hence,
89 the present study seeks to evaluate the impact of the CYP2C19 genotype on the Platelet reactivity
90 index (PRI) using a rapid genetic testing approach among CCS patients undergoing PCI.

91 **Subjects and Methods**

92 **Study Design**

93 This investigation employed a prospective, single-center, parallel design, and randomized
94 approach, encompassing chronic coronary syndromes (CCS) patients scheduled for percutaneous
95 coronary intervention (PCI) or those undergoing diagnostic coronary angiography with the
96 intention of ad hoc PCI. Informed consent was diligently obtained from all subjects and, if
97 applicable, their legal guardian(s). The study was equipped with an early termination clause for
98 serious safety concerns. Ethical oversight was provided by the Medical Research and Ethics
99 Committee (MREC) of the Ministry of Health Malaysia, and the study adhered to the principles
100 outlined in the Declaration of Helsinki and Good Clinical Practice guidelines (NMRR-
101 20241756795). The study protocol was duly registered on ClinicalTrials.gov under the reference
102 number NCT05516784.

103 The study cohort encompassed all patients receiving follow-up care at the Cardiology department
104 of a public hospital situated in the northern region of Malaysia who had previously undergone PCI
105 and were under management with either clopidogrel or ticagrelor as antithrombotic therapy.
106 Notably, all participants had been receiving adjunctive aspirin therapy at 100 mg before PCI.

107 Patient assessments were conducted before admission to the intervention ward and on the day of
108 the procedure. Comprehensive inclusion and exclusion criteria were established and are delineated
109 in Appendix 1. In brief, patients aged between 18 and 80 years who were scheduled for
110 individualized PCI and had no contraindications for treatment with either ticagrelor or clopidogrel
111 were considered eligible for the CYP2C19 genetic test. Patients undergoing emergency or urgent
112 PCI, wherein genetic test results were unattainable before the procedure, were excluded from
113 participation.

114 Patients meeting the study's entry criteria underwent rapid genetic testing utilizing the Nested
115 Allele-Specific Multiplex PCR (NASM-PCR) method, as detailed in Appendix 2. This genetic

116 analysis aimed to identify carriers of the CYP2C19*2 and *3 alleles, representing the most
117 prevalent loss-of-function (LOF) alleles. Carriers of this variant *2 or *3 LOF alleles included
118 both homozygotes (*2/*2) and heterozygotes (*1/*2, *1/*3), classified as poor and intermediate
119 metabolizers. In contrast, carriers of the wild type *2 or *3 non-LOF alleles included individuals
120 possessing the *1/*1 allele, characterized as normal metabolizers.

121 Employing a computer-based randomization system, patients identified as CYP2C19 LOF or non-
122 LOF allele carriers and scheduled for PCI were allocated randomly into two groups at a 1:1 ratio.
123 These groups were assigned to receive either ticagrelor (with a loading dose of 180 mg followed
124 by a maintenance dose of 90 mg twice daily) or clopidogrel (with a loading dose of 600 mg
125 followed by a daily maintenance dose of 75 mg). The loading dose was administered promptly
126 during PCI in adherence to local care standards. Subsequently, patients underwent platelet
127 reactivity index (PRI) assessments using the vasodilator-associated stimulated phosphoprotein
128 assay (VASP) ELISA kit (CY-QUANT VASP/ P2Y12). PRI measurements were conducted once,
129 precisely 4 hours after the loading dose administration. A schematic representation of the study
130 design is depicted in Fig. 1.

131 **Genetic testing using NASM-PCR rapid genotyping**

132 The extraction of genomic DNA was executed using commercially available kits (NucleoSpin
133 Blood Quick Pure, Macherey, Duren, Germany). The NASM-PCR method was employed to
134 determine the status of CYP2C19*2 and *3 alleles, accomplishing this within a rapid timeframe
135 of 3 hours.

136 Two-Stage PCR Amplification: Each genomic DNA sample underwent a two-stage PCR
137 amplification process. The initial PCR was conducted to amplify exons 4 and 5 segments
138 simultaneously. Subsequently, the products from the initial PCR were suitably diluted and utilized

139 as templates for the second PCR amplification. The second amplification stage employed specific
140 primers meticulously designed to detect variants within the two amplified regions. These primers
141 were strategically designed to differentiate between non-LOF and LOF carriers (refer to Fig. S1
142 and S2).

143 The outcomes obtained through this process were subjected to thorough confirmation, and the
144 reliability of the genotyping method was ascertained by dispatching the initial PCR products
145 encompassing the amplification of exons 4 and 5 for direct sequencing analysis (see Fig. S3 and
146 Fig. S4).

147 **Platelet function test (PFT) using VASP assay**

148 Platelet function tests (PFT) were conducted using the VASP assay (Biocytex, Inc., Marseille,
149 France) [39]. This assay, cleared by the FDA [40], serves as a point-of-care test for assessing the
150 platelet reactivity index (PRI) [39, 41]. Established criteria defined high platelet reactivity (HPR)
151 as a PRI exceeding 50% in the VASP assay [41, 42]. VASP phosphorylation status was evaluated
152 using the ELISA (CY-QUANT VASP/P2Y12) assay within 24 hours of blood collection.

153 **Blood sample collection**

154 Blood samples were procured using two distinct vacuum tubes. The first tube, containing
155 ethylenediaminetetraacetic acid (EDTA), was utilized for venous blood collection dedicated to
156 CYP2C19 genotyping. The second tube, containing sodium citrate (0.109 mol, 3.2%), was
157 designated for PFT assessment by the manufacturer's recommendations. Both tubes were gently
158 inverted 3–5 times to ensure thorough mixing of the anticoagulant. Blood sample collection was
159 conducted 4 hours after administering the antiplatelet medication. Subsequently, the samples were
160 transported from the hospital to the laboratory in an icebox maintained at room temperature until
161 testing.

162 **Statistical analysis**

163 Utilizing a PRI>50 cut-off value, non-responders to ticagrelor and clopidogrel were classified. The
164 frequencies of antiplatelet response and CYP2C19 genotypes were examined and reported for the
165 overall sample and each ethnic group. Continuous variables were expressed as either the median
166 with interquartile range or the mean with standard deviation, and comparisons between two groups
167 were conducted using independent sample t-tests. Categorical variables were presented as
168 frequencies and percentages, with comparisons between two groups assessed using the Pearson
169 chi-square or Fisher's exact test.

170 P-values denote superiority tests, with statistical significance set at a 2-tailed p-value of 0.05. Data
171 from all patients were analyzed for primary variables, and statistical analysis was executed using
172 IBM® SPSS® v26.0 (SPSS Inc., Chicago, Illinois).

173 **Results**

174 **Patient Enrollment and Genotyping**

175 Between August 12, 2021, and August 22, 2022, a comprehensive total of 94 chronic coronary
176 syndromes (CCS) patients who were scheduled for percutaneous coronary intervention (PCI)
177 underwent genetic testing. Among these, 54 patients (57.45%) were identified as non-LOF
178 carriers. Specifically, 24 were assigned to receive clopidogrel, while the remaining 30 were
179 designated to receive ticagrelor. This allocation was determined through a randomization process.
180 Conversely, 40 patients (42.55%) were identified as carriers of at least one loss-of-function (LOF)
181 allele. Among the LOF carriers, 25% were homozygotes (*2/*2, n = 10), while 75% were
182 heterozygotes (*1/*2, n = 20; *1/*3, n = 10). Like the non-LOF group, these patients were
183 randomly assigned, with 20 receiving clopidogrel and another 20 receiving ticagrelor (see Fig. 2).

184 Out of the total patient cohort, ten individuals did not undergo post-dose sampling due to their
185 voluntary withdrawal before the scheduled post-dose sampling. Consequently, the platelet function
186 test (PFT) results were derived from the data of 84 patients.

187 **Baseline Characteristics**

188 Both treatment groups exhibited comparable demographics, clinical profiles, and concurrent
189 medication usage. Notably, all patients reported abstaining from alcohol consumption.
190 Additionally, approximately half of the patients in both groups had a documented history of
191 diabetes mellitus (see Table 1).

192 **Baseline laboratory and genotype characteristics**

193 At the study's outset, haematological assessments revealed no significant disparities between the
194 treatment groups, as detailed in Table 2. Among the patient cohort, 53 individuals (56.4%) were
195 identified as non-LOF carriers for both alleles (*1/1). Notably, the CYP2C192 allele was detected
196 in 20 patients (21.3%) who were heterozygous carriers (*1/*2) and in 10 patients (10.6%) who
197 were homozygous carriers (*2/2). Additionally, ten patients (10.6%) exhibited heterozygous
198 carrier status for the CYP2C93 allele. Intriguingly, one patient was found to be a carrier of both
199 heterozygous and homozygous genotype alleles, specifically (*2/*2) and (*1/*3).

200 **Platelet function test Results**

201 Platelet function test (PFT) results were successfully obtained from 84 patients, with 44 patients
202 belonging to the clopidogrel group and 40 patients to the ticagrelor group, all screened for response
203 outcomes. Notably, within the entire patient cohort, 23 individuals (52.3%) in the clopidogrel
204 group exhibited a PRI \geq 50% (indicative of a non-responsive status). In contrast, only one patient
205 (2.5%) in the ticagrelor group demonstrated this condition (as depicted in Table 3). Importantly,

206 patients administered ticagrelor achieved significantly lower PRI values than those receiving
207 clopidogrel (51.4 ± 17.7 vs. 26.9 ± 11.8 , respectively; $P < 0.001$).

208 **Association Between Antiplatelet Therapy and Platelet Reactivity Based on CYP2C19** 209 **Genotypes**

210
211 We analyzed the distribution of responder and non-responder patients within the non-LOF
212 and LOF carrier subgroups of the clopidogrel and ticagrelor treatment groups. We employed a chi-
213 square test for association to establish associations while utilizing two PRI cut-off points for
214 response categorization. The tables presenting the cross-tabulation of response classifications,
215 considering the cut-off points and genotypes, can be found in Table 4.

216 Notably, the proportions of patients falling into the categories of $PRI < 50\%$ or $PRI \geq 50\%$ exhibited
217 significant differences when comparing the clopidogrel and ticagrelor groups within the subsets of
218 non-LOF and LOF carriers ($P = 0.005$ and < 0.001 , respectively). Specifically, among non-LOF
219 carriers, 33.3% were non-responders in the clopidogrel group, while no non-responders were
220 observed in the ticagrelor group based on the PRI cut-off point classifications. In contrast, 25.0%
221 of respondents in the clopidogrel group were LOF carriers. In comparison, a substantial 95.0% of
222 responders belonged to the LOF carrier category in the ticagrelor group, based on the same PRI
223 cut-off point classifications. Notably, 75.0% of non-responders in the clopidogrel group were LOF
224 carriers. In contrast, only 5.0% of non-responders were LOF carriers in the ticagrelor group, again
225 based on the PRI cut-off points between the two treatment groups.

226 **Discussion**

227 This present study sought to explore the impact of the CYP2C19 genotype on PRI antiplatelet
228 therapy among Malaysian chronic coronary syndromes (CCS) patients following percutaneous
229 coronary intervention (PCI). The distinct distribution of CYP2C19 genotypes within Asian

230 populations, as compared to existing data from other populations, underscores the importance of
231 assessing the relevance of specific gene polymorphisms in different ethnic groups.

232 The implementation of genotype-guided selection for platelet-inhibiting therapy has needed to be
233 improved in clinical practice due to the time-consuming assays required for determining CYP2C19
234 genotypes, which can take several days [4, 37, 43, 44]. However, our study successfully addressed
235 this challenge by introducing the novel Nested Allele-Specific Multiplex PCR (NASM-PCR)
236 method, offering rapid genetic testing within a mere 3 hours, thus enabling efficient screening of
237 CYP2C19 genotypes among CCS patients post-PCI.

238 The prevalence of CYP2C19*2 and *3 alleles exhibits considerable variability among different
239 ethnicities. Notably, Asian populations, with an approximate range of 55.0% to 70.0%, tend to
240 have a higher prevalence of CYP2C19 loss-of-function (LOF) variant alleles (CYP2C19 *2 and
241 *3) compared to Caucasian populations (approximately 25.0% to 35.0%) and African populations
242 (approximately 35.0% to 45.0%) [45, 46].

243 Our study findings align with this trend, with 54 patients (57.45%) identified as non-LOF carriers
244 and 40 patients (42.55%) as carriers of at least one LOF allele. Among these, 25% were
245 homozygotes (*2/*2, n=10), while 75% were heterozygotes (*1/*2, n=20; *1/*3, n=10). These
246 results closely mirror the reported frequencies of CYP2C19 gene polymorphisms in Asian
247 populations [4, 5, 47, 48].

248 Consistent with previous research by Xu et al., our study demonstrates congruent findings in CCS
249 patients undergoing antiplatelet therapy [49]. Specifically, a significant reduction in the platelet
250 reactivity index (PRI) was observed in CCS patients treated with ticagrelor compared to the
251 clopidogrel group. Furthermore, the ALPHEUS study, conducted across 49 hospitals in France
252 and the Czech Republic, confirmed the superiority of ticagrelor over clopidogrel, as indicated by

253 PRI values obtained four hours post-loading dose, with over half of the patients in the clopidogrel
254 group displaying elevated P2Y12-mediated platelet reactivity [41].

255 Our study elucidates a substantial association between antiplatelet therapy and PRI based on
256 CYP2C19 genotypes, aligning with the growing literature on this topic [5, 41, 50-52]. The adoption
257 of genetic testing for personalized antiplatelet therapy has been under investigation for more than
258 a decade [20, 36, 53-55]. A genotype-guided approach supersedes generic treatment allocation
259 endorsed by expert consensus recommendations [53]. his study and previous research provide
260 compelling support for adopting a genotype-guided strategy for personalized oral antiplatelet
261 therapy [50, 56, 57].

262 It is essential to acknowledge that the CYP2C19 enzyme does not exclusively govern the
263 metabolism of clopidogrel. Other isoenzymes, including CYP2B6 and CYP1A2 in the initial stage,
264 and CYP3A4, CYP2C9, and CYP2B6 in the subsequent stage of metabolic activation, play
265 contributory roles [4, 58-60]. Consequently, some individuals may still respond to clopidogrel
266 therapy even when classified as LOF carriers of CYP2C19. Conversely, genetic variations in these
267 additional enzymes could influence clopidogrel metabolism, although our study did not explore
268 this aspect.

269 Drawing from prior randomized and non-randomized studies, as well as meta-analyses, the
270 Personalized antiplatelet therapy approach, with the utilization of ticagrelor in LOF allele carriers,
271 has exhibited a reduction in atherothrombotic events compared to conventional treatment strategies
272 [21, 61-65]. Nevertheless, the TAILOR-PCI trial, encompassing patients with acute or chronic
273 coronary syndrome undergoing PCI, did not yield a definitive conclusion. While the reduction in
274 major atherothrombotic events between the groups at 12 months did not reach statistical

275 significance (HR, 0.66; 95% CI 0.43–1.02, P = 0.06), a post hoc analysis demonstrated a
276 significant decrease in events at 90 days (HR, 0.21, 95% CI 0.08–0.54, P = 0.001) [66].

277 The most recent study evaluating a genotype-guided strategy employing replacement therapy
278 (ticagrelor or prasugrel) versus clopidogrel was conducted across nine medical centers, with
279 genotyping performed in the context of PCI. Results revealed a lower rate of atherothrombotic
280 events among LOF allele carriers treated with replacement therapy compared to clopidogrel at
281 both 90 days (HR, 0.40; 95% CI 0.23–0.71, P = 0.002) and 12 months (HR, 0.56; 95% CI 0.39–
282 0.82). Additionally, there were no discernible differences in clinically significant bleeding events
283 among LOF carriers treated with replacement therapy compared to clopidogrel [21].

284 The findings from this study, corroborated by previous investigations, emphasize the advantages
285 of determining CYP2C19 genotypes before initiating antiplatelet therapy. Such an approach has
286 demonstrated its efficacy in influencing the platelet reactivity index and reducing the risk of
287 recurrent CCS in CYP2C19 LOF carrier patients treated with ticagrelor instead of clopidogrel
288 without introducing additional bleeding risks.

289 Historically, the implementation of genotype-guided selection for platelet inhibition therapy in
290 PCI patients has been hamstrung by the need for more assays capable of delivering rapid CYP2C19
291 genotype results [36, 38]. Although prior studies have underscored the therapeutic benefits of
292 selecting platelet inhibitors based on CYP2C19 genotype [43, 67], conventional genotyping
293 methods were often expensive and time-consuming, rendering results unavailable during the
294 crucial period of antiplatelet initiation [43, 50, 68]. This limitation carries substantial implications,
295 including the need to transition to alternative antiplatelet treatments post-hospital discharge,
296 potentially elevating the risk of thrombotic complications post-PCI [69, 70]. However, our study

297 transcends these limitations by demonstrating the feasibility of implementing rapid genetic testing
298 in CCS patients undergoing diagnostic coronary angiography with subsequent PCI intent.

299 **Study limitations**

300 Despite the Significance of Our Findings on Personalizing Antiplatelet Therapy for Malaysian
301 CCS Patients Undergoing PCI, Our Study Has Several Limitations. Our study is constrained by its
302 single-center design, a relatively small sample size, and a relatively short duration of follow-up.
303 To address these limitations comprehensively, we recommend the execution of multicenter studies
304 encompassing more substantial participant cohorts and extended follow-up periods. It is important
305 to note that our study did not incorporate Prasugrel as an antiplatelet therapy option. Our study
306 didn't assess the PRI after 24 h or another period time, because in many cases, elective PCI patients
307 indeed be discharged on the same day of the procedure, making later assessment challenging. We
308 used only one indicator (PRI) to evaluate the response of patients with different genotypes. So, we
309 recommend the importance of considering additional indicators in future research to provide a
310 more complete assessment of the genotypic influence on antiplatelet response. The exclusion
311 criteria, which precluded patients who had recently taken clopidogrel within two weeks before
312 recruitment, excluded a noteworthy segment of screened patients from our study. This omission
313 may have implications for a more comprehensive assessment of antiplatelet therapy outcomes. The
314 NASM-PCR method, like any scientific technique, has its limitations, and the effectiveness of the
315 method can be affected by the quality of primer design. Inaccurate or poorly designed primers may
316 lead to negative results. Cross-contamination can lead to inaccurate results, so strict laboratory
317 practices are necessary to minimize this risk. Subject to allele-specific bias, where one allele is
318 preferentially amplified over another. Careful optimization of the assay is required to minimize
319 this bias. These limitations underscore the need for future research endeavors to provide a more

320 comprehensive understanding to personalised antiplatelet therapy in the context of East Asian CCS
321 patients undergoing PCI.

322 **Conclusions**

323 In conclusion, our findings emphasize the relevance of CYP2C19 genotype determination before
324 initiating antiplatelet therapy. This approach impacts platelet reactivity and reduces the risk of
325 recurrent CCS among CYP2C19 LOF carriers treated with clopidogrel. The application of
326 genotype test to select the platelet-inhibiting therapy has faced challenges in clinical practice due
327 to the time-consuming assays required for CYP2C19 genotype determination, often taking several
328 days. However, our study has effectively addressed this limitation by introducing the innovative
329 Nested Allele-Specific Multiplex PCR (NASM-PCR) method, enabling rapid genetic testing
330 within a mere 3 hours, facilitating efficient screening of CYP2C19 genotypes in CCS patients post-
331 PCI.

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334 of this manuscript.

335 **Conflict of Interest**

336 The authors declare no competing interests.

337 **Data availability statement**

338 The raw data supporting the conclusions of this article will be made available by the authors,
339 without undue reservation.

340 **Ethics Approval and Consent to Participate**

341 The study protocol was submitted to the National Institute of Health (NIH), Ministry of Health of
342 Malaysia, and had been reviewed and granted ethical approval by the Medical Research and

343 Ethics Committee (MREC) (NMRR-20-2417-56795). All patients provided written informed
344 consent for participation before study entry.

345 **Consent for Publication**

346 All authors reviewed the manuscript and approved its submission.

347 **Author contributions**

348 M.A.A., N.A.A.D., and B.I.: Conceptualization; M.A.A., N.A.A.D., D.A.M.N., M.A.S, and B.I.:
349 Methodology; M.A.A. did the analysis, and wrote the original draft as well as the final manuscript;
350 N.A.A.D., A.S., D.A.M.N., M.A.S, M.J.A.W., and B.I.: critically reviewed the manuscript;
351 N.A.A.D., and B.I.: Supervision. All authors have read and agreed to the published version of the
352 manuscript

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