Preliminary Study of Genome-Wide Association Identified Novel Susceptibility Genes for Hemorheological Indexes in a Chinese Population

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**Abstract**

**Background:** Genome-wide association studies for various hemorheological characteristics have not been reported. We aimed to identify genetic loci associated with hemorheological indexes in a cohort of healthy Chinese Han individuals.

**Methods:** Genotyping was performed using Applied Biosystems Axiom™ Precision Medicine Diversity Array in 838 individuals, and 6,423,076 single nucleotide polymorphisms were available for genotyping. The relations were examined in an additive genetic model using mixed linear regression and combined with identical by descent matrix.

**Results:** We identified 38 genetic loci ($p < 5 \times 10^{-6}$) related to hemorheological traits. In which, \textit{LOC102724502-OLIG2} rs28371438 was related to the levels of nd30 ($p = 8.58 \times 10^{-07}$), nd300 ($p = 1.89 \times 10^{-06}$), erythrocyte rigidity ($p = 1.29 \times 10^{-06}$), assigned viscosity ($p = 6.20 \times 10^{-08}$) and whole blood high cut relative ($p = 7.30 \times 10^{-08}$). The association of \textit{STK32B} rs4689231 for nd30 ($p = 3.85 \times 10^{-06}$) and nd300 ($p = 2.94 \times 10^{-06}$) and \textit{GTSCR1}-\textit{LINC01541} rs11661911 for erythrocyte rigidity ($p = 9.93 \times 10^{-09}$) and whole blood high cut relative ($p = 2.09 \times 10^{-07}$) was found. \textit{USP25-MIR99AHG} rs1297329 was associated with erythrocyte rigidity ($p = 1.81 \times 10^{-06}$) and erythrocyte deformation ($p = 1.14 \times 10^{-06}$). Moreover, the association of \textit{TMEM232-SLC25A46} rs3985087 and \textit{LINC00470-METTL4} rs9966987 for fibrinogen ($p = 1.31 \times 10^{-06}$ and $p = 4.29 \times 10^{-07}$) and plasma viscosity ($p = 1.01 \times 10^{-06}$ and $p = 4.59 \times 10^{-07}$) was found.

**Conclusion:** These findings may represent biological candidates for hemorheological indexes and contribute to hemorheological study.

**Introduction**

Most metabolic diseases are always accompanied by disorders of blood rheology, such as increased blood and plasma viscosity, decreased red blood cell deformability, and increased cell aggregation [1, 2]. Hemorheology, the study of deformation and blood flow, has more focused on the deformation and aggregation of erythrocytes since erythrocytes are the main components in blood [3–5]. Blood viscosity and erythrocytes deformability are the main factors for maintaining and regulating microcirculation. Blood and plasma viscosity are risk factors for atherosclerosis; and erythrocytes rheological changes, such as erythrocytes rigidity, have been observed in patients with hypertension, diabetes mellitus, and obesity [6–8]. Caprari et al. [1] reported that the hemorheological characteristics of SCA subjects showed high blood viscosity,
increased erythrocytes aggregation, and decreased erythrocytes deformability. Hemorheological variations of the erythrocyte behavior and blood plasma can help in the clinical diagnosis [9].

Recently, the identification of various susceptibility loci and genes related to hematological traits in diverse ethnic groups may provide important insights into the hematology [10]. Christiansen et al. [11] have demonstrated that ABO locus was related to the increased platelet aggregation in patients with stable coronary artery disease. Krause et al. [12] reported that rs17114036, a common noncoding polymorphism at 1p32.2, is located in the endothelial enhancer dynamically regulated by hemodynamics. Seiki et al. [13] displayed the association of ABO, PDGFRα-KIT, USP49-MED20-BSYL-CCHN3, C6orf182-CD164, TERT, and TMPRSS6 variants with erythrocyte traits in Japanese population. Qayyum et al. [14] demonstrated that six single nucleotide polymorphisms (SNPs) were associated with platelet aggregation (p < 5 × 10⁻⁸). So far, a number of loci associated with quantitative hematological traits have been discovered [15], genome-wide association studies (GWASs) for various hemorheological characteristics have not been reported. Therefore, it is necessary to determine the genetic variation related to hemorheological traits. Here, we performed a genome-wide association study to identify imputed genetic loci associated with hemorheological indexes in a cohort of healthy Chinese Han individuals by the Applied Biosystems Axiom® Precision Medicine Diversity Array Chip.

**Table 1. Characteristics of samples used in the GWAS**

| Characteristics                  | N/median (IQR) |
|----------------------------------|----------------|
| n                                | 838            |
| Age, years                       | 44.00 (36.00–50.00) |
| Female, n (%)                    | 413 (49.3)     |
| BMI, kg/m²                       | 23.53 (21.22–25.95) |
| nd1, mPas                        | 20.51 (18.38–22.91) |
| nd30, mPas                       | 5.43 (4.86–6.08)  |
| nd300, mPas                      | 4.14 (3.69–4.60)  |
| Relative index of whole blood hyposectomy | 14.85 (13.13–16.48) |
| Erythrocyte aggregation index    | 4.92 (4.60–5.29)  |
| Erythrocyte rigidity index       | 4.59 (4.02–5.23)  |
| Erythrocyte deformation index    | 0.82 (0.77–0.87)   |
| Assigned viscosity, mPas         | 3.39 (3.03–3.78)   |
| Fibrinogen, g/L                  | 3.09 (2.97–3.15)   |
| Whole blood high cut reduction viscosity | 6.31 (5.56–7.22)   |
| Whole blood high cut relative index | 2.98 (2.67–3.33)   |
| Whole blood low cut reduction viscosity | 44.87 (41.02–48.39) |
| Plasma viscosity, mPas           | 1.41 (1.36–1.43)   |
| Hematocrit, L/L                  | 0.42 (0.40–0.46)   |

GWAS, genome-wide association study; IQR, interquartile range; BMI, body mass index.

**Material and Method**

**Study Cohorts**
A total of 838 participants (413 women and 425 men) visited the Health Care Center of Henan Provincial People’s Hospital for annual checkup. All individuals were healthy Chinese Han population. Subjects with tumors, known disease, or pregnant women were excluded. To evaluate genetic association, 14 hemorheological indexes were examined (Table 1), including nd1 (mPas), nd30 (mPas), nd300 (mPas), relative index of whole blood hyposectomy, erythrocyte aggregation index, erythrocyte rigidity index, erythrocyte deformation index, assigned viscosity (mPas), fibrinogen (g/L), whole blood high cut reduction viscosity, whole blood high cut relative index, whole blood low cut reduction viscosity, plasma viscosity (mPas), and hematocrit (L/L). Demographic and hemorheology data were obtained from questionnaires or medical records. Written informed consent was obtained from all of the participating cohort. The protocols were approved by the Institutional Research Ethics Committee of Henan Provincial People’s Hospital and complied with the Declaration of Helsinki.

**GWAS Genotyping and Genotype Imputation**
A peripheral blood sample (5 mL) of each participant was collected in EDTA-coated tubes, and genomic DNA was purified using the GoldMag DNA Isolation Kit (GoldMag Co., Ltd., Xi’an, China). A total of 796,288 loci were available for GWAS analysis based on the following: (1) sample calling rate >0.95, marker calling rate >0.90, and Hardy-Weinberg equilibrium >5 × 10⁻⁶ for quality control and (2) removing indels, copy number variation, duplication, and loci from sex chromosome. Genome-wide genotyping of the subjects was carried out using the Applied Biosystems Axiom® Precision Medicine Diversity Array on the GeneTitan™ Multi-Channel Instrument (Thermo Fisher, CA, USA). Genotype clustering was conducted using Axiom Analysis Suite 6.0 software. Genome-wide data were imputed from the third phase of 1,000 genomes haplotype reference panels through IMPUTE2 software, and loci with minor allele frequency <1%, the correlation coefficient (r²) linkage disequilibrium <0.5, and non-biallelic were deleted. Taking into account the uncertainty of imputation, the association analysis was performed by Gold Helix SNP & Variation Suite 8.7 software. After quality control and imputation, 6,423,076 loci were included for final analysis.

**Data Analysis**
Continuous variables were evaluated for normality using the Kolmogorov-Smirnov test. Continuous variables with non-normal distribution as median with interquartile range were compared using the Mann-Whitney U test. Relations of SNPs to hemodynamic phenotypes were examined in the additive genetic model using mixed linear regression adjusting with age and gender by the PLINK software and combined with identical by descent matrix. The levels of hemodynamic indexes were normalized using rank-based inverse normal transformations. Manhattan plots and quantile-quantile plots were conducted by −log10 (p value) using R-package version 3.32. Locus regional plots were constructed by LocusZoom 1.1 software. The values of p < 5 × 10⁻⁸ means that the genetic polymorphism is genome-wide significantly associated with hemorheological indexes. The values of p < 5 × 10⁻⁸ suggests a suggestively significant genome-wide association with hemorheological indexes.

**Bioinformatics Analysis**
GWAS catalog (https://www.ebi.ac.uk/gwas/) and Clinvar (https://www.ncbi.nlm.nih.gov/clinvar/) were used to see if some of the SNPs were already associated with clinical phenotypes and

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if these relate somehow to the parameters. HaploReg v4.1 database (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) is a database used to predict the potential functions of selected SNPs.

### Results

The Manhattan plot (Fig. 1) displayed the chromosome location of significantly associated loci with hemodynamic indexes, including nd1 (1 loci), nd30 (2 loci), nd300 (3 loci), relative index of whole blood hyposectomy (5 loci), erythrocyte aggregation index (4 loci), erythrocyte rigidity index (3 loci), erythrocyte deformation index (3 loci), assigned viscosity (1 loci), fibrinogen (4 loci), whole blood high cut reduction viscosity (5 loci), whole blood high cut relative index (3 loci), whole blood low cut reduction viscosity (6 loci), plasma viscosity (6 loci), and hematocrit (1 loci). A quantile-quantile plot for hemodynamic index levels is shown in Figure 2, and the distribution of p values for the association tests showed no evidence of systematic bias.

Table 2 displays the details of 38 loci with p values <5 × 10^{-6} for the levels of hemodynamic indexes. Of the 63 identified loci, LOC730100 rs117580912 (p = 9.56 × 10^{-07}) was associated with the nd1 level (online suppl. Fig. 1; see www.karger.com/doi/10.1159/000524849 for all online suppl. material). The significant association with nd30 concentration was for rs4689231 in STK32B (p = 3.85 × 10^{-06}) and rs28371438 in LOC102724502-OLIG2 (p = 8.58 × 10^{-07}, online suppl. Fig. 2). Three SNPs (STK32B rs4689231: p = 2.94 × 10^{-06}, CCSR2-LINC01519 rs115646937: p = 3.90 × 10^{-06} and LOC102724502-OLIG2 rs28371438: p = 1.89 × 10^{-06}, respectively) were considered significant markers for nd300 level (online suppl. Fig. 3). Rs34469348 in LSAMP (p = 4.87 × 10^{-06}), rs9361295 in MEI4 (p = 2.44 × 10^{-06}), rs2410367 in TUSC3-MSR1 (p = 4.24 × 10^{-07}), rs10977588 in PTPRD (p = 2.88 × 10^{-06}), and rs745439 in TSHZ3-THEG5 (p = 3.14 × 10^{-06}) were associated with the level of the relative index of whole blood hyposectomy (online suppl. Fig. 4). The significant association with the concentration of erythrocyte aggregation index was for SFMBT2 rs11255044 (p = 4.83 × 10^{-06}).
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10^{−07}), LINC01493-LRRC4C rs67538811 (p = 5.21 \times 10^{−07}), NELL1-ANO5 rs12420881 (p = 2.21 \times 10^{−06}) and C12orf42 rs56670740 (p = 1.53 \times 10^{−06}, online suppl. Fig. 5). Three genome-level significant SNPs (p value of 9.93 \times 10^{−09} for rs11661911 in GTSCR1-LINC01541, 1.81 \times 10^{−06} for rs1297329 in USP25-MIR99AHG and 1.29 \times 10^{−06} for rs28371438 in LOC102724502-OLIG2) associated with erythrocyte rigidity index level were identified (online suppl. Fig. 6). We also found that TMEM232 rs2900050 (p = 3.72 \times 10^{−08}) and USP25-MIR99AHG rs1297329 (p = 1.14 \times 10^{−06}) were significant loci for the circulating level of erythrocyte deformation index (online suppl. Fig. 7). Rs28371438 in LOC102724502-OLIG2 (p = 6.20 \times 10^{−08}, online suppl. Fig. 8) was a significant marker related to the level of assigned viscosity. Four loci were associated with fibrinogen concentration, including rs78314456 in CNIH3 (p = 1.95 \times 10^{−06}), rs3985087 in TMEM232-SLC25A46 (p = 1.31 \times 10^{−06}), rs34247085 in LINC00917-FENDRR (p = 1.80 \times 10^{−06}), and rs9966987 in LINC00470-METTL4 (p = 4.29 \times 10^{−07}, online suppl. Fig. 9). For whole blood high cut reduction viscosity, the significant association was with rs144907988 in ADGRA3-GBA3 (p = 1.74 \times 10^{−06}), rs6827644 in C4orf22 (p = 3.18 \times 10^{−08}), rs1030490 in IRX1-LINC02114 (p = 2.53 \times 10^{−08}),
| Characteristics                          | SNP ID      | Chr | Position     | REF/ALT | SNP function | RefGene          | MAF | β    | SE   | p value   | GWAS catalog | HaploReg       |
|-----------------------------------------|-------------|-----|--------------|---------|--------------|------------------|-----|------|------|-----------|--------------|----------------|
| nd1                                     | rs117580912 | 2   | 51,343,364   | C/T     | ncRNA intronic | LOC730100        | 0.005 | 0.011 | 0.039 | 9.56E-07  | –            | Motifs changed |
| nd30                                    | rs4689231   | 4   | 5,437,107    | G/A     | Intronic     | STK32B           | 0.370 | 0.162 | 0.330 | 3.85E-06  | –            | Motifs changed |
|                                           | rs28371438  | 21  | 32,986,517   | C/T     | Intergenic   | LOC102724502;OLIG2 | 0.478 | 0.231 | 0.331 | 8.58E-07  | Height        | Motifs changed |
| nd300                                   | rs4689231   | 4   | 5,437,107    | G/A     | Intronic     | STK32B           | 0.370 | 0.200 | 1.107 | 2.94E-06  | –            | Motifs changed |
|                                           | rs115646937 | 10  | 84,813,648   | A/G     | Intergenic   | CCSER2;LINCO1519 | 0.092 | 0.031 | 1.107 | 3.90E-06  | –            | Motifs changed |
|                                           | rs28371438  | 21  | 32,986,517   | C/T     | Intergenic   | LOC102724502;OLIG2 | 0.478 | 0.193 | 1.107 | 1.89E-06  | Height        | Motifs changed |
| Relative index of whole blood           | rs34469348  | 3   | 115,815,531  | G/T     | Intronic     | LSAMP            | 0.094 | 0.359 | 0.140 | 4.87E-06  | –            | Motifs changed |
| hyposectomy                            | rs9361295   | 6   | 77,831,550   | C/A     | Intronic     | MEH              | 0.259 | 0.388 | 0.141 | 2.44E-06  | –            | Motifs changed |
|                                          | rs2410367   | 8   | 15,931,636   | T/C     | Intergenic   | TUSC3;MSR1       | 0.222 | 0.194 | 0.140 | 4.24E-07  | –            | Motifs changed |
|                                          | rs10977588  | 9   | 9,218,157    | C/T     | Intronic     | PPPRD            | 0.010 | 0.266 | 0.139 | 2.88E-06  | –            | Motifs changed |
|                                          | rs745439    | 19  | 31,537,088   | C/T     | Intergenic   | TSH23;THEG5      | 0.386 | 0.450 | 0.143 | 3.14E-06  | –            | Motifs changed |
| Erythrocyte aggregation index           | rs11255044  | 10  | 7,221,420    | G/C     | Intronic     | SFMBT2           | 0.033 | 0.063 | 1.433 | 4.83E-07  | –            | Motifs changed |
|                                          | rs67538811  | 11  | 39,184,039   | G/A     | Intergenic   | LINCO1493;LRC4C  | 0.140 | 0.030 | 1.436 | 5.21E-07  | –            | Motifs changed |
|                                          | rs12420881  | 21  | 21,664,423   | C/A     | Intergenic   | NELL1;ANO5       | 0.007 | 0.006 | 1.433 | 2.21E-06  | –            | Motifs changed |
|                                          | rs56670740  | 12  | 103,353,181  | T/A     | Intronic     | C12orf42         | 0.247 | 0.149 | 1.435 | 1.53E-06  | –            | Enhancer histone marks, motifs changed |
| Erythrocyte rigidity index              | rs11661911  | 18  | 70,983,826   | A/C     | Intergenic   | GTSCR1;LINCO1541 | 0.135 | 0.104 | 0.952 | 9.93E-09  | –            | Motifs changed |
|                                          | rs1297329   | 21  | 15,939,669   | G/A     | Intergenic   | USP25;MIR99AHG   | 0.013 | 0.018 | 0.957 | 1.81E-06  | –            | Motifs changed |
|                                          | rs28371438  | 21  | 32,986,517   | C/T     | Intergenic   | LOC102724502;OLIG2 | 0.478 | 0.220 | 0.958 | 1.29E-06  | Height        | Motifs changed |
| Erythrocyte deformation index           | rs2900050   | 5   | 110,721,671  | G/A     | Intronic     | TMEM232           | 0.008 | 0.092 | 0.998 | 3.72E-06  | –            | Motifs changed, selected eQTL hits |
|                                          | rs1297329   | 21  | 15,939,669   | G/A     | Intergenic   | USP25;MIR99AHG   | 0.013 | 0.057 | 0.996 | 1.14E-06  | –            | Motifs changed |
| Assigned viscosity                      | rs28371438  | 21  | 32,986,517   | C/T     | Intergenic   | LOC102724502;OLIG2 | 0.479 | 0.200 | 1.559 | 6.20E-08  | Height        | Motifs changed |
| Characteristics                        | SNP ID          | Chr | Position     | REF/ALT | SNP function | RefGene          | MAF   | β      | SE   | p value     | GWAS catalog                                                                 |
|---------------------------------------|-----------------|-----|--------------|---------|--------------|------------------|-------|--------|------|-------------|------------------------------------------------------------------------------|
| Fibrinogen                            | rs78314456      | 1   | 224,640,188 | C/T     | Intron       | CNIH3            | 0.008 | −0.024 | 0.407| 1.95E-06    | − Enhancer histone marks, DNase, motifs changed                             |
|                                       | rs3985087       | 5   | 110,736,955 | C/A     | Intergenic   | TMEM232;SLC25A46 | 0.025 | −0.052 | 0.407| 1.31E-06    | − Promoter histone marks, enhancer histone marks, DNase, proteins bound, motifs changed, selected eQTL hits |
|                                       | rs34247085      | 16  | 86,472,977  | G/A     | Intergenic   | LINC00917;FENDRR | 0.008 | 0.033  | 0.408| 1.80E-06    | − Enhancer histone marks, motifs changed                                   |
|                                       | rs9966987       | 18  | 1,918,849   | C/A     | Intergenic   | LINC00470;METTL4 | 0.330 | 0.153  | 0.408| 4.29E-07    | − Enhancer histone marks, motifs changed                                   |
| Whole blood high cut reduction viscosity | rs144907988     | 4   | 22,589,091  | C/T     | Intergenic   | ADGRA3;GBA3     | 0.002 | −0.229 | 0.150| 1.74E-06    | −                                                                            |
|                                       | rs6827644       | 4   | 80,874,656  | C/T     | Intron       | C4orf22          | 0.066 | −0.311 | 0.151| 3.18E-06    | − Motifs changed                                                             |
|                                       | rs1030490       | 5   | 4,574,832   | T/G     | Intergenic   | IRX1;LINC02114  | 0.401 | −0.037 | 0.156| 2.53E-06    | − Motifs changed                                                             |
|                                       | rs11911466      | 21  | 32,983,986  | T/C     | Intergenic   | LOC102724502;OLIG2 | 0.416 | −0.397 | 0.153| 7.37E-07    | − Enhancer histone marks, motifs changed                                   |
|                                       | rs6519816       | 22  | 43,905,383  | C/T     | Intergenic   | PNPLA5;PNPLA3   | 0.155 | −0.165 | 0.151| 3.37E-06    | − Enhancer histone marks, motifs changed                                   |
| Whole blood high cut relative index   | rs11990599      | 8   | 126,350,838 | T/C     | Intergenic   | LOC101927657;FAM84B | 0.120 | −0.091 | 0.042| 3.11E-06    | − Motifs changed                                                             |
|                                       | rs11661911      | 18  | 70,983,826  | A/C     | Intergenic   | GTSCR1;LINC0154I | 0.134 | −0.105 | 0.043| 2.09E-07    | − Motifs changed                                                             |
|                                       | rs28371438      | 21  | 32,986,517  | C/T     | Intergenic   | LOC102724502;OLIG2 | 0.479 | −0.257 | 0.060| 7.30E-08    | − Motifs changed                                                             |
| Whole blood low cut reduction viscosity | rs2842173       | 1   | 43,493,328  | T/C     | Intergenic   | SZT2;PTPRF      | 0.217 | −0.280 | 0.159| 4.56E-07    | − Enhancer histone marks, selected eQTL hits                               |
|                                       | rs74525620      | 3   | 67,290,451  | C/A     | Intergenic   | MIR4272;SUCLG2  | 0.003 | −0.155 | 0.157| 4.41E-06    | − Promoter histone marks, enhancer histone marks, motifs changed, selected eQTL hits |
|                                       | rs73872706      | 3   | 151,645,475 | G/C     | Intergenic   | MIRS186;AADACL2 | 0.138 | −0.056 | 0.157| 1.90E-06    | − Enhancer histone marks, motifs changed                                   |
|                                       | rs246509        | 5   | 52,968,323  | A/C     | Intergenic   | ITGA1;ITGA2     | 0.219 | −0.242 | 0.159| 2.05E-06    | − Motifs changed                                                             |
|                                       | rs117016320     | 6   | 81,831,622  | T/A     | Intergenic   | LINC01526;IBTK  | 0.012 | −0.142 | 0.157| 2.09E-06    | − Promoter histone marks, enhancer histone marks, DNase, proteins bound, motifs changed |
|                                       | rs10977641      | 9   | 9,291,862   | A/G     | Intron       | PTPRD           | 0.009 | −0.140 | 0.157| 3.02E-06    | − Motifs changed                                                             |
| Characteristics       | SNP ID   | Chr  | Position     | REF/ALT | SNP function | RefGene                  | MAF       | β         | SE    | p value       | GWAS catalog | HaploReg                        |
|-----------------------|----------|------|--------------|---------|--------------|--------------------------|-----------|-----------|-------|---------------|--------------|--------------------------------------------------------------------|
| Plasma viscosity      | rs3985087| 5    | 110,736,955  | C/A     | Intergenic   | TMEM232;SLC25A46         | 0.025     | -0.048    | 0.917 | 1.01E−06      |              | Promoter histone marks, enhancer histone marks, DNAse, proteins bound, motifs changed, selected eQTL hits |
|                       | rs118096558| 5    | 180,197,694  | G/A     | Intronic     | RASGEF1C                 | 0.009     | -0.016    | 0.917 | 1.06E−06      |              | Enhancer histone marks, motifs changed                             |
|                       | rs11794922| 9    | 18,789,607   | T/G     | Intronic     | ADAMTSL1                 | 0.002     | -0.029    | 0.917 | 4.96E−06      |              | Enhancer histone marks, motifs changed                             |
|                       | rs76126204| 12   | 64,983,618   | C/T     | ncRNA intronic| LINC02231                | 0.030     | 0.076     | 0.919 | 8.66E−07      |              | Motifs changed                                                    |
|                       | rs9966987 | 18   | 1,918,849    | C/A     | Intergenic   | LINC00470;METTL4         | 0.269     | 0.093     | 0.917 | 4.59E−07      |              | Enhancer histone marks, motifs changed                             |
|                       | rs140700208| 6    | 139,497,581  | T/C     | Intergenic   | LINC01625;LOC100132735   | 0.331     | 0.160     | 0.917 | 1.06E−06      |              | Promoter histone marks, enhancer histone marks, DNAse, proteins bound, motifs changed |
| Hematocrit            | rs78577937| 4    | 92,146,844   | A/C     | Intergenic   | CCSER1;LINCRESS2         | 0.168     | -0.509    | 0.135 | 1.43E−06      |              |                                                                      |

GWAS, genome-wide association study; SNP, single nucleotide polymorphism; REF/ALT, reference/alternates; MAF, minor allele frequency.
rs11911466 in LOC102724502-OLIG2 (p = 7.37 × 10^{-6}), and rs6519816 in PNPLA5-PNPLA3 (p = 3.37 × 10^{-06}, online suppl. Fig. 10). Rs11990599 in LOC101927657-FAM84B (p = 3.11 × 10^{-06}), rs11661911 in GTSCR1-LINC01541 (p = 2.09 × 10^{-06}), and rs28371438 in LOC102724502-OLIG2 (p = 7.30 × 10^{-08}, online suppl. Fig. 11) were related to the level of whole blood high cut relative index. Six SNPs also achieved significant association with the level of whole blood low cut reduction viscosity, with p values of 4.56 × 10^{-07}, 4.41 × 10^{-06}, 1.90 × 10^{-06}, 2.05 × 10^{-06}, 2.09 × 10^{-06}, and 3.02 × 10^{-06} for SRT2-PTPRF rs2842173, MIR4227-SUCLG2 rs74525620, MIR5186-AADACL2 rs73872706, ITGA1-ITGA2 rs246509, LINC01526-IBTK rs117016320, and PTPRD rs10977641, respectively (online suppl. Fig. 12). Rs3985087 in TMEM232-SLC25A46 (p = 1.01 × 10^{-06}), rs118096558 in RASGEF1C (p = 1.06 × 10^{-06}), rs11794922 in ADAMTS1L (p = 4.96 × 10^{-06}), rs76126204 in LINC02231 (p = 8.66 × 10^{-06}), and rs9966987 in LINC00470-METTL4 (p = 4.59 × 10^{-06}), and rs140700208 in LINC01625-LINC00132735 (p = 1.06 × 10^{-06}) showed suggestive associations with the level of plasma viscosity (online suppl. Fig. 13). Moreover, the significant association of hematocrit was with rs78577937 in the intergenic region of the CCSE1-LNC-PRESS2 gene (p = 1.43 × 10^{-06}, online suppl. Fig. 14).

Based on GWAScat database (Table 2), we found that rs28371438 was associated with height trait. The results of HaploReg v4.1 displayed that these SNPs were associated with the regulation of promoter and/or enhancer histones, DNase, changed motifs, and selected eQTL hits.

### Discussion

Hemorheology (also named blood rheology) is the study of the flow characteristics of blood and its elements. Hemorheology indicators, such as whole blood viscosity, plasma viscosity, erythrocyte aggregation, erythrocyte rigidity, erythrocyte deformation, fibrinogen, and hematocrit, play fundamental roles in maintaining microcirculation [16, 17]. In our study of 14 hemorheological traits, a total of 38 SNPs were significantly related to hemorheological traits (p < 5 × 10^{-8}). In which, six SNPs, including rs28371438 for nd30, nd300, erythrocyte rigidity index, assigned viscosity and whole blood high cut relative index; rs4689231 of STK32B for nd30 and nd300; rs11661911 for erythrocyte rigidity index and whole blood high cut relative index; rs1297329 for erythrocyte rigidity index and erythrocyte deformation index; rs3985087 and rs9966987 for fibrinogen and plasma viscosity, were identified as multiple hematological markers. This is the first GWAS examining the genetic loci of hemorheological indexes in a normal Chinese Han population.

Blood viscosity and its major determinants (hematocrit and plasma viscosity) are related to increased risks of cardiovascular disease and cardiovascular-related premature mortality [16]. In our study, LOC730100 rs117580912 was associated with the nd1 level. LOC730100 on chromosome 2p16.3 was increased expression in glioma tissues and cell lines, and enhanced proliferation and invasion of glioma cells [18]. The significant association with nd30 and nd300 levels was for STK32B and LOC102724502-OLIG2 rs28371438. CCSE1-LINC01519 rs115646937 was also a significant marker for the nd300 level. The effects of overexpressed STK32B (chromosome 4p16.2) might be involved in relevant essential tremor pathways [19]. OLIG2, located on chromosome 21q22.1, is the key transcription factor that maintains the neural progenitor cells of the pMN domain [20]. CCSE2 on chromosome 10q23.1 was identified a reference gene, also called novel housekeeping gene [21].

Moreover, rs28371438 in LOC102724502-OLIG2, rs3985087 in TMEM232-SLC25A46, rs118096558 in RASGEF1C, rs11794922 in ADAMTS1L, rs76126204 in LINC02231, rs9966987 in LINC00470-METTL4, and rs140700208 in LINC01625-LINC00132735 showed suggestive associations with the level of plasma viscosity. TMEM232 associated with atopic dermatitis in the Chinese Han population [22] and SLC25A46 related to patients with Parkinson’s disease and optic atrophy [23] are located on chromosome 5q22.1. ADAMTS1L (chromosome 9p22.2-p22.1) protein was lower expressed in intracranial aneurysm tissue than in the control cerebral artery [24]. LINC00470 (chromosome 18p11.32) promoted the proliferation and invasion of glioma cell by LINC00470/miR-134/Myc/ABCC1 axis [25]. METTL4, located on chromosome 18p11.32, was identified as a candidate of N6-adenine methylase [26]. The function of long intergenic nonprotein coding RNA (LOC102724502, LINC01519, LINC02231, LINC01625, and LOC100132735) and RASGEF1C needs further study.

Hematocrit and fibrinogen are important determinants of whole blood viscosity. McMullin et al. reported that red cell mass measurement along with hemoglobin and hematocrit cut-offs as a major diagnostic criterion for the diagnosis of polycythemia Vera [27]. In this study, the significant association of hematocrit was with rs78577937 in the intergenic region of CCSE1-LNC-PRESS2. CCSE1 (chromosome 4q22.1), also known as FAM190A, was reported to be associated with type 1 diabetes [28]. LNC-PRESS2 is a long intergenic nonprotein coding RNA, whose function needs further study. Fibrinogen is an acute phase protein with proinflammatory and anti-inflammatory properties. Its secretion in the liver is upregulated during inflammation [29]. Previous study has shown that elevated fibrinogen is associated with an increased risk of lung, colorectal, and breast cancers [30].
Four loci were associated with fibrinogen concentration, including CNH3 rs78314456, TMEM232-SC25A46 rs3985087, LINC00917-FENDRR rs34247085, and LINC00470-METTL14 rs9966987. Cornichon 3 (CNH3, chromosome 1q42.12) enhanced the glutamate sensitivity, single-channel conductance, and calcium permeability of CP-AMPARs while decreasing their block by intracellular polyamines [31]. FENDRR (chromosome 16q24.1) is lower expressed in colorectal cancer tissue and cells, which is responsible for inhibiting of cell proliferation, migration, and invasion [32].

The aggregation of red blood cells may be enhanced during various pathophysiological processes, including circulatory and metabolic disorders, infection, blood patholgy, and several other disease states [33]. Altered erythrocyte aggregation may be a factor that affects the clinical process and also an indicator for the development and prognosis of disease. We found that the significant association with the concentration of erythrocyte aggregation index was for SFMBT2 rs11255044, LINC01493-LRRCA4 rs67538811, NELL1-ANOS1 rs12420881, and C12orf42 rs56670740. SFMBT2, a circRNA located on chromosome 10p14, had an increased expression level in gastric cancer tissues and was associated with the proliferation of gastric cancer cells [34]. LRRCA4 (chromosome 11p12) was a novel candidate susceptibility gene for pediatric central nervous system tumors [35]. NELL1 (chromosome 11p15.1) was associated with bone formation and osteoclast differentiation [36], and ANOS1 (chromosome 11p15.1) was related to myopathy. Hypothetical gene C12orf42, located on chromosome 12q23.2-q23.3, was associated with T-lymphoblastic lymphoma [37].

Erythrocyte aggregation is reversible and shear-dependent (i.e., disperses at high shear and reforms at low shear), and the degree of erythrocyte aggregation is the main determinant of low-shear blood viscosity [33]. Our study displayed that LSAMP rs34469348, MEH4 rs9361295, TUSC3-MSR1 rs2410367, PTPRD rs10977588, and TSHZ3-THEG5 rs745439 were associated with the level of relative index of whole blood hyposectomy. Rs11990599 of chromosome 10p14, had an increased expression level in gastric cancer tissues and was associated with the proliferation of gastric cancer cells [34]. LRRCA4 (chromosome 11p12) was a novel candidate susceptibility gene for pediatric central nervous system tumors [35]. NELL1 (chromosome 11p15.1) was associated with bone formation and osteoclast differentiation [36], and ANOS1 (chromosome 11p15.1) was related to myopathy. Hypothetical gene C12orf42, located on chromosome 12q23.2-q23.3, was associated with T-lymphoblastic lymphoma [37].

The rigidity of erythrocyte is the main rheological characteristic of the blood of Sickle Cell Anemia patients and several pathologies [38]. Three genome-level significant SNPs (rs11661911 in GTSCR1-LINC01541, rs1297329 in USP25-MIR99AHG and rs28371438 in LOC102724502-OLIG2) associated with erythrocyte rigidity index level were identified. LINC01541 (chromosome 18q22.3) plays a key role in 17β-estradiol (17β-E2)-stimulated endometrial stromal cells [39]. USP25 (chromosome 21q21.1) suppresses the degradation of BCR-ABL protein in cells harboring the Philadelphia chromosome (Ph) in chronic myelogenous leukemia [40]. LncRNA host gene MIR99AHG (alias MONC) interfered with hematopoietic lineage decisions and enhanced the proliferation of immature erythroid progenitor cells in acute megakaryoblastic leukemia [41]. Erythrocyte deformation is determined mainly by the fluidity of the membrane and the viscosity of the cytoplasm, but extracellular factors may have an irreversible effect on the erythrocyte membrane [42]. Fornal et al. [43] reported a statistically significant correlation between left ventricular mass index, erythrocyte deformability, and aggregability. We also found that TMEM232 rs2900050 and USP25-MIR99AHG rs1297329 were significant loci for the circulating level of erythrocyte deformation index.

Several potential limitations of this study cannot be ignored. First, all subjects were recruited from the same hospital; therefore, there was selection bias. Second, all participants were only from populations of Chinese Han ancestry, suggesting our finding couldn’t be generalized to other ethnic groups. Therefore, replication studies in other Chinese Han populations or other ethnic groups are required to confirm the association of the identified loci with hemorheological phenotypes. Third, the potential function of identified loci has not been assessed. Further functional analysis is required to reveal the biological mechanism behind the observed associations. Four, the most commonly accepted threshold of a genome-wide association study is $p < 5 \times 10^{-8}$. After consulting the literature on disease GWAS, we found that when the sample size is small, a relatively relaxed threshold will be selected [44–46]. Therefore, we chose a relatively relaxed threshold as the suggestive threshold for significant genome-wide association ($p < 5 \times 10^{-6}$). However, to the best of our knowledge, this is the first GWAS examining the genetic loci of hemorheological indexes in a normal Chinese Han population. The identification of susceptibility loci and genes related to hemorheological indexes may provide important insight into the regulation of hemorheological indexes.
In summary, we reported 38 suggestive loci associated with hemorheological indexes in the Chinese Han population. In particular, six SNPs (rs28371438 in LOC102724502-OLIG2, rs4689231 of STK32B, rs11661911 in GTSCR1-LINC01541, rs1297329 in USP25-MIR99A-HG, rs3985087 in TMEM232-SLC25A46, rs9966987 in LINC00470-METTL4) were identified as multiple hematological markers. The results of our genome-wide association study may represent biological candidates for hemorheological indexes and contribute to hemorheological study.

Contribution

Written informed consent was obtained from all of the participating cohort. The protocols were approved by the Institutional Research Ethics Committee of Henan Provincial People’s Hospital and complied with the Declaration of Helsinki.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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