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Research paper

Association of genetic polymorphisms with erythrocyte traits: Verification of SNPs reported in a previous GWAS in a Japanese population

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ABSTRACT

Erythrocyte count and volume are the commonly used hematological indices for anemia that change in various diseases. To date, however, only one study ever exists that addressed erythrocyte trait-associated single nucleotide polymorphisms (SNPs) in a Japanese population. Because that study was performed in patients with various diseases, we confirmed the reported associations in a general population. Participants in the current study were from the Shizuoka component of the Japan Multi-Institutional Collaborative Cohort Study, which included 4971 men and women aged 35 to 69 years who were recruited between 2006 and 2007. We analyzed the association of seven selected SNPs with the following erythrocyte traits: red blood cell count, hemoglobin (Hb) and hematocrit (Ht) levels, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. The erythrocyte traits were regressed on a number of minor alleles of selected SNPs. Then we compared our findings with those from a genome-wide association study performed in a Japanese population. We replicated the association of *ABO* rs495828, *PDGFRA-KIT* rs218237, *USP49-MED20-BSYL-CCND3* rs3218097, *C6orf182-CD164* rs11966072, *TERT* rs2736100, and *TMPRSS6* rs5756504 with erythrocyte traits in our independent Japanese population. In addition, we found a significant interaction between *TERT* rs2736100 and smoking habit that affected Hb and Ht levels.

1. Introduction

Erythrocyte count and volume are commonly used as indices for anemia, and important clinical biomarkers and modifiers of disease severity that are often measured to assess the general health of patients as well. Numerous diseases manifest with an increase or decrease in erythrocyte count and/or blood hemoglobin (Hb) concentration (Sagone and Balcerzak, 1975). In addition, variations in erythrocyte traits, even within a clinically normal range, correlate with mortality and risk of non-hematological diseases such as hypertension, embolism, and other cardiovascular diseases (Strazzullo et al., 1990; Kobayashi et al., 2001; Braekkan et al., 2010; Coglianese et al., 2012).

While erythrocyte traits are influenced by altitude and dietary intake such as vitamins and iron, their heritability reportedly range from 40% to 90% (Whitfield and Martin, 1985; Evans et al., 1999; Lin et al., 2007), indicating the importance of genetic factors. Because genetic polymorphisms greatly differ between populations, the evidence in one population may not be applicable to other ethnic groups (Patel et al., 2009). There are several reports on erythrocyte trait-associated genetic polymorphisms (Ganesh et al., 2009; Soranzo et al., 2009; Kamatani et al., 2010; Chen et al., 2013); however, to the best of our knowledge, only one study has been performed in a Japanese population (Kamatani et al., 2010). Because that study was performed among patients with various diseases, it is necessary to confirm the associations between erythrocyte traits and genetic polymorphisms in a general population.

Abbreviations: J-MICC, Japan Multi-Institutional Collaborative Cohort; SNP, single nucleotide polymorphism; GWAS, genome-wide association study; MAF, minor allele frequency; LD, linkage disequilibrium; RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PDGFRA, platelet derived growth factor receptor alpha; KIT, KIT proto-oncogene receptor tyrosine kinase; CCND2, cyclin D2; USP49, ubiquitin specific peptidase 49; MED20, mediator complex subunit 20; BYSL, bystin-like; CCND3, cyclin D3; CD164, cluster of differentiation 164; TERT, telomerase reverse transcriptase; TMPRSS6, transmembrane protease, serine 6; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase

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Therefore, in this study, we analyzed the associations of erythrocyte traits (red blood cell [RBC] count, Hb and hematocrit [Ht] levels, mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], and mean corpuscular hemoglobin concentration [MCHC]) with selected genetic polymorphisms to verify previous findings (Kamatani et al., 2010) in an independent Japanese population. In addition, because erythrocyte traits are influenced by smoking habit (Okuno, 1973; Sagone and Balcerzak, 1975; Tirlapur et al., 1983), we also examined the interactive effects between the genetic polymorphisms and smoking on erythrocyte traits.

2. Material and methods

2.1. Study subjects

Subjects were participants of the Shizuoka component of the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study, who visited the Seirei Preventive Health Care Center in Shizuoka, Japan (Hamajima et al., 2007). Details of this study have been described elsewhere (Asai et al., 2009). Briefly, participants included 5040 men and women aged 35 to 69 years who were recruited between 2006 and 2007. All of the participants completed a self-administered questionnaire regarding their lifestyles and medical history, and donated venous blood samples after providing informed consent. RBC count, Hb and Ht levels were measured using the Sysmex XE-2100 hematology automated analyzer (Sysmex; Kobe, Japan), and MCV, MCH, and MCHC were calculated with RBC, Hb, and Ht measurements using the following equations: MCV (fl) = Ht (%) × 1000 / RBC (10^4/μL); MCH (pg) = Hb (g/dL) × 1000 / RBC (10^4/μL); and MCHC (g/dL) = Hb (g/dL) / Ht (%) × 100. We excluded 32 subjects due to withdrawal from the study, 13 due to genotyping error in the seven single nucleotide polymorphisms (SNPs) included in the analysis, and 24 subjects who were taking drugs for anemia or iron supplements; the remaining 4971 subjects (3390 men and 1581 women) were included in the study. Written informed consent, including that for genotyping, was obtained from all of the participants. The protocol for the Shizuoka component of the J-MICC Study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine (Nagoya, Japan; Approval No. 1248-10).

2.2. SNP determination

We determined the genotypes using DNA extracted from buffy coat samples. First, we chose 29 SNPs associated with erythrocyte measures, according to a previous genome-wide association study (GWAS) in a Japanese population (Kamatani et al., 2010). Among them, we selected seven SNPs that were previously analyzed in the Shizuoka component of the J-MICC study with a genotype call rate ≥ 99%. Eventually, we genotyped the subjects for *ABO* rs495828, *PDGFRA-KIT* rs218237, *USP49-MED20-BSYL-CCND3* rs3218097, *CCND2* rs11611647, *C6orf182-CD164* rs11966072, *TERT* rs2736100, and *TMPRSS6* rs5756504 using the DigiTag2 method (Nishida et al., 2007).

2.3. Statistical analysis

Because erythrocyte traits greatly differ by sex, we also summarized the characteristics of the study subjects by sex. Smoking was assessed as a dichotomous variable (current smoking: yes/no). Since the life span of erythrocytes is about 120 days, the influence of smoking on erythrocytes would be much smaller after smoking stopped; thus, we included former smokers in the non-smoker group. The genotype of each SNP was scored using an additive model: 0, homozygous for a major allele; 1, heterozygous for a major allele; and 2, homozygous for a minor allele. To confirm accordance of the genotype frequencies with Hardy–Weinberg equilibrium, we performed the chi-squared test for each SNP. To examine the association of each genetic polymorphism with each erythrocyte trait, we conducted multiple linear regression analyses including sex, age (continuous), smoking habit, and the aforementioned SNP scores as independent variables and RBC count, Hb and Ht levels, MCV, MCH, and MCHC as dependent variables. Regression coefficients (β) were estimated using the *t*-test. Furthermore, the association between genetic polymorphism and erythrocyte trait was examined by smoking habit (i.e., non-smokers and smokers). Moreover, the interactive effects of SNPs and smoking on erythrocyte measurements were statistically evaluated by incorporating interaction terms (SNP score × current smoking habit [yes: 1, no: 0]) in the regression model, because erythrocyte traits are influenced by smoking habit (Sagone and Balcerzak, 1975). All statistical analyses were performed using STATA software, version 13.1 (STATA, College Station, TX, USA).

Table 1
Background characteristics of the study participants.

	Men	Women
Number of participants (n)	3390	1581
Age (years)	52.75 ± 8.67	51.25 ± 8.64
RBC (10^4/μL)	483.55 ± 37.30	437.27 ± 32.44
Hb (g/dL)	14.81 ± 1.01	12.78 ± 1.17
Ht (%)	45.67 ± 2.82	40.39 ± 3.13
MCV (fl)	94.65 ± 4.38	92.50 ± 5.48
MCH (pg)	30.69 ± 1.56	29.27 ± 2.20
MCHC (g/dL)	32.43 ± 0.83	31.61 ± 1.00
Smoking status		
Never (%)	1072 (31.6)	1414 (89.4)
Former (%)	1518 (44.8)	91 (5.8)
Current (%)	796 (23.5)	71 (4.5)
Unknown (%)	4 (0.1)	5 (0.3)

Values are presented as means ± SD or n (percentage).
SD, standard deviation; RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

3. Results

The participants' characteristics are shown by sex in Table 1. Men accounted for two-thirds of the study subjects. The proportion of former and current smokers was much greater in men than in women, as were RBC count and Hb and Ht levels. We confirmed that the genotype distribution of the SNPs was in accordance with Hardy–Weinberg equilibrium; according to the chi-squared test, the *p* values were 0.549 for rs495828, 0.628 for rs218237, 0.327 for rs3218097, 0.805 for rs11611647, 0.178 for rs11966072, 0.244 for rs2736100, and 0.286 for rs5756504.

We examined the associations of selected SNPs with erythrocyte traits (Table 2), and found that their minor allele frequencies (MAFs) were similar to those reported in the GWAS by Kamatani et al. (2010). The SNPs associated with RBC count included rs495828 (*ABO*, $p = 8.49 \times 10^{-7}$, $\beta = -3.888$), rs218237 (*PDGFRA-KIT*, $p = 2.07 \times 10^{-9}$, $\beta = -4.674$), rs3218097 (*USP49-MED20-BYSL-CCND3*, $p = 1.99 \times 10^{-4}$, $\beta = 3.427$), rs11966072 (*C6orf182-CD164*, $p = 0.047$, $\beta = -2.466$), and rs2736100 (*TERT*, $p = 0.037$, $\beta = 1.509$); those associated with Hb were rs495828 (*ABO*, $p = 4.51 \times 10^{-5}$, $\beta = -0.097$), rs218237 (*PDGFRA-KIT*, $p = 0.047$, $\beta = -0.047$), and rs5756504 (*TMPRSS6*, $p = 0.017$, $\beta = 0.051$); and SNPs associated with Ht were rs495828 (*ABO*, $p = 1.12 \times 10^{-5}$, $\beta = -0.287$) and rs218237 (*PDGFRA-KIT*, $p = 0.004$, $\beta = -0.185$). SNPs associated with MCV were rs218237 (*PDGFRA-KIT*, $p = 3.33 \times 10^{-8}$, $\beta = 0.554$), rs3218097 (*USP49-MED20-BYSL-CCND3*, $p = 7.42 \times 10^{-8}$, $\beta = -0.637$), rs11966072 (*C6orf182-CD164*, $p = 0.035$, $\beta = 0.338$), rs2736100 (*TERT*, $p = 4.83 \times 10^{-4}$, $\beta = -0.324$), and rs5756504 (*TMPRSS6*, $p = 0.001$, $\beta = 0.288$); and those associated with MCH were the same as those that correlated with