Relationship between CYP2C19 Polymorphism and Clopidogrel Resistance in Patients with Coronary Heart Disease and Ischemic Stroke in China

Rong Chang (1,2), Wenqin Zhou (3), Yi Ye (3), Xiaofei Zhang (3), Yanmin Liu (3), and Jinchun Wu (3)

1Department of Cardiology, Shenzhen Longhua District Central Hospital, Shenzhen, China
2The Affiliated Central Hospital of Shenzhen Longhua District, Guangdong Medical University, Shenzhen, Guangdong 518109, China
3Department of Cardiology, Qinghai Provincial People’s Hospital, Xining, Qinghai 810007, China

Correspondence should be addressed to Rong Chang; changrong6617@163.com and Jinchun Wu; wujinchun117@sina.com

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Objective. Clopidogrel is widely used for preventing ischemic complications related to cardiovascular diseases. However, many patients experience clopidogrel resistance (CR). The polymorphisms of CYP2C19 have been implicated in CR, but CYP2C19 polymorphism considerably varies with both ethnic group and geographical location. This study aimed to investigate the association between CYP2C19 polymorphisms and clopidogrel resistance (CR) in patients with coronary heart disease and ischemic stroke among Han and Tibetan populations in Qinghai Province, China. Methods. From June 2019 to January 2020, patients who were diagnosed with coronary heart disease or cerebral infarction in internal medicine of Qinghai Provincial People’s Hospital and had taken dual antiplatelet drugs were included in this study. Blood was collected and routine items were completed. Whole exome sequencing was performed for CYP2C19 genetic polymorphisms of CYP2C19*2 (rs4244285), CYP2C19*3 (rs4986893), and CYP2C19*17 (rs12248560). Results. A total of 91 patients with coronary heart disease or cerebral infarction (67 Han people (65.99 ± 12.25 years old) and 24 Tibetan (63.63 ± 24 years old)) including 52 cases with CR and 39 cases with non-CR were enrolled in this study. For the Han population, the differences in age, glycosylated hemoglobin, activated partial thromboplastin time (APTT), gender, aspirin resistance, and diabetes were significant between the CR and non-CR groups. For the Tibetan population, the two groups showed no significant difference in all indicators. There was no significant difference between CR and non-CR groups for all genotypes (CYP2C19*2, *3, and *17) in either Han or Tibetan populations. For the Han populations, age, APTT, and aspirin resistance were significantly correlated with CR. Conclusion. The present study indicated that CYP2C19*2, CYP2C19*3, and CYP2C19*17 alleles were not correlated with CR for both Han and Tibetan populations in Qinghai Province, while age, APTT, and aspirin resistance were independent risk factors of CR in this region.

1. Introduction

Cardiac-cerebral disease is common in clinical practice and is the leading cause of long-term disability and death around the world [1]. The global challenges of cardiac-cerebral diseases present an enormous health burden. Coronary heart disease and ischemic stroke are two common cardiac-cerebral diseases, which demonstrates a high-frequency of emergency department visits to manage acute and chronic symptoms [2]. Antiplatelet therapy with aspirin and clopidogrel is frequently used for the secondary prevention of acute coronary syndrome, ischemic stroke, and other related ischemic cardiac-cerebral diseases to reduce recurrent ischemic events [3, 4]. Clopidogrel is an irreversible P2Y12 inhibitor, which is usually used for preventing ischemic complications related to cardiovascular diseases [5]. However, many patients experience relapse or bleeding [6], which is associated with increased late mortality [7, 8]. Clopidogrel
2.2. Inclusion and Exclusion Criteria. The inclusion of patients with coronary heart disease was in line with the “coronary heart disease guidelines and expert consensus” in 2019, and patients with stroke or transient ischemic attacks met the “practical diagnosis and expert consensus of ischemic stroke in China” in 2020. The patients were permanent residents or long-time (10 years or more) residents in the Qinghai region. All patients received routine doses of aspirin (100 mg once daily) and clopidogrel (75 mg once daily) for 5–7 days.

The patients with one or more of the following conditions were excluded: allergic or intolerant; contraindications of antiplatelets therapy; rupture and defect of gastrointestinal mucous membrane; inflammation of endocardium or heart valve due to microbial invasion of the body; serious decline in the ability of glomeruli to expel toxins and waste; continuous growth of cancer cells; decreased ejection fraction; combined with pulmonary congestion and inadequate peripheral perfusion with contemplated surgical operation; severe liver disease and/or abnormal coagulation function; and incomplete clinical case data.

2.3. Sample Collection, DNA Extraction, and Whole Exome Sequencing. Blood was collected on an empty stomach the next morning after admission for patients who met the inclusion and exclusion criteria, and routine items such as blood routine, biochemistry, thyroid function, and saccharification were completed. Basic clinical data, such as previous history, medication history, personal history, and others, were collected.

A total of 4 ml fasting venous blood was taken and placed in an anticoagulant tube containing ethylenediamine tetra-acetic acid (EDTA), and the specimen was stored at ~80°C. DNA was extracted using a TianGen DNA extraction kit (TianGen Ltd, Beijing, China). The DNA concentration was determined by fluorescence quantification. Genomic DNA (1 μg) was sheared by sonication, and the fragments with an average size of 150–250 bp were selected by magnetic beads. Then, the fragments were ligated to adapters and amplified by pre-PCR. PCR products were then hybridized and washed with Agilent SureSelect or BGI Hybridization and Wash kits (Agilent, CA, USA), followed by PCR amplification. The reaction conductions were 18 cycles of 98°C for 10 s, 65°C for 30 s, and 72°C for 30 s, followed by a final incubation at 72°C for 5 min, and then hold at 4°C. The primers were CYP2C19 1: forward 5′-ATT ACAACCAGAGCTTGGCAT-3′, reverse 5′-TTTGATGTCCATGATTCTTG-3′; CYP2C19 3: forward 5′-CTGCAATGTGATCTGCTCAT-3′, reverse 5′-TTCCAGGGCTTGGTCAATAG-3′; and CYP2C19 17: forward 5′-GATGAAATGTGGTATAATTCA-3′, reverse 5′-GAGAACAGGACACTGTGGT-3′. The library concentration was measured using the Qubit kit (Invitrogen, USA). Sequencing was performed by the combinatorial probe-anchor synthesis method [19]. CYP2C19 genetic polymorphisms of CYP2C19 2 (rs4244285), CYP2C19 3 (rs4986893), and CYP2C19 17 (rs12248560) were recorded.

2.4. Statistical Analysis. Statistical analysis was performed using SPSS 22.0 (IBM, Armonk, New York, USA). Quantitative data were assessed for normality by the Shapiro–Wilk test [20]. If the data were normally distributed, they were expressed as the mean ± standard deviation, and the differences were compared by independent sample t-test. On the contrary, the data were expressed as median (interquartile range) and the differences were analyzed by Mann–Whitney Test [21]. Qualitative data were represented in the form of N (%) and the difference between groups was analyzed by chi-square test [22]. P < 0.05 was considered significant.
3. Results

3.1. Baseline Information. Finally, 91 patients (67 Han people and 24 Tibetan) meeting the inclusion and exclusion criteria were enrolled in this study. Patient demographics, and clinical and laboratory findings are shown in Table 1. Except for prothrombin time, urea nitrogen, and smoking history ($P < 0.05$), there was no significant difference in the other detection indexes between ethnic Han and Tibetan.

For all patients, according to the definition of CR, there were 52 cases with CR and 39 cases with non-CR. The differences between CR and non-CR groups were significant for age, glycosylated hemoglobin (HBALC), activated partial thromboplastin time (APTT), gender, aspirin resistance, diabetes, and coronary heart disease classification ($P < 0.05$), and the other indicators were not significantly different between the two groups (Table 2).

For the Han population, the differences in age, HBALC, APTT, gender, aspirin resistance, and diabetes were statistically significant between CR and non-CR groups ($P < 0.05$), and the differences in the other indicators were not significant (Table 3). For the Tibetan population, the two groups showed no significant difference in all indicators (Table 4).

3.2. Comparative Analysis of CYP2C19 *2 and *3 Loci in Different Ethnic Groups. The genotypes of the CYP2C19 *2 locus in all participants included GG (67.0%), GA (26.4%), and AA (6.6%), respectively. The genotypes of the CYP2C19 *3 locus were GG (48.4%), GA (26.6%), and AA (23.0%), respectively. The genotypes of the CYP2C19 *17 locus were GG and GA, accounting for 65.1% and 34.9%, respectively. For the Han population, the genotypes of the CYP2C19 *2 locus were GG (67.2%), GA (23.9%), and AA (9.0%), respectively. The genotypes of CYP2C19 *3 locus were GG (50.7%), GA (25.4%), and AA (23.9%), respectively. The genotypes of the CYP2C19 *17 locus were GG and GA, accounting for 65.7% and 34.3%, respectively. For the Tibetan population, there were 66.7% GG and 33.3% GA for the genotypes of CYP2C19 *2, respectively. The genotypes of the CYP2C19 *3 locus were GG (41.7%), GA (37.5%), and AA (20.8%), respectively. For the genotypes of the CYP2C19 *17 locus, GG accounted for 54.2 and GA accounted for 45.8% (Table 1). There was no significant difference between ethnic Han and Tibetan.

3.3. Comparative Analysis of Different Locus between CR and Non-CR Groups. For all patients, the genotypes of CYP2C19 *2, CYP2C19 *3, and CYP2C19 *17 for CR and non-CR groups are shown in Table 2. The genotypes of CYP2C19 *2, CYP2C19 *3, and CYP2C19 *17 for the Han population in two groups are shown in Table 3 and for the Tibetan population in two groups are shown in Table 4. There was no significant difference between CR and non-CR groups for all genotypes in either Han or Tibetan populations.

3.4. Logistic Analysis of Risk Factors for CR. Variables with significant differences in baseline information were included for univariate and multivariate logistic regression analyses to explore the significant related factors of CR. As shown in Table 5, for all patients, age was a significant risk factor for CR, with an odds ratio (OR) (95% confidence interval (CI)) = 1.08 (1.02, 1.13), $P = 0.005$. The older the patients, the higher the risk of CR. APTT was a significant negative correlation factor for CR (OR (95% CI) = 0.81 (0.69, 0.95), $P = 0.011$), and the risk of CR decreased with the increase of APTT. Aspirin resistance was a significant positive correlation factor for CR (OR (95% CI) = 6.47 (2.02, 20.67), $P = 0.002$). Patients with aspirin resistance were at a significantly increased risk of developing CR. There was no significant association between coronary heart disease type and CR. For the Han populations, age, APTT, and aspirin resistance were significantly correlated with CR ($P < 0.05$) (Table 6).

4. Discussion

Clopidogrel combined with aspirin is usually recommended for preventing ischemic events in patients with cardiovascular [23]. Despite the standard treatment, there are still a lot of adverse cardiovascular events, and CR is considered to be the main reason [24]. In this study, we investigated the association between *2, *3, and *17 allelic variants of the CYP2C19 gene and CR in patients with coronary heart disease and ischemic stroke among Han and Tibetan populations. The results showed that three alleles were not statistically correlated with CR, while age, APTT, and aspirin resistance were significantly correlated with CR.

Presently, the mechanisms underlying CR have not been fully elucidated. The CYP2C19 genotype is the most important determinant of the pharmacodynamic and pharmacokinetic responses to clopidogrel [25]. It has been reported that CYP2C19 *2 and CYP2C19 *3, the main mutant alleles, are the most common genotypes in Asian populations [26]. CYP2C19 *2 or CYP2C19 *3 allelic variants increase the risk of CR [27]. CYP2C19 *17 allele is correlated with an increased risk of bleeding [28]. However, the present study showed that CYP2C19 *2, CYP2C19 *3, and CYP2C19 *17 alleles were not significantly different between Han or Tibetan populations as well as between CR and non-CR groups, which suggested that the three alleles were not statistically correlated with CR in this study.

Our result is in accordance with a recent study that investigated the association between CYP2C19 *2, CYP2C19 *3, and CYP2C19 *17 variants of the CYP2C19 gene and CR in patients with acute coronary syndromes in Morocco, and demonstrated that none of the three alleles showed a statistical correlation with CR. Different from the results of our study, that study identified a synergic effect among the three alleles on CR [29]. In fact, the correlation between polymorphisms of CYP2C19 and platelet responsiveness to clopidogrel has been widely recognized among patients with acute coronary syndrome and percutaneous coronary intervention, but the association with other indications, such as arterial fibrillation and stable angina, is negative [30, 31]. The inconsistent
Table 1: Baseline information for participants.

| Characteristics                          | Total (N=91) | Han population (N=67) | Tibetan population (N=24) | P value |
|-----------------------------------------|--------------|-----------------------|---------------------------|---------|
| Age, years                              | 65.36 ± 12.44 | 65.99 ± 12.25         | 63.63 ± 13.06             | 0.428   |
| Height (m)                              | 1.68 ± 0.08   | 1.68 ± 0.09           | 1.66 ± 0.08               | 0.251   |
| Weight (Kg)                             | 68.12 ± 10.75 | 68.88 ± 11.05         | 66.00 ± 9.80              | 0.262   |
| BMI (Kg/m²)                             | 24.11 ± 2.71  | 24.19 ± 2.74          | 23.91 ± 2.68              | 0.663   |
| Oxygen saturation (%)                   | 90.0 (89.0, 93.0) | 91.0 (89.0, 93.0)     | 90.0 (90.0, 92.0)         | 0.816   |
| SBP (mmHg)                              | 126.9 ± 24.4  | 128.7 ± 22.3          | 121.2 ± 29.4              | 0.328   |
| DBP (mmHg)                              | 74.0 ± 13.5   | 73.9 ± 12.3           | 74.1 ± 16.8               | 0.960   |
| Heart rate                              | 76.8 ± 15.5   | 78.5 ± 15.0           | 72.1 ± 16.2               | 0.080   |
| White blood cell                        | 7.20 (5.70, 11.09) | 7.05 (5.55, 10.96)   | 7.88 (6.64, 11.33)        | 0.471   |
| Red blood cell                          | 4.77 (4.26, 5.31) | 4.75 (4.40, 5.39)    | 4.78 (4.03, 5.22)         | 0.365   |
| Hemoglobin                              | 151.1 ± 24.2  | 153.2 ± 21.5          | 145.0 ± 30.2              | 0.156   |
| Glucoseylated hemoglobin                | 5.81 (5.47, 6.70) | 5.78 (5.46, 6.70)    | 5.95 (5.59, 6.95)         | 0.418   |
| PT                                      | 12.0 (11.5, 12.6) | 11.9 (11.4, 12.5)   | 12.2 (11.9, 13.4)         | 0.010   |
| APTT                                    | 27.6 (25.4, 31.2) | 27.5 (25.4, 30.8)   | 30.7 (25.9, 32.8)         | 0.072   |
| ALAT                                    | 30.0 (19.0, 54.0) | 29.0 (19.0, 54.0)    | 38.0 (17.0, 66.0)         | 0.496   |
| AST                                     | 38.0 (22.0, 159.0) | 34.0 (21.0, 159.0)   | 44.5 (22.3, 258.5)        | 0.438   |
| AST/ALT                                 | 1.2 (0.8, 2.4) | 1.3 (0.9, 2.8)       | 1.1 (0.7, 2.1)            | 0.355   |
| Urea nitrogen                           | 6.07 (4.98, 8.10) | 5.87 (4.97, 7.58)   | 7.16 (5.99, 10.68)        | 0.014   |
| Creatinine                              | 84.0 (67.0, 94.0) | 82.0 (67.0, 93.0)   | 84.5 (75.3, 114.5)        | 0.242   |
| Glomerular filtration rate (mL/min × 1.73 m²) | 78.2 ± 25.2 | 80.6 ± 24.8          | 70.9 ± 25.1               | 0.109   |
| Albumin                                 | 37.8 (34.8, 40.6) | 38.0 (35.2, 40.7)    | 36.6 (32.7, 38.3)         | 0.085   |
| Total cholesterol (mmol/L)              | 4.13 ± 1.12  | 4.13 ± 1.03           | 4.02 ± 1.37               | 0.699   |
| Triglyceride                            | 1.42 (0.90, 2.14) | 1.59 (0.90, 2.18)   | 1.32 (0.88, 1.87)         | 0.418   |
| TSH                                     | 1.60 (0.86, 2.82) | 1.62 (0.85, 2.70)   | 1.30 (0.88, 2.91)         | 0.571   |
| Sex, n (%)                              | Male 63 (69.2) | 45 (67.2)             | 18 (75.0)                 | 0.475   |
|                                           | Female 28 (30.8) | 22 (32.8)            | 6 (25.0)                  | 0.660   |
| Aspirin resistance, n (%)                | No 42 (46.2) | 30 (44.8)             | 12 (50.0)                 | 0.039   |
|                                           | Yes 49 (53.8) | 37 (55.2)            | 12 (50.0)                 | 0.039   |
| Smoking history, n (%)                  | No 52 (57.1) | 34 (50.7)             | 18 (75.0)                 | 0.291   |
|                                           | Yes 39 (42.9) | 33 (49.3)            | 6 (25.0)                  | 0.291   |
| Coronary heart disease type, n (%)      | STEMI 42 (46.2) | 31 (46.3)            | 11 (45.8)                 | 0.932   |
|                                           | NSTEMI 12 (13.2) | 8 (11.9)            | 4 (16.7)                  | 0.932   |
|                                           | SAP 15 (16.5) | 11 (16.4)            | 4 (16.7)                  | 0.932   |
|                                           | UA 22 (24.2) | 17 (25.4)            | 5 (20.8)                  | 0.932   |
| Hypertension, n (%)                     | No 30 (33.0) | 20 (29.9)             | 10 (41.7)                 | 0.291   |
|                                           | Yes 61 (66.7) | 47 (70.1)            | 14 (58.3)                 | 0.291   |
| Diabetes, n (%)                         | No 45 (49.5) | 35 (52.2)             | 10 (41.7)                 | 0.374   |
|                                           | Yes 46 (50.5) | 32 (47.8)            | 14 (58.3)                 | 0.374   |
| Stroke, n (%)                           | No 59 (64.8) | 42 (62.7)             | 17 (70.8)                 | 0.473   |
|                                           | Yes 32 (35.2) | 25 (37.3)            | 7 (29.2)                  | 0.473   |
| Hyperlipidaemia, n (%)                  | No 54 (59.3) | 40 (59.7)             | 14 (58.3)                 | 0.907   |
|                                           | Yes 37 (40.7) | 27 (40.3)            | 10 (41.7)                 | 0.907   |
| CYP2C19 * 2, n (%)                      | GG 61 (67.0) | 45 (67.2)             | 16 (66.7)                 | 0.120   |
|                                           | GA 24 (26.4) | 16 (23.9)             | 8 (33.3)                  | 0.120   |
|                                           | AA 6 (6.6)  | 6 (9.0)               | 0 (0.0)                   | 0.120   |
| CYP2C19 * 3, n (%)                      | GG 44 (48.4) | 34 (50.7)             | 10 (41.7)                 | 0.527   |
|                                           | GA 26 (28.6) | 17 (25.4)             | 9 (37.5)                  | 0.527   |
|                                           | AA 21 (23.0) | 16 (23.9)             | 5 (20.8)                  | 0.527   |
| CYP2C19 * 17, n (%)                     | GG 57 (62.6) | 44 (65.7)             | 13 (54.2)                 | 0.317   |
|                                           | GA 34 (37.4) | 23 (34.3)             | 11 (45.8)                 | 0.317   |

*mean ± sd, P value, Han population vs. Tibetan population.
Table 2: Comparison of differences in baseline information between CR and non-CR for all participants.

| Characteristics                        | Non-CR (N = 39) | CR (N = 52) | P value |
|----------------------------------------|-----------------|-------------|---------|
| Age, years<sup>±</sup>                 | 59.46 ± 11.01   | 69.79 ± 11.67 | <0.001  |
| Height (m)<sup>±</sup>                 | 1.70 ± 0.08     | 1.66 ± 0.09  | 0.062   |
| Weight (Kg)<sup>±</sup>                | 68.56 ± 9.64    | 67.79 ± 11.60 | 0.736   |
| BMI (Kg/m<sup>2</sup>)<sup>±</sup>     | 23.71 ± 2.43    | 24.41 ± 2.89 | 0.225   |
| Oxygen saturation (%)                  | 91.0 (89.0, 93.0)| 90.0 (90.0, 92.8) | 0.827   |
| SBP (mmHg)<sup>±</sup>                 | 126.3 ± 27.2    | 127.4 ± 22.3 | 0.837   |
| DBP (mmHg)<sup>±</sup>                 | 74.9 ± 14.9     | 73.2 ± 12.5  | 0.553   |
| Heart rate<sup>±</sup>                 | 76.5 ± 14.8     | 77.1 ± 16.2  | 0.854   |
| White blood cell                       | 7.32 (5.55, 10.06) | 7.17 (5.71, 11.33) | 0.721   |
| Red blood cell                         | 4.89 (4.40, 5.54) | 4.70 (4.25, 5.22) | 0.342   |
| Hemoglobin<sup>±</sup>                 | 153.2 ± 28.7    | 149.5 ± 20.3 | 0.500   |
| Glycosylated hemoglobin                | 5.63 (5.38, 6.19) | 6.06 (5.52, 7.17) | 0.005   |
| PT                                     | 12.0 (11.7, 12.7) | 11.9 (11.4, 12.5) | 0.271   |
| APTT                                   | 28.7 (26.7, 32.3) | 26.9 (24.3, 31.0) | 0.008   |
| ALT                                    | 37.0 (19.0, 82.0) | 28.5 (19.3, 42.0) | 0.183   |
| AST                                    | 48.0 (22.0, 221.0) | 33.5 (21.3, 109.5) | 0.195   |
| AST/ALT                                | 1.3 (0.9, 3.4)  | 1.2 (0.8, 2.3)  | 0.431   |
| Urea nitrogen                          | 5.91 (4.97, 10.04) | 6.28 (5.25, 7.99) | 0.745   |
| Creatinine                             | 83.0 (67.0, 96.0) | 84.0 (67.3, 92.8) | 0.591   |
| Glomerular filtration rate (mL/min × 1.73 m<sup>2</sup>)<sup>±</sup> | 80.7 ± 28.3 | 76.0 ± 22.7 | 0.389   |
| Albumin                                | 38.2 (34.7, 41.1) | 37.8 (34.9, 39.7) | 0.510   |
| Total cholesterol (mmol/L)<sup>±</sup> | 4.00 ± 1.22     | 4.17 ± 1.05  | 0.478   |
| Triglyceride                           | 1.32 (0.90, 1.97) | 1.59 (0.90, 2.38) | 0.432   |
| TSH                                    | 1.19 (0.54, 2.68) | 1.68 (1.20, 2.89) | 0.156   |

Sex, n (%)

|            | Non-CR (N = 39) | CR (N = 52) | P value |
|------------|-----------------|-------------|---------|
| Male       | 32 (82.1)       | 31 (59.6)   |         |
| Female     | 7 (17.9)        | 21 (40.4)   |         |

Aspirin resistance, n (%)

|            | Non-CR (N = 39) | CR (N = 52) | P value |
|------------|-----------------|-------------|---------|
| No         | 28 (71.8)       | 14 (26.9)   | <0.001  |
| Yes        | 11 (28.2)       | 38 (73.1)   |         |

Smoking history, n (%)

|            | Non-CR (N = 39) | CR (N = 52) | P value |
|------------|-----------------|-------------|---------|
| No         | 20 (51.3)       | 32 (61.5)   | 0.328   |
| Yes        | 19 (48.7)       | 20 (38.5)   |         |

Coronary heart disease type, n (%)

|            | Non-CR (N = 39) | CR (N = 52) | P value |
|------------|-----------------|-------------|---------|
| STEMI      | 19 (48.7)       | 23 (44.2)   | 0.041   |
| NSTEMI     | 8 (20.5)        | 4 (7.7)     |         |
| SAP        | 2 (5.1)         | 13 (25.0)   |         |
| UA         | 10 (25.6)       | 12 (23.1)   |         |

Hypertension, n (%)

|            | Non-CR (N = 39) | CR (N = 52) | P value |
|------------|-----------------|-------------|---------|
| No         | 16 (41.0)       | 14 (26.9)   | 0.157   |
| Yes        | 23 (59.0)       | 38 (73.1)   |         |

Diabetes, n (%)

|            | Non-CR (N = 39) | CR (N = 52) | P value |
|------------|-----------------|-------------|---------|
| No         | 26 (66.7)       | 19 (36.5)   | 0.004   |
| Yes        | 13 (33.3)       | 33 (63.5)   |         |

Stroke, n (%)

|            | Non-CR (N = 39) | CR (N = 52) | P value |
|------------|-----------------|-------------|---------|
| No         | 25 (64.1)       | 34 (65.4)   | 0.899   |
| Yes        | 14 (35.9)       | 18 (34.6)   |         |

Hyperlipidaemia, n (%)

|            | Non-CR (N = 39) | CR (N = 52) | P value |
|------------|-----------------|-------------|---------|
| No         | 21 (53.8)       | 33 (63.5)   | 0.355   |
| Yes        | 18 (46.2)       | 19 (36.5)   |         |

CYP2C19 * 2, n (%)

|            | Non-CR (N = 39) | CR (N = 52) | P value |
|------------|-----------------|-------------|---------|
| GG         | 24 (61.5)       | 37 (71.2)   | 0.412   |
| GA         | 11 (28.2)       | 13 (25.0)   |         |
| AA         | 4 (10.3)        | 2 (3.8)     |         |

CYP2C19 * 3, n (%)

|            | Non-CR (N = 39) | CR (N = 52) | P value |
|------------|-----------------|-------------|---------|
| GG         | 24 (61.5)       | 37 (71.2)   | 0.334   |
| GA + AA    | 15 (38.5)       | 15 (28.8)   |         |

CYP2C19 * 3, n (%)

|            | Non-CR (N = 39) | CR (N = 52) | P value |
|------------|-----------------|-------------|---------|
| GG         | 24 (61.5)       | 37 (71.2)   | 0.387   |
Table 2: Continued.

| Characteristics | Non-CR (N = 39) | CR (N = 52) | P value |
|-----------------|-----------------|-------------|---------|
| GG              | 22 (56.4)       | 22 (42.3)   |         |
| GA              | 10 (25.6)       | 16 (30.8)   |         |
| AA              | 7 (17.9)        | 14 (26.9)   |         |
| CYP2C19 * 3, n (%) |               |             | 0.183   |
| GG              | 22 (56.4)       | 22 (42.3)   |         |
| GA + AA         | 17 (43.6)       | 30 (57.7)   |         |
| CYP2C19 * 17, n (%) |              |             | 0.532   |
| GG              | 23 (59.0)       | 34 (65.4)   |         |
| GA              | 16 (41.0)       | 18 (34.6)   |         |

Table 3: Comparison of differences in baseline information between CR and non-CR for Han populations.

| Characteristics                             | Non-CR (N = 27) | CR (N = 40) | P value |
|---------------------------------------------|-----------------|-------------|---------|
| Age, years                                 | 59.33 ± 10.48   | 70.48 ± 11.38 | <0.001  |
| Height (m)                                 | 1.70 ± 0.08     | 1.67 ± 0.09  | 0.115   |
| Weight (Kg)                                 | 69.04 ± 9.93    | 68.78 ± 11.86 | 0.925   |
| BMI (Kg/m²)                                 | 23.60 ± 2.13    | 24.59 ± 3.04 | 0.151   |
| Oxygen saturation (%)                       | 91.0 (89.0, 93.0) | 90.5 (89.3, 93.0) | 0.892   |
| SBP (mmHg)                                 | 129.6 ± 28.1    | 128.1 ± 17.7 | 0.809   |
| DBP (mmHg)                                 | 75.0 ± 14.1     | 73.2 ± 11.0  | 0.549   |
| Heart rate (%)                              | 77.7 ± 14.2     | 79.1 ± 15.7  | 0.699   |
| White blood cell                            | 7.17 (5.55, 10.53) | 6.93 (5.48, 11.06) | 0.898   |
| Red blood cell                              | 5.10 (4.55, 5.55) | 4.60 (4.25, 5.26) | 0.120   |
| Hemoglobin†                                 | 158.5 ± 22.1    | 149.7 ± 20.6 | 0.098   |
| Glycosylated hemoglobin                     | 5.57 (5.38, 5.97) | 6.09 (5.49, 7.24) | 0.009   |
| PT                                          | 11.9 (11.6, 12.7) | 11.7 (11.2, 12.5) | 0.169   |
| APTT                                        | 27.9 (26.7, 30.9) | 26.5 (24.5, 30.0) | 0.031   |
| ALT                                         | 33.0 (20.0, 70.0) | 27.0 (18.3, 39.5) | 0.125   |
| AST                                         | 60.0 (22.0, 209.0) | 31.0 (20.0, 121.5) | 0.179   |
| AST/ALT                                     | 1.4 (1.0, 3.4)  | 1.2 (0.8, 2.4)  | 0.315   |
| Urea nitrogen                               | 5.41 (4.86, 7.54) | 6.11 (5.25, 7.63) | 0.201   |
| Creatinine                                  | 78.0 (67.0, 90.0) | 84.5 (67.0, 93.0) | 0.908   |
| Glomerular filtration rate (mL/min × 1.73 m²) | 87.4 ± 28.3     | 75.9 ± 21.5  | 0.064   |
| Albumin                                     | 38.6 (36.1, 41.4) | 37.8 (35.2, 40.0) | 0.457   |
| Total cholesterol (mmol/L)                  | 4.07 ± 1.02     | 4.16 ± 1.04  | 0.731   |
| Triglyceride                                | 1.29 (0.90, 1.97) | 1.77 (0.90, 2.51) | 0.315   |
| TSH                                         | 1.79 (0.66, 2.65) | 1.62 (1.15, 2.81) | 0.498   |
| Sex, n (%)                                  |                |             | 0.010   |
| Male                                        | 23 (85.2)       | 22 (55.0)   |         |
| Female                                      | 4 (14.8)        | 18 (45.0)   |         |
| Aspirin resistance, n (%)                   |                |             | <0.001  |
| No                                          | 20 (74.1)       | 10 (25.0)   |         |
| Yes                                         | 7 (25.9)        | 30 (75.0)   |         |
| Smoking history, n (%)                      |                |             | 0.065   |
| No                                          | 10 (37.0)       | 24 (60.0)   |         |
| Yes                                         | 17 (63.0)       | 16 (40.0)   |         |
| Coronary heart disease type, n (%)          |                |             | 0.206   |
| STEMI                                       | 14 (51.9)       | 17 (42.5)   |         |
| NSTEMI                                      | 5 (18.5)        | 3 (7.5)     |         |
| SAP                                         | 2 (7.4)         | 9 (22.5)    |         |
| UA                                          | 6 (22.2)        | 11 (27.5)   |         |
| Hypertension, n (%)                         |                |             | 0.110   |
| No                                          | 11 (40.7)       | 9 (22.5)    |         |
| Yes                                         | 16 (59.3)       | 31 (77.5)   |         |
| Diabetes, n (%)                             |                |             | 0.003   |
| No                                          | 20 (74.1)       | 15 (37.5)   |         |
| Yes                                         | 7 (25.9)        | 25 (62.5)   |         |
| Stroke, n (%)                               |                |             | 0.969   |
| No                                          | 17 (63.0)       | 25 (62.5)   |         |
### Table 3: Continued.

| Characteristics                  | Non-CR (N = 27) | CR (N = 40) | P value |
|----------------------------------|----------------|------------|---------|
| Yes                              | 10 (37.0)      | 15 (37.5) | 0.570   |
| No                               | 15 (55.6)      | 25 (62.5) |         |
| Yes                              | 12 (44.4)      | 15 (37.5) |         |
| Hyperlipidaemia, n (%)           |                |            | 0.570   |
| CYP2C19 * 2, n (%)               |                |            | 0.333   |
| GG                               | 16 (59.3)      | 29 (72.5) |         |
| GA                               | 7 (25.9)       | 9 (22.5)  |         |
| AA                               | 4 (14.8)       | 2 (5.0)   |         |
| CYP2C19 * 2, n (%)               |                |            | 0.258   |
| GG                               | 16 (59.3)      | 29 (72.5) |         |
| GA                               | 6 (22.2)       | 11 (27.5) |         |
| AA                               | 4 (14.8)       | 12 (30.0) |         |
| CYP2C19 * 3, n (%)               |                |            | 0.216   |
| GG                               | 17 (63.0)      | 17 (42.5) |         |
| GA                               | 6 (22.2)       | 11 (27.5) |         |
| AA                               | 4 (14.8)       | 12 (30.0) |         |
| CYP2C19 * 3, n (%)               |                |            | 0.100   |
| GG                               | 17 (63.0)      | 17 (42.5) |         |
| GA + AA                          | 10 (37.0)      | 23 (57.5) |         |
| CYP2C19 * 17, n (%)              |                |            | 0.888   |
| GG                               | 18 (66.7)      | 26 (65.0) |         |
| GA                               | 9 (33.3)       | 14 (35.0) |         |

### Table 4: Comparison of differences in baseline information between CR and non-CR for Tibetan populations.

| Characteristics                  | Non-CR (N = 12)   | CR (N = 12)   | P value |
|----------------------------------|------------------|---------------|---------|
| Age, years^#                     | 59.75 ± 12.63    | 67.50 ± 12.82 | 0.150   |
| Height (m)^#                     | 1.68 ± 0.08      | 1.64 ± 0.08   | 0.227   |
| Weight (Kg)^#                    | 67.50 ± 9.26     | 64.50 ± 10.49 | 0.466   |
| BMI (Kg/m^2)^#                   | 23.97 ± 3.11     | 23.84 ± 2.31  | 0.911   |
| Oxygen saturation (%)            | 90.5 (89.0, 92.8) | 90.0 (90.0, 92.0) | 0.630 |
| SBP (mmHg)^#                     | 119.1 ± 24.6     | 125.2 ± 34.4  | 0.623   |
| DBP (mmHg)^#                     | 74.8 ± 17.3      | 73.4 ± 17.1   | 0.851   |
| Heart rate^#                     | 73.8 ± 16.4      | 70.3 ± 16.5   | 0.607   |
| White blood cell                 | 7.35 (5.36, 9.55) | 9.08 (6.86, 15.20) | 0.266 |
| Red blood cell                   | 4.55 (3.78, 5.49) | 4.79 (4.19, 5.19) | 0.731 |
| Hemoglobin^#                     | 141.1 ± 38.3     | 149.0 ± 20.1  | 0.533   |
| Glycosylated hemoglobin          | 5.69 (5.26, 7.04) | 6.01 (5.81, 6.91) | 0.291 |
| PT                               | 12.1 (11.9, 13.3) | 12.3 (11.9, 13.5) | 0.799 |
| APTT                             | 31.8 (27.2, 35.0) | 29.4 (23.6, 32.0) | 0.114 |
| ALT                              | 45.0 (12.0, 134.8) | 35.5 (20.3, 31.8) | 0.887 |
| AST                              | 43.0 (20.0, 771.8) | 44.5 (23.3, 106.0) | 0.843 |
| AST/ALT                          | 1.0 (0.7, 4.7)   | 1.1 (0.6, 2.1) | 0.887   |
| Urea nitrogen                    | 8.56 (5.99, 13.66) | 7.16 (5.13, 8.80) | 0.443 |
| Creatinine                       | 93.5 (76.3, 127.5) | 82.5 (72.8, 88.5) | 0.319 |
| Glomerular filtration rate (mL/min × 1.73 m^2)^# | 65.5 ± 22.6   | 76.4 ± 27.3   | 0.297   |
| Albumin                          | 37.7 (32.6, 41.0) | 36.2 (33.2, 38.3) | 0.833   |
| Total cholesterol (mmol/L)^#     | 3.84 ± 1.62      | 4.20 ± 1.10   | 0.528   |
| Triglyceride                     | 1.38 (0.92, 1.95) | 1.16 (0.89, 2.09) | 0.799 |
| TSH                              | 1.12 (0.36, 2.94) | 2.04 (1.26, 2.90) | 0.211 |
| Sex, n (%)                       |                 |               | 1.000   |
| Male                             | 9 (75.0)         | 9 (75.0)      |         |
| Female                           | 3 (25.0)         | 3 (25.0)      |         |
| Aspirin resistance, n (%)        |                 |               | 0.102   |
| No                               | 8 (66.7)         | 4 (33.3)      |         |
| Yes                              | 4 (33.3)         | 8 (66.7)      |         |
| Smoking history, n (%)           |                 |               | 0.342   |
| No                               | 10 (83.3)        | 8 (66.7)      |         |
| Yes                              | 2 (16.7)         | 4 (33.3)      |         |
results may be due to the magnitude of the influence of CYP2C19 on the effectiveness of clopidogrel and may be consistent with the influence of this molecule on specific clinical indications [32, 33].

In this study, APTT, age, and aspirin resistance were significantly correlated with CR. The APTT is a widely available test used to screen for hypercoagulable states in bleeding disorders [34]. Shortened APTT is an independent predictor for CR. The findings suggest that genetic variations in CYP2C19 have a significant impact on clinical outcomes, highlighting the importance of individualized treatment strategies based on genetic profiling.
risk factor for ischemic stroke [35], but its role in CR has not been reported to our knowledge. Age was a positively correlated factor of CR, which was inconsistent with previous studies. Prabhakaran et al. [36] have reported that being older than 55 years contributed to a low response to clopidogrel loading. It has been reported that patients with aspirin resistance have increased platelet reactivity [37]. High on-treatment platelet reactivity has become the most important factor inhibiting the antiplatelet effect of clopidogrel, resulting in the ineffectiveness of this agent [38]. Clopidogrel’s high on-treatment platelet reactivity could negatively influence the clinical course of a stroke and increase the risk of recurrent vascular events [39]. Therefore, platelet function testing is necessary for stroke individuals, especially those predisposed to CR.

There were several limitations in the present study. First, there was a lack of sequence analysis that could provide more robust information on the investigated CYP2C19 polymorphisms. Second, the study only comprised Chinese patients, while multicentric investigation might have been more informative in terms of data robustness. Third, there was a lack of functional correlation between examined gene polymorphisms and enzyme activity in patients. At last, no control group represented by healthy individuals was included in the analysis. Furthermore, studies including larger sample sizes and control groups may help to better understand the phenomenon of heterogeneity in clopidogrel response.

5. Conclusion

In conclusion, the present study indicated that CYP2C19*2, CYP2C19*3, and CYP2C19*17 alleles were not correlated with CR for both Han and Tibetan populations in Qinghai Province, while age, APTT, and aspirin resistance were independent risk factors of CR in this region. Our results may provide useful data for precision medicine based on individual gene sequencing results.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

This study was approved by the Ethics Committee of Qinghai Provincial People’s Hospital.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

RC and JW were responsible for conception and design of the research, YY, WZ, and XZ were responsible for acquisition of data, YY and WZ were responsible for analysis and interpretation of data, RC and JW were responsible for statistical analysis, RC and JW were responsible for obtaining funding, RC and JW were responsible for drafting the manuscript, and YL was responsible for revision of the manuscript for important intellectual content. All authors have read and approved the final manuscript.

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