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Single nucleotide polymorphisms of the *DGKB* and *VCAM1* genes are associated with granulocyte colony stimulating factor-mediated peripheral blood stem cell mobilization

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ABSTRACT

We previously reported the association between LDL cholesterol level (LDL-C) and granulocyte colony stimulating factor (G-CSF)-mobilized peripheral blood (PB) hematopoietic stem cells (HSC). In this study, we investigated the association between gene single nucleotide polymorphisms (SNPs) involved in hematopoiesis and lipid level and PBHSC mobilization. In 46 patients who underwent peripheral blood stem cell harvest (PBSCH), we measured CD34-positive cells in PB and PBSCH, and the patients were classified into good, intermediate, or poor mobilizer groups based on the CD34-positive cell counts. And SNPs of the OR4C12, ENO1, RERE, DGKB, DSC3, VCAM1, CD44, and FADS1 genes were investigated. The frequency of the TT type of the DGKB gene was higher in the poor mobilizer group compared to other groups ($p < 0.05$), whereas that of the CC type of the VCAM1 gene was high in the good mobilizer group ($p < 0.05$). Association with the efficiency of HSC mobilization to PB were found in the SNPs of the DGKB gene involved in cell transport and SDF-1-induced migration ability and of the VCAM1 gene which is essential for HSC homing, suggesting that SNPs involved in cell migration ability might be partly involved in HSC mobilization to PB.

1. Introduction

Many factors are influencing hematopoietic stem cell (HSC) mobilization, including gender, age, frequency of chemotherapy and radiotherapy, and type of disease [1–5]. In addition, mobilization of HSCs to peripheral blood (PB) in mice has been induced by cholesterol [6], and we previously reported that low density lipoprotein-cholesterol (LDL-C) may serve as a favorable biomarker of HSC mobilization to PB in human [7].

Many single nucleotide polymorphisms (SNPs) involved in hematopoiesis and cell migration are reported to affect HSC mobilization. SNPs of *VCAM1* and *CD44* have been reported to relate with CD34-positive cell number in PB after granulocyte colony stimulating factor (G-CSF) administration [8].

LDL-C level involved in HSC mobilization has been also associated with *fatty acid desaturase* (*FADS*) SNPs in a survey of Japanese males [9], and the association between the HSC count and *OR4C12*, *ENO1/RERE*, *DGKB*, and *DSC3* SNPs has also been reported [10]. No factor accurately predicting the mobilization efficiency, however, has been established yet.

The purpose of this study is to investigate whether the SNPs involved in the steady-state PB HSC count, LDL-C level, and adhesion molecules influence the efficiency of HSC mobilization to PB.

2. Materials and methods

2.1. Subjects

The subjects were 46 patients who underwent autologous peripheral blood stem cell harvest (PBSCH). Their PBs and PBSCHs were used (Table 1). The median age was 59 years (range, 20 to 71). The subjects included patients with non-Hodgkin's lymphoma (n = 27) and multiple myeloma (n = 9), acute leukemia (n = 4), and others (n = 6). This study was performed after approval by

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Table 1
Patients' characteristics.

Characteristics	Value
Age (year)	59 (20–71)
Sex (female/male)	19/27
Disease	
Non-Hodgkin lymphoma	27
Hodgkin lymphoma	1
Multiple myeloma	9
Acute leukemia	4
Amyloidosis	2
Pancreatic cancer	1
Testicular cancer	1
Osteosarcoma	1
Number of treatment lines before mobilization	3 (0–8)
Number of apheresis	2 (0–4)
White cell counts ($\times 10^3/\mu L$)	2.3 (1.0–62.7)
$CD34^+$ cells in peripheral blood ($/\mu L$)	11.2 (0.0–523.2)
Peak $CD34^+$ cells in peripheral blood ($/\mu L$)	27.9 (0.0–523.2)
$CD34^+$ cells after first apheresis ($\times 10^6/kg$)	1.1 (0.1–27.3)
Total $CD34^+$ cells ($\times 10^6/kg$)	3.1 (0.2–27.3)
LDL-C (mg/dL)	114 (65–356)
HDL-C (mg/dL)	60 (26–101)

Data presented are median (range).

the Medical Ethics Committee of Shimane University Faculty of Medicine. Written informed consent was obtained from all participants.

2.2. Mobilization and collection of peripheral blood stem cells

PBSCH was performed by inducing HSC mobilization to PB by G-CSF administration initiated in the neutropenic period after completion of cancer chemotherapy in the patients. HSC were mobilized using several different chemotherapy regimens, according to disease-specific protocols. The CD34-positive cells were counted from the day the white blood cell count exceeded 1,000/μL, and PB stem cells were collected when the white blood cell count reached 5,000/μL. G-CSF was subcutaneously administered as the dose of 10 μg/kg of Lenograstim (Chugai Pharmaceutical Co. Tokyo, Japan) or 400 $\mu g/m^2$ of Filgrastim (Kyowa Kirin Co. Tokyo, Japan).

For harvest, COMTEC (Fresenius SE & Co. KGaA, Homburg, Germany) and C4Y kit (Fresenius SE & Co. KGaA) were used, and the blood volume processed in one harvest was 10,000 mL. When the number of collected CD34-positive cells was lower than 2×10^6/kg donor body weight, harvest was repeated under the same conditions on the following day until reach to the number.

For blood removal and retransfusion, a puncture needle with an outer diameter of 16 gauge and inner diameter of 18 gauge, Cannula with Clamping Tube (Covidien Japan Co. Tokyo, Japan), was inserted into veins in the bilateral anterior cubital regions and used as blood removal and retransfusion routes, respectively. When securing a peripheral blood vessel was difficult, the Blood Access UK Catheter Kit (Covidien Japan Co.) was inserted into the femoral vein for blood removal and retransfusion.

2.3. CD34-positive cell counting

CD34-positive cells were counted using the Stem-Kit (Beckman Coulter, Miami, FL, USA) and a flow cytometer, Navios (Beckman Coulter), following the ISHAGE guidelines [11].

2.4. Detection of SNP

DNA was extracted from the patients' PB or harvests using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). SNPs of *OR4C12* (rs2183383), *ENO1/RERE* (rs11121242), *DGKB* (rs976760), *DSC3* (rs2591119), *VCAM1* (rs1041163), *CD44* (rs13347), and *FADS1* (rs174546) were investigated using the TaqMan method. A reaction solution with a total volume of 20 μL was prepared by combining 10 μL of Lightcycler 480 Probes Master (Roche Diagnostics, Basel, Switzerland), 0.5 μL of ×40 TaqMan SNP Genotyping Assays (Life Technologies, Carlsbad, CA, USA), 7.5 μL of PCR-grade H_2O, and 2 μL of the target DNA. PCR reactions were activated and held at 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 10 s and 60 °C for 1 minute, then cooled down at 40 °C for 30 s. Allele-specific signals were generated and acquired upon the completion of PCR using the dye-labeled probes. The genotypes were called using the endpoint genotyping LightCycler 480 Software version 1.5 [12].

2.5. Statistical analysis

The data are presented as the median (lowest-highest) or number of subjects (n). The subjects were classified into 3 groups based on the CD34-positive cell counts in PB and harvests: poor (the peak PB CD34-positive cell count was less than 20/μL or the total CD34-positive cell yield of 3 continuous harvests was less than 2×10^6/kg) [13], good (the CD34-positive cell count in one harvest was 2×10^6/kg or higher), and intermediate mobilizer groups, and subjected to statistical analysis.

The rates were analyzed using Fisher's exact probability test, differences were analyzed using one-way analysis of variance or the Mann–Whitney U-test, and the significance level was set at less than 5 % ($p < 0.05$). Regarding SNP, Hardy–Weinberg equilibrium was analyzed using Fisher's exact probability test. Analyses were performed using SPSS statistics 22 (IBM, Chicago, IL, USA).

3. Results

3.1. Mobilization efficiency

The median time of harvest was 3, the peak PB CD34-positive cell count was 27.9/μL, and the total CD34-positive cell yield was 3.1×10^6/kg (Table 1). The number of good, intermediate and poor mobilizers is 20, 17, and 9, respectively. No significant difference was noted in the age, gender, frequency of harvest, LDL-C, or high density lipoprotein-cholesterol (HDL-C) among the groups (Table 2).

3.2. SNPs allele frequencies and the relationship between mobilizer groups

All polymorphisms were in the Hardy–Weinberg equilibrium. We showed genotype distribution frequencies of the *OR4C12*, *ENO1*, *RERE*, *DGKB*, *DSC3*, *VCAM1*, *CD44*, and *FADS1* genes (Table 3).

When the allele frequency was compared among the 3 mobilizer groups, the frequency of the *DGKB* SNPs, TT type was higher in the good group than other groups. The TT type were 9 of 20 (45 %) patients in the good group, 3 of 17 (18 %) patients in the intermediate group, and 0 of 9 (0%, $P = 0.022$) patients in the poor group. Its odds ratio for good mobilizer was 6.3 (Fig. 1). The frequency of the CC type of *VCAM1* SNPs was high in the good group, the CC type were 0 of 20 (0%) patients in the good group, 0 of 17 (0%) patients in the intermediate group, and 2 of 9 (22%, $P = 0.035$) patients in the poor group. No significant difference was found in any other genes (Table 4).

3.3. DGKB *SNPs*

The CD34-positive cell count per patient body weight in the first harvest (2.6×10^6/kg versus 1.1×10^6/kg, $P = 0.040$), and CD34-positive cell count in the first harvest ($27.5 \times 10^7/\mu L$ versus $12.9 \times 10^7/\mu L$, $P = 0.020$) were significantly higher in TT than in the types with the C allele. No significant difference was detected in