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Sex and Body Mass Index but Not CXCL12 801 G/A Polymorphism Determine the Efficacy of Hematopoietic Cell Mobilization: A Study in Healthy Volunteer Donors

Juliane Lenk [1], Martin Bornhauser [1,2,*], Michael Kramer [1], Kristina Hölig [1], Kirsten Poppe-Thiede [3], Helmuth Schmidt [4], Markus Wiesneth [5], Kerstin Schaefer-Eckart [6], Peter Schlenke [7], Michael Punzel [4], Sonja Martin [8], Frank Kroschinsky [1], Alexander H. Schmidt [9], Gerhard Ehninger [1], Christian Thiede [1]

[1] *Medizinische Klinik und Poliklinik I, University Hospital, Dresden, Germany*
[2] *Center for Regenerative Therapies, Dresden, Germany*
[3] *Cellex, Zentrum für Zellgewinnung, Dresden, Germany*
[4] *Cellex, Zentrum für Zellgewinnung, Köln, Germany*
[5] *Institut für Klinische Transfusionsmedizin und Immungenetik, University Hospital, Ulm, Germany*
[6] *Medizinische Klinik 5, Klinikum Nürnberg, Germany*
[7] *Institut für Transfusionsmedizin und Transplantationsimmunologie, University Hospital Münster, Germany*
[8] *Hämatologie, Onkologie und Palliativmedizin, Robert Bosch Hospital Stuttgart, Germany*
[9] *DKMS German Bone Marrow Donor Center, Tübingen, Germany*

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Analyses of healthy donors of granulocyte colony-stimulating factor (G-CSF) mobilized hematopoietic stem and progenitor cells (HSPCs) and of patients undergoing autologous stem cell transplantation have suggested that individuals harboring the CXCL12-A allele mobilize a higher number of CD34 + HSPCs after G-CSF administration. We typed 463 healthy unrelated donors (376 men and 87 women) who had received daily subcutaneous injections at a mean dose of 7.36 ± 1.71 μg/kg G-CSF for 5 days for CXCL12 801 G/A using a real-time PCR assay. Interestingly, the median concentration of mobilized CD34 + cells on day 5 was almost identical in donors with the A-allele (79/μL; range, 11 to 249/μL) and the G/G-group (82/μL; range, 15 to 268/μL). In addition, the allelic distribution was not different in donors (n = 11) who mobilized less than 20/μL CD34 + cells. No difference in the overall yield of CD34 + cells in the apheresis product and in the number of CD34 + cells/kg recipient could be detected between both groups. In a multivariate regression model for the endpoint CD34 + cells/μL at day 5, only male sex (regression coefficient, 11.5; 95% confidence interval, 1.7 to 21.2, $P = .021$) and body mass index as continuous variables (regression coefficient, 3.5; 95% confidence interval, 2.5 to 4.5, $P = .0001$) but not age, smoking status, or CXCL12 allelic status represented independent variables. Our data derived from a large well-controlled cohort contradict previous analyses suggesting an association between CXCL12 allelic status and the yield of CD34 + HSPC after G-CSF mobilization. Concentration of CD34 + cells in the peripheral blood, the most objective parameter, could not be predicted by CXCL12 genotype.

INTRODUCTION

Peripheral blood stem cells (PBSCs) collected after mobilization with granulocyte colony-stimulating factor (G-CSF) are the most frequently applied source of hematopoietic stem and progenitor cells (HSPCs) for allogeneic transplantation. This preference is mainly due to a faster engraftment of neutrophils and platelets and to a less invasive procedure not requiring in-hospital stays or general anesthesia [1].

In approximately 75% of donors, one single leukapheresis is sufficient to collect the CD34 + cell dose required, whereas

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* Correspondence and reprint requests: Martin Bornhauser, MD, Technische Universität, Medizinische Klinik und Poliklinik I, Universitätsklinikum Carl Gustav Carus, Fetscherstrasse 74, 01307 Dresden, Germany.
E-mail address: martin.bornhaeuser@uniklinikum-dresden.de (M. Bornhauser).
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one fourth of donors needs a second leukapheresis to yield the target cell dose [2]. However, 1% to 5% of healthy volunteer PBSC donors unexpectedly fail to mobilize a sufficient quantity of CD34 + cells [3,4].

To prospectively identify donors in whom G-CSF administration will lead to insufficient mobilization of CD34 + HSPC, predictive markers are warranted. Certain donor characteristics are already considered as predictive markers for the efficacy of PBSC mobilization [5-9]. Particularly, sex, age, and body weight were shown to influence mobilization potential [5-7]. However, donors do present without any negative predictive factor but unexpectedly fail to mobilize the target cell dose, which suggests so far unknown determinants of HSPC amplification and release after G-CSF. Consequently, it seems rational to examine the influence of possible genetic factors associated with mobilization efficacy.

The mobilization of PBSC is a complex process, in which the activation of metalloproteases, cellular components, chemokinetactic gradients of CXCL12 and spingosine-1-phosphate, and β-adrenergic sympathetic nerves are involved [10]. The interaction between the chemokine CXCL12 (formerly stromal cell–derived factor 1, SDF1) and its receptor CXCR4 have been described as playing a pivotal role in G-CSF–induced mobilization of CD34 + progenitor cells from bone marrow into peripheral blood [11].

Winkler et al. [12] revealed a G → A transition at position 801 in the 3′-untranslated region (3′-UTR) of the CXCL12 gene, suggesting a genetic influence on the CXCL12–CXCR4 axis. In the homozygous state, SDF1-3′A allele delays the onset of acquired immunodeficiency syndrome in late stage of human immunodeficiency virus type 1 infection [12]. Benboubker et al. [13] were the first to demonstrate a significant influence of the CXCL12-3′A allele on HSPC mobilization. In addition, genetic variants of VCAM1 and CD44, molecules involved in adhesive and chemotactic interactions of CD34 + cells within the bone marrow niche, have been associated with the efficacy of progenitor cell mobilization [14].

The aim of our study was to investigate the assumed association of the *CXCL12* gene polymorphism and progenitor cell mobilization after G-CSF administration in a larger cohort of unrelated healthy volunteer donors in a retrospective multicenter study. This setting was not biased by background disease or chemotherapy.

METHODS

Donor Characteristics

The CXCL12-3′-UTR-801-G-A polymorphism, as well as the CD34 + cell count/μL peripheral blood on day 5 of G-CSF treatment, CD34 + absolute cell count in the first leukapheresis product, and CD34 + cells/kg recipient, were analyzed in 463 healthy volunteer donors of allogeneic transplants in an unrelated setting. PBSC collections were performed at 8 different centers in Germany. Informed consent was obtained from all 463 donors studied. This study was approved by the local Ethics Committee in accordance with the Declaration of Helsinki.

Clinical data were collected retrospectively. DNA samples had been stored at the repository of the DKMS (German Bone Marrow Donor Center) after obtaining informed consent. The current analysis was approved by the Institutional Review Board of the University Hospital Dresden.

Mobilization and Collection of CD34 + Cells

Most donors (97%) received G-CSF (lenograstim; mean, 7.36 ± 1.71 μg/kg/d) for 5 days. Within specific protocols, 3% of the donors received filgrastim (10 μg/kg/d). The first leukapheresis was performed on day 5. If the required number of CD 34 + cells per recipient body weight was not collected, rhG-CSF administration was repeated (depending on the donor's leukocyte count) and a second PBSC collection was performed on day 6.

Administration of rhG-CSF was performed either daily by the donor's family doctor (15.3%) or by the donor or a family member every 12 hours (81.9%). PBSCs were collected by different continuous-flow blood cell separators (Table 1) via bilateral (forearm) peripheral venous access, whenever possible, or otherwise via a central line in the femoral vein. In donors who needed a central line, only 1 leukapheresis was performed and the venous line was removed immediately thereafter. During the first leukapheresis, the donor's total blood volume was processed 4 to 5 times at 50 to 110 mL/min over 3 to 4.5 hours.

Anticoagulation was performed with ACD-A (ratio, 1:12 to 20), and most donors received heparin (5000 U per donor). During the second leukapheresis, if necessary, the maximum volume processed was 3 times the donor's blood volume, and heparin was used only if the platelet count was $>100 \times 10^9$/L. The desired yield of CD34 + cells was 4 to 10×10^6/kg of recipient body weight, depending on the disease and treatment protocol. Two leukapheresis procedures were performed in 34 donors (7.3%).

Flow Cytometric CD34 Measurement

CD34 cells in peripheral blood and apheresis samples were quantified by flow cytometry. Participating centers used either single- or dual-platform technologies. Measurements of CD34 + cells and analysis procedure thus followed published guidelines [15]. All laboratories had successfully participated in trans-European interlaboratory tests focusing on the accuracy of CD34 + progenitor cell enumeration and had been licensed by national authorities.

Analysis of the CXCL12 3′-UTR Polymorphism

Genotyping of the *CXCL12*-3′-UTR Single Nucleotide Polymorphism (SNP) (rs1801157) was performed based on a commercially available, allele-specific real-time PCR assay (Invitrogen Assays on Demand, Darmstadt, Germany) using VIC- and 6-carboxyfluprescein (FAM)-labeled specific probes for both allelic variants. Briefly, 15 ng genomic DNA was amplified in a Roche Lightcycler 480 using the TaqMan Universal Master mix (Life Technologies; Darmstadt, Germany). Reaction conditions and cycle parameters were chosen as recommended by the manufacturer. Results of automated allele calling were verified in a subgroup of 64 patients using an Msp1-based RFLP assay with primers described by Winkler et al. [12] as well as with direct sequencing.

Table 1
Donor Characteristics

	N (Median, Range)	Percent
Sex		
Men	376	81.2
Women	87	18.8
Age, yr		
Median (min-max)	33 (18-54)	
BMI, kg/m^2		
Median (min-max)	25.4 (17.7-44.7)	
Smoking status		
Smokers	187	40.4
Nonsmokers	276	59.6
Daily alcohol intake		
Yes	46	10
No	415	90
Missing n = 2		
G-CSF		
Lenograstim	449	97
Filgrastim	14	3
Separating machines		
Cobe Spectra	352	76
Baxter CS 3000	3	1
Fresenius Comtec	108	23
CD34+/μL peripheral blood on day 5		
Median (min-max)	73 (11-268)	
CD34 + absolute $\times 10^8$ (in first leukapheresis product)		
Median (min-max)	6.2 (1.28-21.89)	
CD34+/kg recipient $\times 10^6$		
Median (min-max)	8.2 (1.3-72.7)	
Number of donors with 2 aphereses	34	7.3
Number of poor mobilizers (CD34+/μL peripheral blood on day 5 of G-CSF treatment <20)	11	2.4
Processed volume in L (first leukapheresis)		
Median (min-max)	17.6 (9.9-31.2)	