

Research Article

Identification of *ITGA2B* and *ITGB3* Single-Nucleotide Polymorphisms and Their Influences on the Platelet Function

Qian Xiang,¹ Shun-Dong Ji,^{2,3} Zhuo Zhang,¹ Xia Zhao,¹ and Yi-Min Cui¹

¹Department of Pharmacy, Base for Clinical Trial, Peking University First Hospital, Beijing 100034, China

²Jiangsu Institute of Hematology, Key Laboratory of Thrombosis and Hemostasis of Ministry of Health, The First Affiliated Hospital of Soochow University, Suzhou 215006, China

³Collaborative Innovation Center of Hematology, Soochow University, Suzhou 215006, China

Correspondence should be addressed to Yi-Min Cui; bdyyyljd@126.com

Received 3 February 2016; Accepted 18 April 2016

Academic Editor: Robert Baiocchi

Copyright © 2016 Qian Xiang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The aim of the study was to investigate *ITGA2B* and *ITGB3* genetic polymorphisms and to evaluate the variability in the platelet function in healthy Chinese subjects. The genetic sequence of the entire coding region of the *ITGA2B* and *ITGB3* genes was investigated. Adenosine diphosphate-induced platelet aggregation, glycoprotein IIb/IIIa content, bleeding time, and coagulation indexes were detected. Thirteen variants in the *ITGA2B* locus and 29 variants in the *ITGB3* locus were identified in the Chinese population. The rs1009312 and rs2015049 were associated with the mean platelet volume. The rs70940817 was significantly correlated with the prothrombin time. The rs70940817 and rs112188890 were related with the activated partial thromboplastin time, and *ITGB3* rs4642 was correlated with the thrombin time and fibrinogen. The minor alleles of rs56197296 and rs5919 were associated with decreased ADP-induced platelet aggregation, and rs55827077 was related with decreased GPIIb/IIIa per platelet. The rs1009312, rs2015049, rs3760364, rs567581451, rs7208170, and rs117052258 were related with bleeding time. Further studies are needed to explore the clinical importance of *ITGA2B* and *ITGB3* SNPs in the platelet function.

1. Introduction

Platelet aggregation plays a central role in the pathogenesis of acute thrombosis in coronary heart disease, stroke, and peripheral arterial disease. The cellular events leading to platelet aggregation are mediated by the binding of fibrinogen to the glycoprotein (GP)IIb/IIIa receptor of platelets as a final common pathway. GPIIb/IIIa is a platelet-specific, surface membrane receptor and also called alpha IIb beta 3 (aIIb β 3) in the integrin nomenclature and thus plays a primary role in both platelet adhesion and thrombus formation at the vascular injury site [1]. A large interindividual number variability for GPIIb/IIIa receptors expressed on the platelet surface is commonly observed [2, 3]. Moreover, a defect in the GPIIb/IIIa complex or a qualitative abnormality of this complex is seen in Glanzmann's thrombasthenia patients with impaired platelet aggregation and increased bleeding [4].

The *ITGA2B* gene encodes the aIIb subunit (GPIIb), whereas the *ITGB3* gene encodes β 3 (GPIIIa). The *ITGA2B*

spanning 17 kb has 30 exons, whereas the *ITGB3* spanning 46 kb has 15 exons; they are closely located on chromosome 17q21.32 without evidence for coordinated expression [5]. A few studies have linked single-nucleotide polymorphisms (SNPs) in *ITGA2B* and *ITGB3* with increased or decreased platelet responses to various agonists and the risk of acute coronary syndrome and atherosclerosis [6–8].

A common polymorphism in the *ITGB3*, known as the human platelet antigen 1 (HPA-1b, PLA2, or rs5918), arises from a single-nucleotide change at position 1565 in Exon2 of *ITGB3*, resulting in a leucine (PLA1) to proline (PLA2) substitution at residue 33 [9]. The SNP has been extensively studied as an inherited risk factor for acute coronary syndrome and for its effect on the platelet function [6–8]. The HPA-3 (rs5910) polymorphism results from a thymine to guanine base change that leads to the replacement of isoleucine (HPA-3a) by serine (HPA-3b) at codon 843 of GPIIb (*ITGA2B*). This polymorphism may potentially influence the activity of the GPIIb/IIIa complex and associate with thrombosis [10].

According to the aforementioned studies, GPIIb/IIIa was believed to be a platelet-platelet contact receptor playing an important role in platelet aggregation, and its SNPs were associated with platelet hyperreactivity and have an effect on the pharmacodynamics of antiplatelet drugs. However, limited information was available on other genetic polymorphisms of *ITGA2B* and *ITGB3* in Asian populations or their association with the platelet function [11, 12]. Moreover, the prevalence of the PLA2 allele (rs5918) is dependent on ethnicity, with a frequency of approximately 15/100 in Caucasian populations [13] falling to less than 1/100 in Oriental populations [12, 14].

In this study, regions of the exons of *ITGA2B* and *ITGB3* genes were sequenced in 86 unrelated healthy Chinese. In addition, the association between genetic variants of *ITGA2B* and *ITGB3* exons and the adenosine diphosphate- (ADP-) induced platelet aggregation, GPIIb/IIIa content, bleeding time, and coagulation indexes was investigated in 55 of the 86 subjects.

2. Materials and Methods

2.1. Study Design. The research was conducted in compliance with the Declaration of Helsinki. The study protocol was approved by the Ethical Review Board of the Peking University First Hospital. All subjects gave their written informed consent prior to participation in the study. Healthy native Chinese subjects ($n = 86$) between 18 and 45 years of age with a body mass index (BMI) of 19–24 kg/m² were included in the study, and their genotypes were unknown.

All the subjects were considered healthy on the basis of their medical history, physical examination, vital signs (blood pressure, pulse rate, and temperature), safety laboratory tests (blood chemistry, hematological tests, and urinalysis), and 12-lead electrocardiography.

The ADP-induced platelet aggregation (transmission, $T\%$ max), GPIIb/IIIa content (GPIIb/IIIa per platelet), bleeding time, and coagulation indexes, including the mean platelet volume (MPV), platelet count (PLT), prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen (FIB), and thrombin time (TT) were detected in 55 of the 86 subjects. None of the donors had taken any medication for 2 weeks before blood collection.

2.2. ADP-Induced Platelet Aggregation. Blood was collected in 3.8% sodium citrate tubes, and platelet-rich plasma (PRP) was obtained by centrifuging blood at 1000 revolutions per minute (rpm) for 10 minutes at room temperature. The PRP was collected in a fresh tube and platelet was counted by Platelet Counter PL100 (Sysmex, Kobe, Japan). Platelet-poor plasma (PPP) was obtained by centrifuging the remaining blood at 3000 rpm for 10 minutes at room temperature, and platelet numbering PRP was adjusted to $250 \times 10^9/L$ by PPP. Detection was completed within 1 hour after sampling.

Platelet aggregation was determined by the turbidimetric method using 20 $\mu\text{mol/L}$ ADP as the agonist. After zero setting with the PPP, assays were performed in platelet-rich plasma in a Chrono-Log aggregometer. Platelet aggregation was quantified as the maximum change in light transmission occurring within 5 minutes of addition of agonist.

2.3. GPIIb/IIIa Content. GPIIb/IIIa on the platelet surface was evaluated in the PRP as the maximal binding of a ¹²⁵I-labeled GPIIb/IIIa integrin antiplatelet antibody F(ab)₂ [15]. The PRP was fixed with equal volume of anticoagulant-fixative solution (10 mmol/L EDTA-Na₂, 0.2% glutaraldehyde, and 0.02% sodium azide dissolved in PBS, pH 7.4). After stored at 4°C, overnight, platelet was washed by Tyrode's solution twice and resuspended in Tyrode solution, containing 0.35% bovine serum albumin (concentration adjusted to 1×10^{11} platelets/L), and sodium azide was added at final concentration of 0.02% (V/V) to preserve the platelet at 4°C for a week-long before assay. For detection, the platelet suspension (100 μL) was added to a 0.5 mL Eppendorf tube and then incubated with 100 μL of antibody (containing 20,000 cpm of labeled monoclonal antibody (McAb) and 0.8 μg of unlabeled McAb) at 37°C for 1 hour, after washed with 0.35% bovine serum albumin/Tyrode's solution for three times. The radioactivity count of the precipitate in the tube was measured. Assuming that one monoclonal antibody would only bind one GP molecule on the platelet membrane, the number of GP molecules on the platelet membrane was calculated as number of GP molecules/platelet = (binding rate \times amount of antibody (g) \times Avogadro's number)/(molecular weight of antibody \times platelet count).

2.4. Bleeding Time. The skin bleeding time was carried out using the Simplate-II device (General Diagnostics, NJ, USA). A sphygmomanometer cuff was inflated on the patient's arm to 40 mmHg. The arm was supported at the level of the heart, and a muscular area on the volar aspect distal to the antecubital area was identified and swabbed with alcohol. The Simplate-II device was used to perform the incision perpendicular to the antecubital fossa. Blood was blotted with sterile filter paper every 30 seconds until blood no longer stained the paper. The time from incision to stopping of bleeding was recorded.

2.5. Genetic Analysis. Genomic DNA was extracted from peripheral whole blood samples of each subject using a DNA Purification Kit (Wizard; Promega, WI, USA). After the polymerase chain reaction (PCR) products were obtained, all samples were directly sequenced to determine the SNPs in *ITGA2B* and *ITGB3* (Life Technologies Biotechnology Co., Ltd., China).

Primers required for PCR amplification and sequencing were designed according to the wild-type *ITGA2B* and *ITGB3* sequences reported in the GenBank (NG_008331.1 and NG_008332.1, resp.). Tables 1 and 2 describe the primer pairs used to amplify the promoters, the 5'- and 3'-untranslated regions (UTRs), the entire coding regions and the intron-exon junctions of the *ITGA2B* and *ITGB3* genes.

2.6. Data Analysis. The Variant Reporter v1.1 (Life Technologies Biotechnology Co., Ltd.) software suite was used for the initial analysis of the sequence, including base calling, fragment assembly, SNP, and sequence insertions/deletions detection. Polymorphisms of *ITGA2B* and *ITGB3* genes were named according to the genomic reference sequences

TABLE 1: Summary of GPIIb (ITGA2B) variations detected in this study.

| Sequence number obtained in this study | dbSNP | Location | NC_000017.11 | NM_000419.3 | Nucleotide change | NP_000410.2 amino acid change | MA(F) | H-W P |
|--|-------------|-----------------|--------------|-------------|---------------------------|-------------------------------|----------|-------|
| ITGA2B-01 | rs3760364* | 5' upstream end | 44390436 | -963 | ctccaagg A/T ctattaca | | T(0.052) | 0.609 |
| ITGA2B-02 | New | 5' upstream end | 44390152 | -679 | tagaccaagg T/C ccattacca | | C(0.006) | 0.957 |
| ITGA2B-03 | New | 5' upstream end | 44390081 | -608 | caagacggag G/A aggagtgagg | | A(0.006) | 0.957 |
| ITGA2B-04 | New | Exon3 | 44385910 | 354 | tgatgagacc C/T gaaatgtagg | Arg108Stop | T(0.006) | 0.957 |
| ITGA2B-05 | New | Exon13 | 44380960 | 1344 | ctcccaggtc C/A tggacagccc | Leu438Met | A(0.006) | 0.957 |
| ITGA2B-06 | rs201355504 | Intron13 | 44380821 | 1393+58 | ctggcactt C/T cagcgaatgt | | A(0.006) | 0.957 |
| ITGA2B-07 | New | Exon14 | 44380638 | 1401 | tagaccgat C/G gfgggagctt | Ile467Met | G(0.006) | 0.957 |
| ITGA2B-08 | rs850730 | Intron21 | 44377095 | 2188-7C>G | ccctctcat C/G tcccatagag | | G(0.477) | 0.289 |
| ITGA2B-09 | New | Exon23 | 44376336 | 2320 | cgtgcccgtc C/T gggcagaggg | Arg774Trp | T(0.006) | 0.957 |
| ITGA2B-10 | rs117870452 | Exon23 | 44376322 | 2334 | gcagtccac C/T tgggcctctg | Gln778= | G(0.012) | 0.913 |
| ITGA2B-11 | rs5911 | Exon26 | 44375697 | 2621T>G | ggggctgggg A/C tgggcagccc | Ile874Ser | G(0.477) | 0.289 |
| ITGA2B-12 | rs5910 | Exon30 | 44372421 | 3063C>T | acccccagg C/T ggcttcttca | Vall1021= | T(0.465) | 0.289 |
| ITGA2B-13 | New | 3' -UTR | 44372213 | *151C>A | gctaccccc C/A tcctgtgcc | | A(0.017) | 0.869 |

dbSNP, single nucleotide polymorphism database; H-W P, Hardy-Weinberg equilibrium P value; MA(F), minor allele (frequency); GP, glycoprotein.

* SNPs which related with bleeding time in this study.

TABLE 2: Primer sequences used in this study.

| DNA sequence number | Amplified or sequenced region | Forward primer (5' to 3') | Reverse primer (5' to 3') | Amplified region NC.000017.11 | Length (bp) |
|---------------------|-------------------------------|---------------------------|---------------------------|-------------------------------|-------------|
| ITGA2B-1 | | TCCTCCTCTTCCGCTTACCG | TACTACCACCGTGCTAGTCC | 44389728–44390630 | 848 |
| ITGA2B-2 | Exon1 | CCAATATGGCTGGTTGAG | AACTTCCCTTACGGCTCA | 44389267–44390059 | 792 |
| ITGA2B-3 | Exon1 | CCAGTGCAGCTCACCTTCTA | GATGAGGGAAATGGAACAGA | 44388253–44389368 | 1115 |
| ITGA2B-4 | Intron1 | TATGAACCACTCCACCCT | TTGGCACTCTTGATTCTG | 44388169–44389006 | 837 |
| ITGA2B-5 | Exon2, Exon3 | ACCGCTGGTTCTTGTTGC | CCTACGGGCGTCTTCTCA | 44385649–44386479 | 830 |
| ITGA2B-6 | Exon4–Exon6 | TACAGGGCACAGGGAACAATC | AGAGGCTCTGGGAGGACACG | 44384990–44385810 | 820 |
| ITGA2B-7 | Exon7, Exon8 | TCCTGGCGGCTATTATTTCT | GCACCGACGACATATTCTGG | 44384339–44385186 | 847 |
| ITGA2B-8 | Exon9–Exon11 | ATTTGCGCCCTTGTCCTC | AGCCGAATCGCCCATAGA | 44383538–44384605 | 1067 |
| ITGA2B-9 | Exon12 | CCCTCTGTCTCCCTTTCC | CATCCAGTCTCCACCAA | 44383189–44383838 | 649 |
| ITGA2B-10 | Exon13, Exon14 | CCTAGTCTCCTGGGATGTTT | TCACGGGTGTCTTGGTCT | 44380390–44381163 | 773 |
| ITGA2B-11 | Exon14–Exon17 | TAATCGCCAATTCTGACCC | CACATCCACCTTCTCCTG | 44379854–44380682 | 828 |
| ITGA2B-12 | Exon18 | ACCCACTGGACTTGTTTCATC | TGTGACTTGGCACTAACCC | 44379610–44379957 | 347 |
| ITGA2B-13 | Exon19, Exon20 | TGGACGACAGAGCGAGAC | GGCCATACCTCGACATTG | 44378354–44378989 | 635 |
| ITGA2B-14 | Exon21 | CATGTGACAGTCCCTTGA | AAAGTCACTACCCAAGGA | 44377551–44377933 | 382 |
| ITGA2B-15 | Exon22 | CTTGGAGGGTGAAGACTGG | CAACTCCTGACCTCCAGTGA | 44376833–44377179 | 346 |
| ITGA2B-16 | Exon23–Exon25 | CCAGGTCTAACTTCAGTGTG | GCTCTGGCAGGAAGATCTGT | 44375629–44376523 | 894 |
| ITGA2B-17 | Exon26 | TCCGACCTGCTCTACATCC | CGGGCTTGCTCACATAGTC | 44375277–44375913 | 636 |
| ITGA2B-18 | Exon27, Exon28 | ATGACCCTCCCTGCATCTC | CACCTTGACACCTGCCTTT | 44374500–44375317 | 817 |
| ITGA2B-19 | Exon29 | GCACGCATGGTTCAACGT | CCTCCCGAGTAGCTGAGATT | 44374025–44374731 | 706 |
| ITGA2B-20 | Exon30 | AAAGGCATCCATTGTGA | TGTTGGTAAAGGCTGGTCTC | 44371896–44372566 | 670 |
| ITGB3-1 | | CAGGAGGTGGAGGATTGT | GCTGGATTCTTGGGACAC | 47252637–47253474 | 837 |
| ITGB3-2 | Exon1 | CGGTTTCAGAGAAGGCATTGAG | GCTCCAAGTCCGCAACTTGA | 47253373–47254015 | 642 |
| ITGB3-3 | Intron1 | TTGGCGTAGGAGGTGAGTGA | CCGCAGGAAGCCAAGTTGAA | 47253929–47254518 | 589 |
| ITGB3-4 | Intron1 | TTGGCGTAGGAGGTGAGTGA | GAAGTTGCAGTGAGCCGAGAA | 47253929–47255063 | 1134 |
| ITGB3-5 | Exon2 | ATTGGGAAAGTTGGGAAGG | GAAAGGGCAGCAGTGGTT | 47274335–47274773 | 438 |
| ITGB3-6 | Exon3 | AGGCTGGTCTTGAACCTCTG | CTCCACCTTGTGCTCTAT | 47283116–47283612 | 496 |
| ITGB3-7 | Exon4 | GGGCTTTCTGGTTTGCTT | CATTTCCCTCCCATTCTC | 47284329–47284978 | 649 |
| ITGB3-8 | Exon5 | TGCTGGGTAACCTGTGGT | CATCTGCCTACTTTGCTG | 47286124–47286725 | 601 |
| ITGB3-9 | Exon6 | TCCAAGGACTGGGACTGA | ATGATGCTGCTGCTATGC | 47286919–47287483 | 564 |
| ITGB3-10 | Exon7, Exon8 | AGCCCAAGCAAGATAAGT | GGAGAAGGCAGTAAGACC | 47289597–47290393 | 796 |
| ITGB3-11 | Exon9 | AAACTGGGCTCCAATAAC | TGAGGGACTGAAGGTAAG | 47290561–47291406 | 845 |
| ITGB3-12 | Exon10 | CAGGGCAGGGAACAACCTT | GGATTGGTCTTATACTCAAAA | 47292050–47292720 | 670 |
| ITGB3-13 | Exon11 | GAGCAAGTCTGCCATAC | TCACAGAGTGTCTCCATAA | 47299101–47299890 | 789 |
| ITGB3-14 | Exon12 | CAGAAATGGCATAGGGTT | TCTTGCTGAGTCTGTGGG | 47300168–47300824 | 656 |
| ITGB3-15 | Exon13 | CTTGAATCTAGGCATCGT | GTATTGAACTCCTGACCC | 47302581–47303050 | 469 |
| ITGB3-16 | Exon14 | CCTCAAGTAGGTCCCAGTG | AACATGACCACCCAAAGC | 47307158–47307721 | 563 |
| ITGB3-17 | Exon15 | CTCATCTCCTCTGTTATTT | TGACATTCTCCCAACCTAC | 47309941–47310337 | 396 |

NG_008331.1 and NG_008332.1, respectively. Novel SNPs were named as *ITGA2B*-number or *ITGB3*-number in the present study.

2.7. Statistical Analysis. The association between two parameters was assessed by Pearson's two-tailed test. Statistical analyses were performed using the Statistical Package for Social Sciences software program for Windows (SPSS version 16.0). A *P* value of 0.05 was considered significant.

3. Results

3.1. *ITGA2B* and *ITGB3* Variations. Within the Chinese sample, 13 and 29 variants in *ITGA2B* and *ITGB3* were identified, respectively. Two novel *ITGB3* SNPs with $MA(F) > 0.02$ were found, which were not reported in the National Center for Biotechnology Information (NCBI) dbSNP database (<https://www.ncbi.nlm.nih.gov/snp/>). The allele frequencies and Hardy–Weinberg equilibrium test results of the identified polymorphic sites are shown in Tables 3 and 4.

TABLE 3: Summary of ITGB3 variations detected in this study.

| Sequence number obtained in this study | dbSNP | Location | NC_000007.11 | NM_000212.2 | Nucleotide change | NP_000410.2 amino acid change | MA(F) | H-W P |
|--|--------------|----------|-------------------|---------------|------------------------------|-------------------------------|-----------------|-------|
| ITGB3-01 | rs147363351 | 5'-UTR | 47253042 | -820 | agcttcaga G/A gttttaagtc | | A(0.012) | 0.913 |
| ITGB3-02 | rs3809862 | 5'-UTR | 47253062 | -800 | ctgggaaga C/T ccagggactc | | T(0.424) | 0.822 |
| ITGB3-03 | rs7208170* | 5'-UTR | 47253393 | -469 | aaggcattca G/A cacagatttg | | A(0.419) | 0.680 |
| ITGB3-04 | New | 5'-UTR | 47253360 | -502 | tgatgaataa T/A aaaggactga | | A(0.023) | 0.913 |
| ITGB3-05 | rs7208055 | 5'-UTR | 47253461 | -401 | gtgaatigt C/A ccaagaatcc | | A(0.221) | 0.273 |
| ITGB3-06 | rs55827077* | 5'-UTR | 47253717 | -145 | tagaagaacc G/C gagggagga | | C(0.448) | 0.494 |
| ITGB3-07 | rs567581451* | 5'-UTR | 47253771 | -91 | accaccgcg -/TCCCC tcccccccc | | insTCCCC(0.058) | 0.567 |
| ITGB3-08 | rs117052258* | 5'-UTR | 47253855 | -7 | cgcgggaggc G/C gacgagatgc | | C(0.227) | 0.771 |
| ITGB3-09 | New | Exon1 | 47253882 | 21 | ggccgagcc C/G cggccgctct | Pro7= | G(0.407) | 0.567 |
| ITGB3-10 | rs11871251 | Intron1 | 47254061 | 79+121 | ctgggaatgc G/A cgtgtccctgg | | A(0.453) | 0.771 |
| ITGB3-11 | New | Intron1 | 47254082 | 79+152 | tggcgggt C/G ggagccggga | | G(0.012) | 0.913 |
| ITGB3-12 | rs112188890* | Intron1 | 47254101 | 79+161 | gagctgggga C/T ctctctggcc | | T(0.116) | 0.864 |
| ITGB3-13 | rs117414137* | Intron1 | 47254192 | 79+252 | aggctgagcg C/G ctctccggcc | | G(0.116) | 0.864 |
| ITGB3-14 | rs11871447 | Intron1 | 47254252 | 79+312 | ccgctctac C/G cggggctg-cg | | G(0.448) | 0.918 |
| ITGB3-15 | New | Intron1 | 47254331 | 79+391 | tggggcttcc G/A ggggtgtctc | | A(0.006) | 0.957 |
| ITGB3-16 | rs1009312* | Intron1 | 47254774 | 79+834 | ggcacagccc G/A ggggtgtctc | | G(0.471) | 0.000 |
| ITGB3-17 | rs2015049 | Intron1 | 47254865 | 79+925 | ggccgctct G/A cctcagagga | | A(0.529) | 0.000 |
| ITGB3-18 | rs56197296* | Intron5 | 47287025-47287029 | 778-45-778-41 | catggctgaa TTTGT/- ttgtctcct | | delTTTGT(0.169) | 0.049 |
| ITGB3-19 | rs5919* | Exon6 | 47287174 | 882 | tgtccagcc T/C aatgacgggc | Pro294= | C(0.262) | 0.022 |
| ITGB3-20 | rs41504748 | Intron7 | 47290145 | 1036-40 | accaccagct T/C cctttggtaa | | C(0.070) | 0.334 |
| ITGB3-21 | rs15908 | Exon9 | 47290971 | 1143 | cttcagctc A/G/T actttagaac | Val381= | C(0.599) | 0.006 |
| ITGB3-22 | New | Exon10 | 47292177 | 1299 | gagcgttcc C/T caggagaagg | Pro433= | T(0.006) | 0.957 |
| ITGB3-23 | rs4642* | Exon10 | 47292411 | 1533A>G | cagcagagca A/G tgcagccccc | Glu511= | G(0.331) | 0.214 |
| ITGB3-24 | rs13306487 | Exon10 | 47292422 | 1544 | tcagccccc A/C/G ggaaggctcag | Arg515Gln | A(0.012) | 0.913 |
| ITGB3-25 | rs4634* | Exon10 | 47292423 | 1545 | gcagccccc G/A gagggctcagc | Arg515= | A(0.331) | 0.214 |
| ITGB3-26 | New | Exon10 | 47292528 | 1659 | gcaggtgtga C/T gacttctct | Asp550= | T(0.006) | 0.957 |
| ITGB3-27 | rs149823724 | Exon11 | 47299519 | 1902 | cagatgccctg C/T acctttaaga | Cys634= | T(0.006) | 0.957 |
| ITGB3-28 | rs11870252 | Intron11 | 47300459 | 1914-19 | ccctaatcac T/C ggtctctc | | C(0.035) | 0.869 |
| ITGB3-29 | rs70940817* | Exon12 | 47300524 | 1960 | ccctaatgac G/A aaaataccctg | Glu654Lys | A(0.076) | 0.432 |

dbSNP, single nucleotide polymorphism database; H-W P, Hardy-Weinberg equilibrium P value; MA(F), minor allele (frequency).

* SNPs which related with ADP induced platelets aggregation, GPIIb/IIIa content, bleeding time, or coagulation indexes in this study.

TABLE 4: Association between individual ITGA2B and ITGB3 SNPs and the ADP induced platelets aggregation, GPIIb/IIIa content, bleeding time, PLT, and MPV (mean \pm SD).

| dbSNP | Genotypes | N | Aggregation max T% | GPIIb-IIIa per platelet | Bleeding time (s) | PLT ($10^9/L$) | MPV (fL) |
|------------------------|-------------|----|--------------------|-------------------------|-------------------|-------------------|-----------------|
| rs3760364 | AA | 48 | 36.6 \pm 15.3 | 45937.1 \pm 15214.0 | 281.3 \pm 97.4 | 277.0 \pm 70.87 | 9.48 \pm 0.84 |
| | AT | 7 | 27.4 \pm 16.1 | 41060.9 \pm 15560.7 | 364.3 \pm 87.3 | 257.7 \pm 83.7 | 9.51 \pm 0.66 |
| <i>P</i> | | | 0.148 | 0.433 | 0.038 | 0.512 | 0.911 |
| rs7208170 | GG | 18 | 38.5 \pm 16.2 | 48835.0 \pm 13746.0 | 326.7 \pm 100.8 | 255.1 \pm 62.1 | 9.14 \pm 0.74 |
| | GA | 24 | 35.1 \pm 14.3 | 45714.9 \pm 15783.1 | 288.8 \pm 86.6 | 290.6 \pm 82.8 | 9.63 \pm 0.80 |
| <i>P</i> | | | 0.229 | 0.105 | 0.030 | 0.428 | 0.051 |
| rs55827077 | AA | 13 | 31.7 \pm 17.1 | 39709.0 \pm 15545.1 | 249.2 \pm 109.1 | 272.1 \pm 60.4 | 9.68 \pm 0.84 |
| | GG | 18 | 39.9 \pm 16.2 | 49297.7 \pm 14047.7 | 315.0 \pm 86.2 | 253.2 \pm 62.6 | 9.24 \pm 0.65 |
| | GC | 22 | 35.1 \pm 13.0 | 47150.7 \pm 13706.9 | 279.5 \pm 102.4 | 291.9 \pm 85.5 | 9.55 \pm 0.85 |
| <i>P</i> | | | 0.091 | 0.021 | 0.401 | 0.251 | 0.141 |
| rs567581451 | -/- | 49 | 35.0 \pm 14.4 | 45317.6 \pm 14892.6 | 282.2 \pm 99.1 | 277.3 \pm 73.7 | 9.56 \pm 0.80 |
| | -/insTCCCC | 6 | 38.5 \pm 24.4 | 45306.8 \pm 19136.0 | 370.0 \pm 64.8 | 252.3 \pm 56.8 | 8.88 \pm 0.62 |
| <i>P</i> | | | 0.609 | 0.999 | 0.040 | 0.428 | 0.054 |
| rs117052258 | GG | 29 | 36.7 \pm 16.3 | 44811.1 \pm 14492.1 | 319.7 \pm 93.6 | 277.1 \pm 79.2 | 9.37 \pm 0.82 |
| | GC | 21 | 37.3 \pm 13.3 | 48026.0 \pm 15188.4 | 268.6 \pm 105.4 | 267.9 \pm 59.9 | 9.58 \pm 0.80 |
| | CC | 4 | 18.3 \pm 14.8 | 31964.5 \pm 18104.4 | 232.5 \pm 61.8 | 309.7 \pm 84.4 | 9.70 \pm 1.01 |
| <i>P</i> | | | 0.165 | 0.539 | 0.028 | 0.764 | 0.288 |
| rs1009312 ^a | GG | 19 | 39.3 \pm 15.9 | 46470.7 \pm 13798.6 | 326.8 \pm 84.2 | 276.4 \pm 95.0 | 9.13 \pm 0.61 |
| | GA | 22 | 34.0 \pm 14.6 | 48883.3 \pm 14927.5 | 287.7 \pm 101.3 | 272.7 \pm 58.6 | 9.64 \pm 0.88 |
| | AA | 14 | 32.3 \pm 16.6 | 38145.0 \pm 16040.8 | 250.7 \pm 105.0 | 275.0 \pm 59.1 | 9.71 \pm 0.82 |
| <i>P</i> | | | 0.189 | 0.160 | 0.027 | 0.944 | 0.029 |
| rs56197296 | TTTGT/TTTGT | 42 | 37.7 \pm 15.9 | 45398.1 \pm 14564.7 | 285.0 \pm 94.7 | 280.5 \pm 76.3 | 9.41 \pm 0.81 |
| | TTTGT/del | 11 | 30.9 \pm 10.2 | 45262.9 \pm 18722.4 | 330.0 \pm 116.2 | 249.6 \pm 57.1 | 9.78 \pm 0.79 |
| | del/del | 2 | 11.5 \pm 7.8 | 43895.5 \pm 16431.0 | 225.0 \pm 63.6 | 288.0 \pm 21.2 | 9.30 \pm 1.13 |
| <i>P</i> | | | 0.014 | 0.916 | 0.695 | 0.433 | 0.416 |
| rs5919 | TT | 31 | 38.8 \pm 16.1 | 45342.5 \pm 14029.1 | 299.0 \pm 96.6 | 283.7 \pm 78.8 | 9.34 \pm 0.84 |
| | TC | 16 | 34.9 \pm 12.5 | 47696.5 \pm 17544.9 | 315.0 \pm 105.6 | 247.7 \pm 58.6 | 9.64 \pm 0.73 |
| | CC | 8 | 23.3 \pm 13.8 | 40455.6 \pm 15470.6 | 217.5 \pm 67.6 | 292.9 \pm 60.5 | 9.73 \pm 0.85 |
| <i>P</i> | | | 0.015 | 0.634 | 0.130 | 0.728 | 0.148 |

Note: Pearson's two-tailed test was used to analyze correlation between genotype and parameter. ^ars1009312 was completely linked with rs2015049.

3.2. Linkage Disequilibrium Analysis. Using the detected polymorphisms greater than 0.01 in frequency [$MA(F) > 0.02$], linkage disequilibrium (LD) was analyzed for $|D'|$ and r^2 values (Figure 1). For *ITGA2B*, rs5911 was completely linked with rs850730 ($r^2 = 1.00$), and rs5910 was strongly linked with rs850730 and rs5911 ($r^2 = 0.95$). For *ITGB3*, complete LD was observed between rs4642 and rs4634 ($r^2 = 1.00$). Other LD results are shown in Figure 1. A new SNP *ITGB3-09* was found for which $MA(F)$ in this study was 0.402.

The Hardy-Weinberg equilibrium P values of rs1009312 and rs2015049 were <0.001 . The possible reason is that the sample in this study may have a Chinese population of other ethnic groups besides the Han population. Those two SNPs were not included in the LD analysis.

3.3. Correlation of ADP-Induced Platelet Aggregation, GPIIb/IIIa Content, Bleeding Time, and Coagulation Indexes. The GPIIb/IIIa contents, present as average numbers of GPIIb/IIIa receptor in each platelet, were associated with ADP-induced platelet aggregation ($r = 0.280$, $P = 0.038$), PLT ($r = -0.522$, $P < 0.001$), PT ($r = 0.453$, $P = 0.001$), and TT ($r = 0.545$, $P < 0.001$) (Figures 1 and 2). The relationship between GPIIb/IIIa content and APTT was not significant ($r = 0.242$, $P = 0.075$), while the association between ADP-induced platelet aggregation and APTT was moderately significant ($r = 0.267$, $P = 0.049$) (Figure S1). The bleeding time had no association with other parameters. It had a negative association trend with MPV but was not significant ($r = -0.244$, $P = 0.073$). The PLT was associated with PT ($r = -0.415$, $P = 0.002$) and TT ($r = -0.363$, $P = 0.007$).

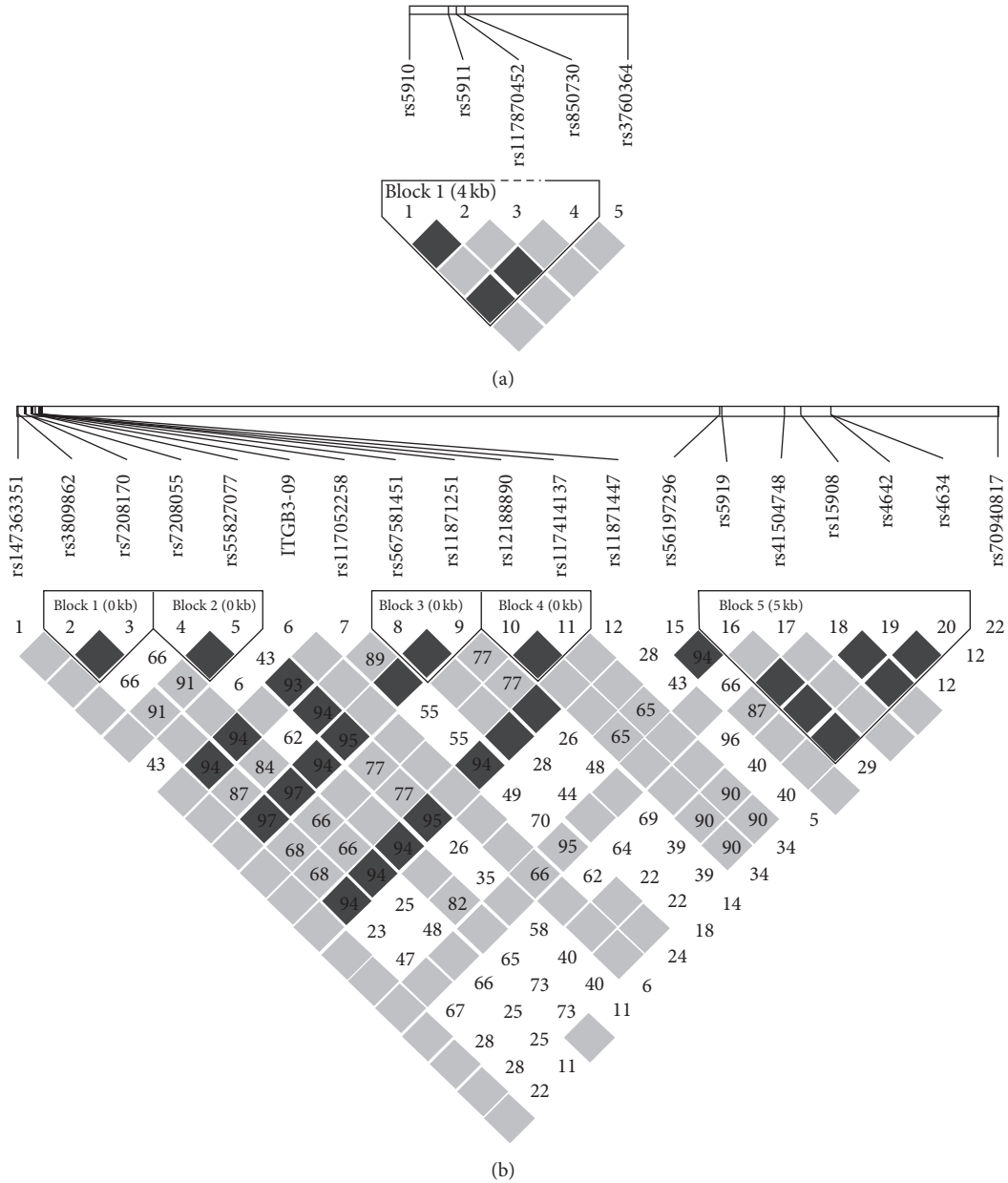


FIGURE 1: Linkage disequilibrium (LD) map of *ITGB2A* and *ITGB3* SNPs along with their locations in the *ITGB2A* and *ITGB3* genes. Variants present at >1% frequency were included in the LD analysis using the statistics $|D'|$ and r^2 . Red depicts a significant linkage between an SNP pair. Numbers inside the square indicate $r^2 \times 100$. (a) *ITGB2A*. (b) *ITGB3*.

The FIB was negatively correlated with TT ($r = -0.409$, $P = 0.002$).

3.4. Impact of *ITGA2B* and *ITGB3* SNPs on ADP-Induced Platelet Aggregation, GPIIb/IIIa Content, Bleeding Time, and Coagulation Indexes. For *ITGA2B*, only one SNP rs3760364 was related with the bleeding time ($P = 0.038$) (Table 4). No significant correlation or trend was observed between *ITGA2B* SNPs and other parameters.

For *ITGB3*, the mean values of ADP-induced platelet aggregation of homozygous mutant genotypes in rs56197296

and rs5919 were lower than those of heterozygous mutations and wild type. Moreover, the GPIIb/IIIa per platelet was associated with rs55827077. A trend of GPIIb/IIIa per platelet was observed among rs70940817 GG (45), GA (9), and AA (1) carriers (43807.7 ± 14157.5 , 51086.8 ± 19368.0 , 61277 , resp., $P = 0.093$), although it did not reach a significant level. The bleeding time was significantly related with rs3760364, rs7208170, rs567581451, and rs117052258 (Table 4).

In the present study, SNPs related with PLT were not found. *ITGB3* rs1009312, which is completely linked with rs2015049, was significantly associated with the MPV

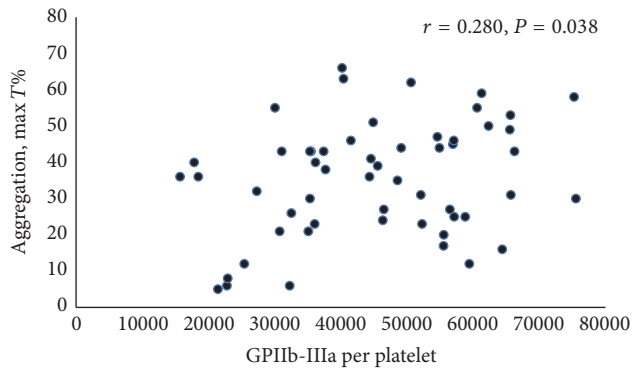


FIGURE 2: Correlations of the maximal level of ADP-induced platelet aggregation and GPIIb/IIIa content in healthy volunteers. r , coefficient of correlation; P , significance of correlation (Pearson's test here and elsewhere).

(Table 4). *ITGB3* rs70940817 was correlated with the PT and APTT ($P = 0.002$ and $P = 0.003$, resp.). The coagulation indexes and their P values are summarized in Table 5.

4. Discussion

In this study, 13 variants in the *ITGA2B* locus and 29 variants in the *ITGB3* locus in the Chinese population were observed. Two of the 29 variants located in *ITGB3* were novel SNPs with $MA(F) > 0.02$. Variants in *ITGA2B* and *ITGB3* genes displayed significant interethnic differences in the global populations (Table 6). The C allele frequency of SNP rs5918 (T/C), located in the *ITGB3* gene, was only inhomogeneously distributed at 0.7% in the Chinese population, while it is common in whites and Africans (allele frequency 13.7% versus 12.8%). The following alleles of SNPs, rs7208170A, rs55827077C, rs11871251A, rs1009312G, and rs2015049G, were found to be more common in the Han Chinese and Africans (37%~50%) than in the European ancestry (10%~20%). In the *ITGA2B* gene, the rs5911 was completely linked with rs850730 and strongly linked with rs5910 in the Chinese population in this study, and all the three SNPs were common in whites and Asians. However, the rs5911 was not found in Africans. According to the ethnicity difference described in the preceding text, resequencing of the *ITGA2B* and *ITGB3* genes in the Chinese population was believed to be very important to reveal the function of SNPs.

Variation in the MPV or PLT can have a profound impact on differences in the platelet function between individuals [16, 17], and these traits have a strong genetic component [18–21]. However, limited information is available for Asians. In the present study, the A alleles of rs1009312 and rs2015049, located in *ITGB3*, were positively associated with the MPV ($P = 0.029$). The MPV had a trend in rs7208170 alleles but did not reach a significant level ($P = 0.051$). The difference between PLT and SNPs in *ITGA2B* or *ITGB3* was not significant.

The APTT and PT are clinical tests commonly used to indicate coagulation factor deficiencies [22, 23], activated coagulation, and risk of venous thromboembolism [24, 25].

To date, two genome-wide association studies (GWAS) of APTT and PT conducted in the European ancestry have been reported. One study identified genome-wide significant associations of APTT and variants at F12 (MIM 610619), KNG1 (MIM 612358), and HRG (MIM 142640) [26]. In the other research, the GWAS for APTT and PT was conducted and replicated genome-wide significant associations at KNG1, HRG, F11, F12, and ABO for APTT and identified significant associations at the F7 and PROCRA/EDEM2 regions for PT. Eight genetic loci accounted for ~29% of the variance in APTT, and two loci accounted for ~14% of the variance in PT [27]. In this study, the association of APTT, PT, FIB, and TT with *ITGA2B* and *ITGB3* SNPs was investigated. The A allele of *ITGB3* rs70940817 was found to significantly correlate with the elevated APTT and PT ($P = 0.003$ and $P = 0.002$, resp.). The T allele of *ITGB3* rs112188890 was related with APTT ($P = 0.029$), and the G allele of *ITGB3* rs4642 was associated with TT and FIB ($P = 0.015$ and $P = 0.029$, resp.).

The ADP-induced platelet aggregation test was often used to identify the platelet function and the efficacy of antiplatelet drugs. Jones et al. detected 1327 SNPs and investigated their correlation with ADP or collagen-related peptide-induced platelet aggregation in 500 healthy northern European subjects. This identified 17 novel associations with the platelet function ($P < 0.005$) accounting for approximately 46% of the variation in response [28]. In this study, the minor allele of rs56197296 and rs5919 was found to be associated with the decreased ADP-induced platelet aggregation ($P = 0.014$ and $P = 0.015$, resp.).

GPIIb/IIIa is the central receptor of platelet aggregation. The variations of GPIIb/IIIa amount expressed on a platelet surface might affect the platelet-aggregating activity. Many studies reported that the rs5918 correlated with the GPIIb/IIIa receptor expression [29]; however, many conflict studies have also been published [3, 30]. O'Halloran et al. investigated whether three polymorphisms of the GPIIIa promoter (−468G/A, −425A/C, and −400C/A) influenced the RNA expression and receptor density in the platelets of patients with cardiovascular disease [3]. They found a threefold variation between the subjects in the number of GPIIb/IIIa receptors expressed per platelet, although no association between the receptor density and the PIA2 or the three promoter polymorphisms was demonstrated. In the present study, rs55827077, which was located at the promoter region of the GPIIIa gene at position −145, was related with GPIIb/IIIa per platelet.

The bleeding time is a test that evaluates the platelet function *in vivo*. A prolonged bleeding time can result from platelet abnormality, Von Willebrand factor deficiency, or vascular disorders such as Ehlers–Danlos syndrome. A disruption of platelet aggregation results in a prolongation of the bleeding time [31]. In this study, the bleeding time was related with rs3760364 of *ITGA2B* and rs7208170, rs567581451, and rs117052258 of *ITGB3* ($P < 0.05$). No previous study reporting on the effect of SNPs on the bleeding time was found. The mechanism of the relationship needs to be further examined. However, a negative trend between the bleeding time and MPV ($r = -0.244, P = 0.073$) was observed. Moreover, rs1009312 and rs2015049 were found to significantly associate

TABLE 5: Association between individual *ITGA2B* and *ITGB3* SNPs and the coagulation indexes (mean \pm SD).

| | Genotypes | N | PT (s) | APTT (s) | TT (s) | FIB (g/L) |
|--------------------------|-----------|----|-----------------|-----------------|-----------------|-----------------|
| rs112188890 ^a | CC | 42 | 10.9 \pm 1.08 | 31.2 \pm 2.13 | 15.7 \pm 2.22 | 2.52 \pm 0.45 |
| | CT | 12 | 11.2 \pm 1.35 | 32.2 \pm 2.73 | 14.7 \pm 1.59 | 2.72 \pm 0.44 |
| | TT | 1 | 12.5 | 36.7 | 16.5 | 2.72 |
| <i>P</i> | | | 0.126 | 0.029 | 0.299 | 0.173 |
| rs4642 ^b | AA | 27 | 10.9 \pm 1.20 | 30.9 \pm 2.25 | 16.1 \pm 2.36 | 2.43 \pm 0.42 |
| | AG | 21 | 11.3 \pm 1.08 | 32.0 \pm 2.34 | 15.2 \pm 1.71 | 2.68 \pm 0.44 |
| | GG | 7 | 10.4 \pm 1.01 | 32.3 \pm 2.79 | 14.2 \pm 1.39 | 2.76 \pm 0.45 |
| <i>P</i> | | | 0.770 | 0.088 | 0.015 | 0.029 |
| rs70940817 | GG | 45 | 10.7 \pm 1.08 | 31.1 \pm 2.19 | 15.2 \pm 2.01 | 2.52 \pm 0.42 |
| | GA | 9 | 11.8 \pm 1.02 | 32.8 \pm 2.26 | 16.8 \pm 2.34 | 2.75 \pm 0.54 |
| | AA | 1 | 12.7 | 37.2 | 15.75 | 3.10 |
| <i>P</i> | | | 0.002 | 0.003 | 0.078 | 0.069 |

MPV, mean platelet volume; PLT, platelet count; PT, prothrombin time; APTT, activated partial thromboplastin time; FIB, fibrinogen; TT, thrombin time. Note: Pearson's two-tailed test was used to analyze correlation between genotype and parameter.

^aStrong LD was observed between rs112188890 and rs117414137 ($r^2 = 0.99$); ^bcompletely LD was observed between rs4642 and rs4634.

TABLE 6: Comparison of *ITGA2B* and *ITGB3* allele frequencies in different ethnic groups.

| dbSNP Number | | MA(F) ^a | | | |
|-----------------|-------------|--------------------|--------------------|--------------------------------|----------------------|
| | | This study Chinese | Asian ^b | European-ancestry ^c | African ^d |
| ITGA2B-01 | rs3760364 | T(0.052) | T(0.012) | T(0) | T(0) |
| ITGA2B-08 | rs850730 | G(0.477) | G(0.467) | G(0.297) | G(0.437) |
| ITGA2B-11 | rs5911 | G(0.477) | G(0.467) | G(0.305) | G(0) |
| ITGA2B-12 | rs5910 | T(0.465) | T(0.442) | T(0.376) | T(0.432) |
| ITGB3-03 | rs7208170 | A(0.419) | A(0.496) | A(0.194) | A(0.431) |
| ITGB3-05 | rs7208055 | A(0.221) | A(0.225) | A(0.117) | A(0.356) |
| ITGB3-06 | rs55827077 | C(0.448) | C(0.417) | C(0.158) | C(0.39) |
| ITGB3-08 | rs117052258 | C(0.227) | C(0.208) | NA | NA |
| ITGB3-10 | rs11871251 | A(0.453) | A(0.482) | A(0.199) | A(0.373) |
| ITGB3-12 | rs12188890 | T(0.116) | T(0) | T(0.023) | T(0) |
| ITGB3-16 | rs1009312 | G(0.417) | A(0.434) | A(0.125) | A(0.432) |
| ITGB3-17 | rs2015049 | G(0.471) | A(0.478) | A(0.138) | A(0.413) |
| NF | rs5918 | C(0) | C(0.007) | C(0.137) | C(0.128) |
| ITGB3-18 | rs56197296 | delTTTGT(0.169) | NA | NA | NA |
| ITGB3-19 | rs5919 | C(0.262) | C(0.232) | C(0.049) | C(0.187) |
| ITGB3-23 | rs4642 | G(0.331) | G(0.31) | G(0.283) | G(0.306) |
| ITGB3-25 | rs4634 | A(0.331) | A(0.350) | A(0.283) | A(0.331) |
| ITGB3-29 | rs70940817 | A(0.076) | NA | NA | NA |

dbSNP, single nucleotide polymorphism database; MA(F), minor allele (frequency); SNP, single nucleotide polymorphisms; NF, not found; NA, not available.

^aResource of MP(F)s was from GenBank and HapMap, <https://www.ncbi.nlm.nih.gov/snp/>; ^bHan Chinese in Beijing, China (CHB), or CHB + Japanese in Tokyo, Japan (JPT), if there is no CHB data in the SNP; ^cUtah residents with Northern and Western European ancestry from the CEPH collection (CEU);

^dYoruba in Ibadan, Nigeria (YRI).

with both bleeding time and MPV. Further, the minor alleles of rs7208170 and rs567581451, which were significantly related with the bleeding time, had a trend with the MPV among genotypes, although they did not reach a significant level ($P = 0.051$ and $P = 0.054$, resp.). However, no relationship was observed among SNPs, bleeding time, and MPV in rs3760364 and rs117052258.

In this study, a positive correlation of the level of ADP-induced aggregation and GPIIb/IIIa content was detected in healthy volunteers. This correlation is consistent with a

previous report. Yakushkin et al. investigated the relationship between the number of GPIIb/IIIa and the level of ADP-induced aggregation in a group of 35 healthy volunteers and found positive and significant correlations between the level of platelet aggregation induced by different ADP doses (from 1.25 to 20 mmol/L) and the number of GPIIb/IIIa [32].

A strong positive correlation was observed between GPIIb/IIIa per platelet and PLT, PT, and TT ($P < 0.01$). Although the TT was strongly correlated with GPIIb/IIIa per platelet ($r = 0.545$, $P < 0.001$), the relationship of SNPs/TT

and SNPs/GP (IIb/IIIa) content is not linked. For example, *ITGB3* rs4642 was significantly related with the TT ($P = 0.015$), while the value of GPIIb/IIIa per platelet had no trend among rs4642 genotypes ($P = 0.789$). The other coagulation indexes were the same. Therefore, the relationship between SNPs and GPIIb/IIIa content cannot be deduced from the correlation of coagulation indexes and SNPs.

Some of the significant correlations between SNPs and platelet function parameters in the present study were not reported previously, which may be due to the reason that SNPs with low MA(F) in the European ancestry were not selected as candidate SNPs in previous studies. For example, the GWAS-genotyping platforms lack the coverage of low frequency/rare variants [33]. Moreover, LD might be another reason, if different genetic patterns (haplotypes) are present in different populations [3].

A possible limitation of the current study is the limited number of subjects. After stratification, the number of subjects in each genotypic group was small, thereby limiting further haplotype association analysis and accession of small effects of an SNP with a small minor allele frequency.

5. Conclusion

In summary, as ethnicity difference might limit the interpretation of the function of SNPs, resequencing the *ITGA2B* and *ITGB3* genes and investigating their functions in the Chinese population are very important. In the present study, nine SNPs were found to associate with indexes of platelet and coagulation haemostasis. Newer studies are needed, particularly, to further assess the clinical importance of the above-discussed SNPs in disease susceptibility and antiplatelet drugs pharmacodynamics. Further studies should pay more attention to the roles of *ITGA2B* and *ITGB3* SNPs in ethnic variations.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This study was supported by grants from the National Natural Science Foundation of China (no. 81273592, no. 81202592, and no. 81373487).

References

- [1] J. J. Calvete, "Clues for understanding the structure and function of a prototypic human integrin: the platelet glycoprotein IIb/IIIa complex," *Thrombosis and Haemostasis*, vol. 72, no. 1, pp. 1–15, 1994.
- [2] C. L. Wagner, M. A. Mascelli, D. S. Neblock, H. F. Weisman, B. S. Coller, and R. E. Jordan, "Analysis of GPIIb/IIIa receptor number by quantification of 7E3 binding to human platelets," *Blood*, vol. 88, no. 3, pp. 907–914, 1996.
- [3] A. M. O'Halloran, R. Curtin, F. O'Connor et al., "The impact of genetic variation in the region of the GPIIIa gene, on PI expression bias and GPIIb/IIIa receptor density in platelets," *British Journal of Haematology*, vol. 132, no. 4, pp. 494–502, 2006.
- [4] S. Bellucci and J. Caen, "Molecular basis of Glanzmann's Thrombasthenia and current strategies in treatment," *Blood Reviews*, vol. 16, no. 3, pp. 193–202, 2002.
- [5] M. Fiore, A. T. Nurden, P. Nurden, and U. Seligsohn, "Clinical utility gene card for: Glanzmann thrombasthenia," *European Journal of Human Genetics*, vol. 20, no. 10, p. 1101, 2012.
- [6] C. N. Floyd, B. H. Ellis, and A. Ferro, "The PLA1/A2 polymorphism of glycoprotein IIIa as a risk factor for stroke: a systematic review and meta-analysis," *PLoS ONE*, vol. 9, no. 7, Article ID e100239, 2014.
- [7] C. N. Floyd and A. Ferro, "The PLA1/A2 polymorphism of glycoprotein IIIa in relation to efficacy of antiplatelet drugs: a systematic review and meta-analysis," *British Journal of Clinical Pharmacology*, vol. 77, no. 3, pp. 446–457, 2014.
- [8] C. N. Floyd, A. Mustafa, and A. Ferro, "The PLA1/A2 polymorphism of glycoprotein IIIa as a risk factor for myocardial infarction: a meta-analysis," *PLoS ONE*, vol. 9, no. 7, article e101518, 2014.
- [9] P. J. Newman, R. S. Derbes, and R. H. Aster, "The human platelet alloantigens, PLA1 and PLA2, are associated with a leucine33/proline33 amino acid polymorphism in membrane glycoprotein IIIa, and are distinguishable by DNA typing," *Journal of Clinical Investigation*, vol. 83, no. 5, pp. 1778–1781, 1989.
- [10] J.-H. Wu, D.-W. Zhang, X.-L. Cheng, H. Shi, and Y.-P. Fan, "Platelet glycoprotein IIb HPA-3 a/b polymorphism is associated with native arteriovenous fistula thrombosis in chronic hemodialysis patients," *Renal Failure*, vol. 34, no. 8, pp. 960–963, 2012.
- [11] M.-P. Li, Y. Xiong, A. Xu et al., "Association of platelet *ITGA2B* and *ITGB3* polymorphisms with ex vivo antiplatelet effect of ticagrelor in healthy Chinese male subjects," *International Journal of Hematology*, vol. 99, no. 3, pp. 263–271, 2014.
- [12] Y. Zhang, Y. Han, L. Dong et al., "Genetic variation of *ITGB3* is associated with asthma in Chinese Han children," *PLoS ONE*, vol. 8, no. 2, article e56914, 2013.
- [13] S. Simsek, N. M. Faber, P. M. Bleeker et al., "Determination of human platelet antigen frequencies in the Dutch population by immunophenotyping and DNA (allele-specific restriction enzyme) analysis," *Blood*, vol. 81, no. 3, pp. 835–840, 1993.
- [14] J. Lim, S. Lal, K. C. Ng, K.-S. Ng, N. Saha, and C.-K. Heng, "Variation of the platelet glycoprotein IIIa PIA1/A2 allele frequencies in the three ethnic groups of Singapore," *International Journal of Cardiology*, vol. 90, no. 2–3, pp. 269–273, 2003.
- [15] Z. Yiming, J. Shundong, D. Ningzheng, H. Yang, and R. Changgeng, "Measurement of affinity constant of humanized monoclonal antibody 813-F(ab)2 binding to platelets of human macaque and dog," *Suzhou University Journal of Medical Science*, vol. 27, no. 1, pp. 8–10, 2007.
- [16] P. M. W. Bath and R. J. Butterworth, "Platelet size: measurement, physiology and vascular disease," *Blood Coagulation and Fibrinolysis*, vol. 7, no. 2, pp. 157–161, 1996.
- [17] T. J. Kunicki, S. A. Williams, D. J. Nugent, and M. Yeager, "Mean platelet volume and integrin alleles correlate with levels of integrins α IIb β 3 and α 2 β 1 in acute coronary syndrome patients and normal subjects," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 32, no. 1, pp. 147–152, 2012.
- [18] K. Shameer, J. C. Denny, K. Ding et al., "A genome- and phenome-wide association study to identify genetic variants

- influencing platelet count and volume and their pleiotropic effects," *Human Genetics*, vol. 133, no. 1, pp. 95–109, 2014.
- [19] R. Qayyum, B. M. Snively, E. Ziv et al., "A meta-analysis and genome-wide association study of platelet count and mean platelet volume in African Americans," *PLoS Genetics*, vol. 8, no. 3, Article ID e1002491, 2012.
- [20] N. Soranzo, A. Rendon, C. Gieger et al., "A novel variant on chromosome 7q22.3 associated with mean platelet volume, counts, and function," *Blood*, vol. 113, no. 16, pp. 3831–3837, 2009.
- [21] C. Meisinger, H. Prokisch, C. Gieger et al., "A genome-wide association study identifies three loci associated with mean platelet volume," *American Journal of Human Genetics*, vol. 84, no. 1, pp. 66–71, 2009.
- [22] G. C. White II, "The partial thromboplastin time: defining an era in coagulation," *Journal of Thrombosis and Haemostasis*, vol. 1, no. 11, pp. 2267–2270, 2003.
- [23] S. Kitchen, A. McCraw, and M. Echenagucia, *Diagnosis of Hemophilia and Other Bleeding Disorders: A Laboratory Manual*, World Federation of Hemophilia (WFH), Montreal, Canada, 2nd edition, 2010.
- [24] G. Hron, S. Eichinger, A. Weltermann, P. Quehenberger, W. M. Halbmayer, and P. A. Kyrle, "Prediction of recurrent venous thromboembolism by the activated partial thromboplastin time," *Journal of Thrombosis and Haemostasis*, vol. 4, no. 4, pp. 752–756, 2006.
- [25] N. A. Zakai, T. Ohira, R. White, A. R. Folsom, and M. Cushman, "Activated partial thromboplastin time and risk of future venous thromboembolism," *American Journal of Medicine*, vol. 121, no. 3, pp. 231–238, 2008.
- [26] L. M. Houlihan, G. Davies, A. Tenesa et al., "Common variants of large effect in F12, KNG1, and HRG are associated with activated partial thromboplastin time," *The American Journal of Human Genetics*, vol. 86, no. 4, pp. 626–631, 2010.
- [27] W. Tang, C. Schwienbacher, L. M. Lopez et al., "Genetic associations for activated partial thromboplastin time and prothrombin time, their gene expression profiles, and risk of coronary artery disease," *American Journal of Human Genetics*, vol. 91, no. 1, pp. 152–162, 2012.
- [28] C. I. Jones, S. Bray, S. F. Garner et al., "A functional genomics approach reveals novel quantitative trait loci associated with platelet signaling pathways," *Blood*, vol. 114, no. 7, pp. 1405–1416, 2009.
- [29] J. S. Bennett, F. Catella-Lawson, A. R. Rut et al., "Effect of the P1A2 alloantigen on the function of β 3-integrins in platelets," *Blood*, vol. 97, no. 10, pp. 3093–3099, 2001.
- [30] O. V. Sirotkina, S. G. Khaspekova, A. M. Zabortina, Y. V. Shimanova, and A. V. Mazurov, "Effects of platelet glycoprotein IIb-IIIa number and glycoprotein IIIa Leu33Pro polymorphism on platelet aggregation and sensitivity to glycoprotein IIb-IIIa antagonists," *Platelets*, vol. 18, no. 7, pp. 506–514, 2007.
- [31] A. Fuentes, A. Rojas, K. B. Porter, G. Saviello, and W. F. O'Brien, "The effect of magnesium sulfate on bleeding time in pregnancy," *American Journal of Obstetrics and Gynecology*, vol. 173, no. 4, pp. 1246–1249, 1995.
- [32] V. V. Yakushkin, I. T. Zyuryaev, S. G. Khaspekova, O. V. Sirotkina, M. Y. Ruda, and A. V. Mazurov, "Glycoprotein IIb-IIIa content and platelet aggregation in healthy volunteers and patients with acute coronary syndrome," *Platelets*, vol. 22, no. 4, pp. 243–251, 2011.
- [33] B. J. Grady and M. D. Ritchie, "statistical optimization of pharmacogenomics association studies: key considerations from study design to analysis," *Current Pharmacogenomics and Personalized Medicine*, vol. 9, no. 1, pp. 41–66, 2011.