Impact of constitutional polymorphisms in *VCAM1* and *CD44* on CD34+ cell collection yield after administration of granulocyte colony-stimulating factor to healthy donors

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The online version of this article has a Supplementary Appendix.

ABSTRACT

Background
The number of CD34+ cells mobilized from bone marrow to peripheral blood after administration of granulocyte colony-stimulating factor varies greatly among healthy donors. This fact might be explained, at least in part, by constitutional differences in genes involved in the interactions tethering CD34+ cells to the bone marrow.

Design and Methods
We analyzed genetic characteristics associated with CD34+ cell mobilization in 112 healthy individuals receiving granulocyte colony-stimulating factor (filgrastim; 10 μg/kg; 5 days).

Results
Genetic variants in *VCAM1* and in *CD44* were associated with the number of CD34+ cells in peripheral blood after granulocyte colony-stimulating factor administration (P=0.02 and P=0.04, respectively), with the quantity of CD34+ cells $\times 10^6$/kg of donor (4.6 *versus* 6.3; P<0.001 and 7 *versus* 5.6; P=0.025, respectively), and with total CD34+ cells $\times 10^6$ (355 *versus* 495; P=0.002 and 522 *versus* 422; P=0.012, respectively) in the first apheresis. Of note, granulocyte colony-stimulating factor administration was associated with complete disappearance of *VCAM1* mRNA expression in peripheral blood. Moreover, genetic variants in granulocyte colony-stimulating factor receptor (*CSF3R*) and in *CXCL12* were associated with a lower and higher number of granulocyte colony-stimulating factor-mobilized CD34+ cells/μL in peripheral blood (81 *versus* 106; P=0.002 and 165 *versus* 98; P=0.02, respectively) and a genetic variant in *CXCR4* was associated with a lower quantity of CD34+ cells $\times 10^6$/kg of donor and total CD34+ cells $\times 10^6$ (5.3 *versus* 6.7; P=0.02 and 399 *versus* 533; P=0.01, respectively).

Conclusions
In conclusion, genetic variability in molecules involved in migration and homing of CD34+ cells influences the degree of mobilization of these cells.

Key words: *VCAM1*, *CD44*, polymorphisms, CD34+ cell mobilization, CD34+ cell yield.

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is used as a curative treatment for life-threatening hematologic diseases. Different sources of CD34+ cells can be used for allogeneic HSCT, including bone marrow (BM), peripheral blood (PB), and umbilical cord blood.[1,2] Currently, more than 70% of allogeneic HSCT are performed using PB as the source of CD34+ cells.[2] Advantages of using PB rather than BM for allogeneic HSCT are easier CD34+ cell collection, quicker post-transplant neutrophil and platelet recovery, a reduction of infectious complications, and lower transplant-related mortality for patients with advanced hematologic malignancies.[3,4]

The precise number of donor CD34+ cells that must be given to the recipient in order to achieve robust myeloid and lymphoid engraftment has not yet been defined, although transplantation of a large quantity of CD34+ cells seems to be associated with better survival.[5] The number of CD34+ cells mobilized into PB following administration of granulocyte colony-stimulating factor (G-CSF) varies significantly among donors.[6] Although different factors may influence CD34+ cell mobilization, such as age, gender, or weight,[7,8] there are currently no reliable predictive factors of poor or good CD34+ cell mobilization in healthy donors. We hypothesized that individual genetic factors might explain, at least in part, the variability and that these factors might be used to predict CD34+ cell mobilization.

G-CSF is the predominant cytokine used for mobilizing CD34+ cells in healthy donors.[1] During the G-CSF-induced mobilization process, the interactions tethering CD34+ cells to the BM stroma are disrupted. Some of these interactions include CXCR4 with CXCL12, very late antigen-4 (VLA-4) with vascular cell adhesion molecule-1 (VCAM-1), and CD44 with hyaluronic acid. G-CSF, through its receptor CSF3R, plays a pivotal role in mobilization of CD34+ cells by releasing proteolytic enzymes, such as neutrophil elastase (NE), catepsin-G (CG), matrix metalloproteinase-9 (MMP-9), and by down-regulating the expression of some of these genes.[1] Other molecules more recently described to be involved in the trafficking and homing of CD34+ cells include β3-adrenergic receptor (ADRB3) and guanine-nucleotide-binding protein stimulatory alpha subunit (GNAS).[9,10]

An increasing number of studies have found evidence that genetic factors known as single nucleotide polymorphisms (SNP) explain, in part, the significant inter-individual variability in responses to drug administration.[11] Identifying SNP predictive of a poor or good response to G-CSF, in terms of number of CD34+ cells mobilized, might be useful when discussing the possibility of using a different mobilizing agent or a different source of CD34+ cells for allogeneic HSCT.

The aim of this study was to evaluate a possible association between SNP in 16 genes involved in adhesive and chemotactic interactions to retain CD34+ cells within the BM with the number of G-CSF-mobilized CD34+ cells/kg of donor in the first apheresis in healthy donors.

Design and Methods

Study population

Two groups of healthy people were studied: group 1 consisted of 112 individuals who were given G-CSF (filgrastim 10 μg/kg subcutaneous for 5 days) to mobilize CD34+ cells for HLA-identical sibling allogeneic HSCT at the Hematology Department of *Universitario Virgen del Rocío* Hospital, Seville, between January 1999 and March 2008. Group 2 consisted of 107 voluntary blood donors from the Regional Centre for Blood Transfusion, Seville. Group 2 was included since the number of CD34+ cells in PB in steady state (i.e. without having received G-CSF) was not available for group 1. All donors were Caucasians from Spain. The local ethics committee of the *Universitario Virgen del Rocío* Hospital provided institutional review board-approval for this study, and informed consent was obtained from all donors in accordance with the Declaration of Helsinki. The main characteristics of the donors in both groups are presented in *Online Supplementary Table S1*.

Peripheral blood cell counts

White blood cell (WBC) and platelet counts were analyzed by a Sysmex XE-2100 auto-analyzer. The number of CD34+ cells in PB was quantified by conventional flow cytometry in a Becton Dickinson FACS-Calibur flow cytometer acquiring a total of 10,000 events and using monoclonal antibody CD34-PE. CD45-FITC monoclonal antibody was used to gate pure leukocyte populations. Both monoclonal antibodies were purchased from BD Biosciences, San Jose, CA (USA).

Selection of genes, single nucleotide polymorphisms and genotyping

Sixteen genes (*CXCL12, CXCR4, VCAM-1, VLA-4, G-CSF, CSF3R, CD34, ADRB3, CXCL2, CXCR2, CD44, Kit ligand, c-Kit, MMP-9, CTSG, GNAS*) were screened at the NCBI database searching for SNP that fulfilled the following criteria: (i) located at either 3'UTR or 5'UTR regions or at coding regions associated with an amino acid change, (ii) frequency of the less frequent homozygous allele more than 10% in Caucasians, and (iii) associated with a functional change of the gene or involved in the pathogenesis of diseases. When no SNP located at either 3'UTR or 5'UTR regions or at coding regions had a frequency higher than 10%, intronic SNP were selected. A total of 28 SNP were selected

Table 1. Correlation analyses of CD34+ cell count/μL of PB and CD34+ cells/kg of donor after G-CSF (Group1) and in steady state (Group 2) with different cell counts and with clinical variables. Pearson's correlation coefficients and *P* values are shown. *P* gender values were calculated by Students' T test.

Cell counts and clinical variables	CD34+ cells in PB Group 1	CD34+ cells in PB Group 2	CD34+ cells/kg of donor after first apheresis (group1)
WBC counts at 5th day of G-CSF	0.542 *P*<0.001		0.475 *P*<0.001
Platelet counts at 5th day of G-CSF	0.275 *P*=0.004		0.258 *P*=0.008
WBC counts at steady state	0.096 *P*=0.359		0.07 *P*=0.5
Platelet counts at steady state	0.343 *P*=0.001	0.339 *P*<0.001	0.248 *P*=0.019
CD34+ cells /kg of donor after first apheresis	0.794 *P*<0.001		
Total CD34+ cells after first apheresis	0.746 *P*<0.001		0.7 *P*<0.001
Age	-0.58 *P*=0.556	-0.039 *P*=0.692	-0.21 *P*=0.029
Gender	*P*=0.25	*P*=0.74	*P*=0.9

WBC: white blood cell.

for genotyping (*Online Supplementary Table S2*). Genomic DNA isolation and allelic discrimination polymerase chain reaction (PCR) methods are described in the *Online Supplementary Design and Methods*.

RNA isolation and gene expression analysis

Details of total RNA isolation and the gene expression analysis are provided in the *Online Supplementary Design and Methods*.

Statistical analysis

SNP were analyzed for deviation from Hardy-Weinberg equilibrium, using a χ^2 test. Allele frequencies and genotype frequencies were formulated by direct counting. In group 1, a univariate analysis was performed for clinical characteristics and each genotype to evaluate possible associations with the CD34$^+$ cell count in PB on the fifth day of G-CSF, with the number of CD34$^+$ cells/kg of donor obtained after the first apheresis and with the total number of CD34$^+$ cells obtained after the first apheresis. In group 2, a univariate analysis was performed to evaluate a possible association of clinical characteristics and genotype with the CD34$^+$ cell steady state count in PB. Pearson's correlation test was used to analyze the effects of age on the CD34$^+$ cell count in PB and with the number of CD34$^+$ cells/kg of donor collected with the first apheresis. A t-test was used to analyze the difference in the CD34$^+$ cell count in PB and the number of CD34$^+$ cells/kg of donor between male and female donors. Three subgroups were established regarding the genotype for each polymorphism (homozygous more frequent, heterozygous, and homozygous less frequent), which were clustered afterwards into two subgroups, regarding the presence or absence (in either homozygosis

Table 2. SNP associated with levels of CD34$^+$ cells after G-CSF: associations of SNP with the number of CD34$^+$ cells in PB, with the CD34$^+$cells/kg of donor and with the total yield of CD34$^+$ cells after the first apheresis.

	Genotype frequencies	CD34$^+$ cells/μL PB Median [range]	*P*	CD34$^+$ cells /kg donor (frequency) Median [range] $\times 10^6$	*P*	CD34$^+$ cells total yield (frequency) Median [range] $\times 10^6$	*P*
	TT (63)	99 [21-267]		(61) 6.2 [1.4-23.6]		(63) 475 [84-2006]	
	TC (41)	105 [38-257]	0.29	(39) 6.8 [2.2-16]	0.3	(41) 525 [141-1074]	0.3
	CC (8)	74 [53-141]		(8) 4.6 [3.6-7]		(8) 355 [244-302]	
rs1041163 (*VCAM1*)							
	CC (8)	74 [53-141]		(8) 4.6 [3.6-7]		(8) 355 [244-546]	
	TT + TC (104)	102 [21-267]	0.018	(100) 6.3 [1.4-23.6]	<0.001	(104) 495 [84-2006]	0.002
	CC (57)	105 [21-265]		(55) 7 [1.4-23.6]		(57) 525 [84-2006]	
	CT (47)	88 [30-267]	0.1	(45) 6 [1.6-16]	0.06	(47) 424 [120-960]	0.07
	TT (7)	92 [43-143]		(7) 5 [2.4-10.2]		(7) 400 [180-765]	
rs13347 (*CD44*)							
	CC (57)	105 [21-265]		(55) 7 [1.4-23.6]		(57) 525 [84-2006]	
	CT+TT (54)	90 [30-267]	0.039	(52) 5.6 [1.6-16]	0.025	(54) 422 [120-960]	0.02
	TT (44)	81 [21-176]		(43) 6[1.4-13.3]		(44) 451 [84-868]	
	TC (57)	106 [40-267]	0.015	(54) 6.3 [2.5-23.6]	0.07	(57) 510 [188-2006]	0.057
	CC (11)	99 [31-252]		(11) 5.9 [2-12.4]		(11) 432 [141-806]	
rs3917924 (*CSF3R*)							
	TT (44)	81 [21-176]		(43) 6 [1.4-13.3]		(44) 451 [84-868]	
	TC+CC (68)	106 [31-267]	0.002	(65) 6.3 [2-23.6]	0.08	(68) 495 [141-2006]	0.146
	GG (59)	95 [21-238]		(59) 6.3 [1.4-23.1]		(59) 491 [84-1884]	
	GA (46)	101 [81-267]	0.042	(44) 6 [2-17.9]	0.28	(46) 433 [161-972]	0.056
	AA (7)	165 [45-265]		(7) 11 [1.6-23.6]		(7) 806 [141-2006]	
rs1801157 (*CXCL12*)							
	AA(7)	165 [45-265]		(7) 11 [1.6-23.6]		(7) 806 [141-2006]	
	GG+GA (105)	98 [21-267]	0.018	(101) 6.2 [1.4-23.1]	0.4	(105) 473 [84-1864]	0.3
	AA (43)	90 [21-257]		(43) 5.3 [1.4-17.3]		(43) 399 [84-1074]	
	AT (53)	108 [30-267]	0.3	(50) 6.9 [2-23.6]	0.06	(53) 546 [120-2006]	0.035
	TT (16)	100 [49-184]		(15) 6.3 [2.2-23.1]		(16) 529 [167-810]	
rs2680880 (*CXCR4*)							
	AA (43)	90 [21-257]		(43) 5.3 [1.4-17.3]		(43) 399 [84-1074]	
	AT + TT (65)	105 [30-267]	0.15	(65) 6.7 [2-23.6]	0.019	(69) 533 [120-2006]	0.012

or heterozygosis) of the SNP allele. Homogeneity and normality in the variables of the study were checked using Levene's test and the Shapiro-Wilk test, respectively. Comparisons between three subgroups were made by one-way analysis of variance, followed by Bonferroni's test for the identification of statistically distinct groups. Comparisons between two subgroups were performed with a t-test, using Levene's test to check for homogeneity of the variances. Age, gender and genetic polymorphisms were considered as independent variables, and CD34+ cell count in PB, number of CD34+ cells/kg of donor and the total number of CD34+ cells after the first apheresis were considered as dependent variables. Multivariate linear and multivariate logistic regression analyses were performed, including those variables showing an association or trend in the univariate analysis (P<0.1). Real time PCR results of mRNA of the different genes were analyzed statistically by either the Kruskal-Wallis test or Mann-Whitney U test, making comparisons among the different subgroups. The effect of G-CSF administration on gene expression was also analyzed by the Mann-Whitney U test. Differences were considered statistically significant when P values were less than 0.05. All statistical analyses were performed using the SPSS software v 15.0 (Chicago, IL, USA).

Results

Clinical and hematologic correlations with the number of CD34+ cells in peripheral blood in steady state and after granulocyte colony-stimulating factor

The number of CD34+ cells/μL in PB after 5 days of G-CSF, CD34+ cells ×10^6/kg of donor and total CD34+ cell count x 10^6 obtained after the first apheresis were: median [range] 99 [21-267], 6.3 [1-24] and 477 [84-2006], respectively. The median number [range] of CD34+ cells/μL in PB in steady state was 5.7 [1-51] (*Online Supplementary Table S3*). There was no correlation between CD34+ cell count in PB before or after G-CSF with age or gender. However, the number of CD34+ cells/kg of donor after the first apheresis was negatively correlated with age (r=-0.21, P=0.03) (Table 1).

Single nucleotide polymorphisms and CD34+ cell numbers

Single nucleotide polymorphisms associated with CD34+ cell count in peripheral blood after granulocyte colony-stimulating factor, with CD34+ cells/kg of donor and with total CD34+ cells after the first apheresis

Two out of 28 SNP tested, one in *VCAM1* and one in *CD44*, were significantly associated with CD34+ cell count in PB after G-CSF (Table 2). Both were in Hardy-Weinberg equilibrium. The genetic variant CC rs1041163 in *VCAM1,* corresponding to homozygous less frequent, was associated with a lower CD34+ cell count in PB after G-CSF (P=0.02), with a lower number of CD34+ cells/kg of donor after the first apheresis (P<0.001), and with the total number of CD34+ cells collected after the first apheresis (P=0.002) (Figure 1A and Table 2). The genetic variant CC rs13347 in *CD44*, corresponding to the homozygous most frequent, was associated with a higher CD34+ cell count in PB (P=0.04), with a higher number of CD34+ cells/kg of donor (P=0.025), and with the total number of CD34+ cells after the first apheresis (P=0.02) (Figure 1B and Table 2). Finally, multivariate logistic regression analysis was performed including the SNP with greatest clinical importance and the age of donor as independent variables, and the median value for CD34+ cells/kg as a dependent variable. rs1041163 in *VCAM1* was the variable with the greatest impact on the number of CD34+ cells/kg of donor (P=0.05).

Single nucleotide polymorphisms associated only with the CD34+ cell count in peripheral blood after granulocyte colony-stimulating factor

Two SNP were associated with CD34+ cell count in PB after G-CSF without influencing the number of CD34+ cells/kg of donor after the first apheresis. The genetic variant TT rs3917924 in *CSF3R*, corresponding to homozygous most frequent, was associated with lower CD34+ cell count after G-CSF (P=0.002), showing a trend to an association with the number of CD34+ cells/kg of donor after the first apheresis (P=0.08) (Figure 1C and Table 2). The genetic variant AA rs1801157 in *CXCL12*, corresponding to homozygous less frequent, was associated with a higher CD34+ cell count in PB after G-CSF (P=0.02) (Figure 1D). Finally, multivariate linear regression analysis including these two SNP, the previous ones (in *VCAM1* and in *CD44*), and the age of donor showed that rs3917924 in *CSF3R* was the variable with the greatest impact on the CD34+ cells in PB (P=0.02, B=-27). None of these SNP was associated with CD34+ cell count in PB in steady state.

Single nucleotide polymorphisms associated with number of CD34+ cells/kg of donor and with total CD34+ cells after the first apheresis

The genetic variant AA rs2680880 in *CXCR4*, corresponding to homozygous most frequent, was significantly associated with a lower number of CD34+ cells/kg of donor after the first apheresis (P=0.02) and with the total number of CD34+ cells collected after the first apheresis (P=0.01). This variant showed a tendency to an association with lower CD34+ cell levels in PB after G-CSF (P=0.15) (Figure 1D and Table 2).

Finally, results obtained for the different SNP associated with the amount of CD34+ cells after G-CSF were validated in a different center (Hospital Clinic, Barcelona). The characteristics and apheresis results of this population are shown in *Online Supplementary Table S6*. The apheresis results in this population were very different, with much lower apheresis yields than in the first population (P<0.001), which may be due to differences in the apheresis process. SNP associated with the number of CD34+ cells in PB/μL after G-CSF in the first cohort (SNP in *VCAM1*, *CSF3R*, *CD44* and *CXCL12*) presented the same pattern in both cohorts for mean values of CD34+ cells in PB/μL after G-CSF (*Online Supplementary Figure S1*).

Association of single nucleotide polymorphisms with gene expression

In order to determine whether CC rs1041163 in *VCAM1*, CC rs13347 in *CD44*, TT rs3917924 in *CSF3R*, AA rs1801157 in *CXCL12*, and AA rs2680880 in *CXCR4* had any influence on gene expression, mRNA levels of these genes were quantified in PB in steady state and after G-CSF. No differences were observed in mRNA expression among the different *VCAM1* genotypes at steady state levels. However, *VCAM1* mRNA was undetected in PB, in any of the different genotypes, after G-CSF.

No differences were observed in mRNA expression among the different *CD44* genotypes at steady state levels. G-CSF caused a global decrease in the expression of CD44

(P<0.001), this effect being significant in subjects with the CC (P=0.02) and CT variants (P=0.009), but not in those with the TT variant, the group with the lowest CD34$^+$ cell yields (Figure 2A).

As regards *CSF3R*, G-CSF increased mRNA expression by 26-fold in the group with the TT genotype (P=0.001), without causing significant changes in the groups with the other *CSF3R* variants. Overall quantification of mRNA levels for CSF3R before and after G-CSF, showed a global increased expression of *CSF3R* (P=0.005) (Figure 2B).

For *CXCL12*, with respect to donors with AG and GG variants, those with the AA genotype showed the lowest expression after G-CSF (P=0.03 for the comparison of the three genotypes and P=0.007 in the recessive model) (*data not shown*). Overall quantification of mRNA levels for CXCL12 showed a global decreased expression for *CXCL12* after G-CSF (P=0.002). The AA genotype showed 14-fold lower mRNA expression after G-CSF (P=0.027), without significant changes in the other rs1801157 *CXCL12* variants (Figure 2C).

In order to examine whether different genotypes in rs2680880 in *CXCR4* influenced the two transcript variants of *CXCR4* to different degrees and whether the effect of G-CSF differed in the two variants, mRNA levels of the two transcript variants were amplified. Variant 1 did not differ among the different genotypes either at steady state or after G-CSF. Variant 2, at steady state, showed a trend towards higher mRNA expression in the TT genotype with respect to AA genotype (P=0.06); this difference was statistically significant after G-CSF (P=0.02) (*data not shown*). Quantification of mRNA levels for the two variants, before and after G-CSF, showed overall decreased expression for both variants, with a more prominent effect in variant 2 (P=0.001 and P<0.001 for variant 1 and variant 2, respectively). A comparison of the effect of G-CSF in the different genotype groups showed a reduction of CXCR4 variant 2 in the TT and AT genotypes (P=0.01 and P<0.001, respectively) but not in the AA genotype which represents the group with the lowest CD34$^+$ cell yield. Furthermore, the effect of G-CSF in variant 1 was only detected in the AT genotype (P=0.004), the group with the highest CD34$^+$ cell yield (Figure 2D).

Association of multiple single nucleotide polymorphisms with the number of CD34$^+$ cells in peripheral blood

A combinatory analysis of SNP was performed with the allelic variants associated with a higher number of CD34$^+$ cells/μL of PB after G-CSF. Results showed a combination of T allele in *VCAM1*, C allele in *CD44*, C allele in *CSF3R*, A allele in *CXCL12* and T allele in *CXCR4* with a difference in the mean values of CD34$^+$ cells/μL PB of 72 (95% CI, 42-104) (P<0.0001) with respect to the most frequent allele combination (*Online Supplementary Figure S2*). The estimated frequency of this allelic combination was 0.052.

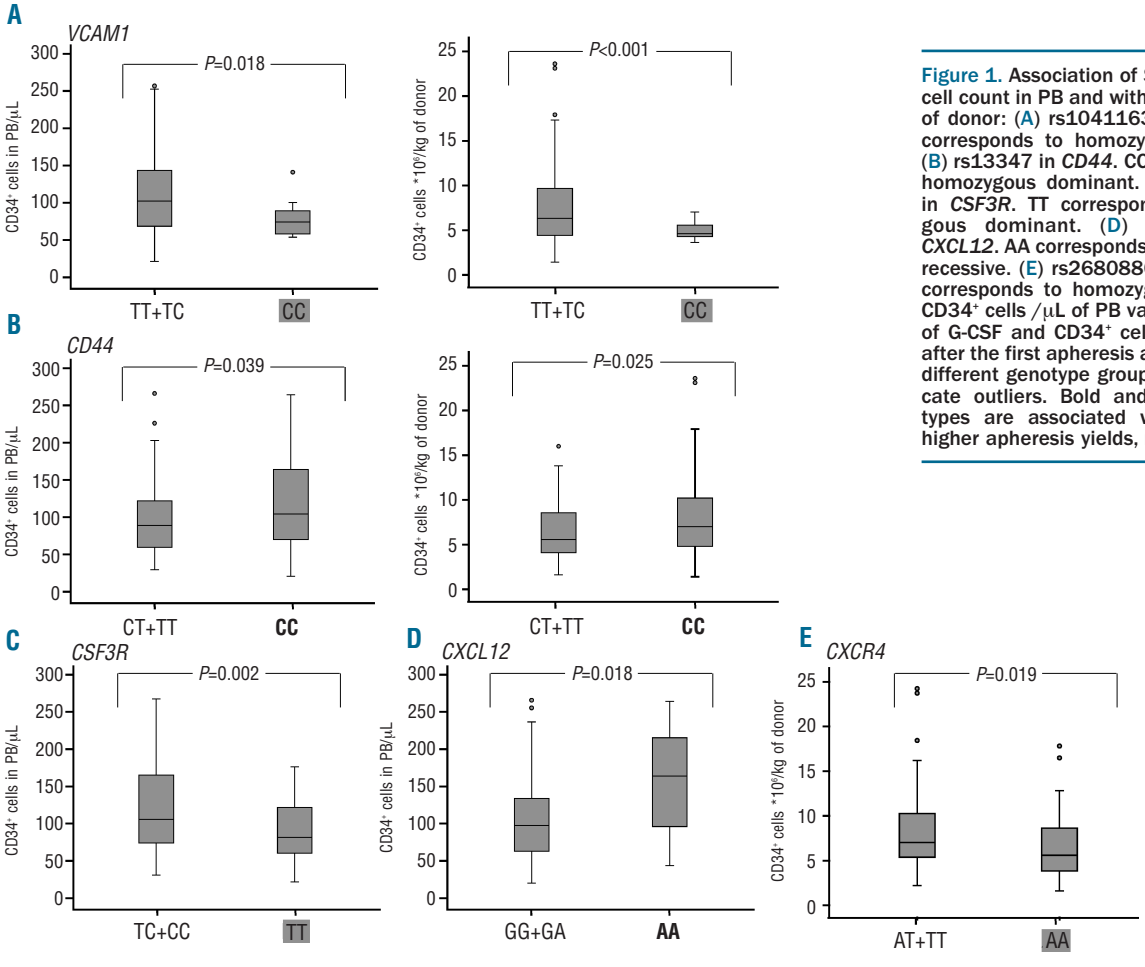

Figure 1. Association of SNP with CD34$^+$ cell count in PB and with CD34$^+$ cells/kg of donor: (A) rs1041163 in *VCAM1*. CC corresponds to homozygous recessive. (B) rs13347 in *CD44*. CC corresponds to homozygous dominant. (C) rs3917924 in *CSF3R*. TT corresponds to homozygous dominant. (D) rs1801157 in *CXCL12*. AA corresponds to homozygous recessive. (E) rs2680880 in *CXCR4*. AA corresponds to homozygous dominant. CD34$^+$ cells /μL of PB values at fifth day of G-CSF and CD34$^+$ cells /kg of donor after the first apheresis are shown in the different genotype groups. * and • indicate outliers. Bold and shaded genotypes are associated with lower and higher apheresis yields, respectively.

Discussion

In the present study, genetic variants in *VCAM1* (VCAM1-1591C), in *CD44* (CD44-2392C), in *CSF3R* (TT rs3917924), and in *CXCL12* (CXCL12-801A) were associated with the number of G-CSF-mobilized CD34+ cells in PB. VCAM1-1591C, CD44-2392C, and CXCR4-40A (rs2680880) were associated with the number of CD34+ cells/kg of donor and with the total number of CD34+ cells obtained with the first apheresis.

VCAM1 is essential for hematopoietic stem cell homing.[12] It is expressed on stromal cells and in a subset of hematopoietic stem cells, the long-term hematopoietic stem cells.[13] VCAM1 and other adhesion molecules and integrins in BM are responsible for dynamic cell-cell interactions which influence the fate of CD34+ cells.[14] G-CSF first increases *VCAM1* expression in BM promoting adhesion of CD34+ cells[12] and then, 5 days after G-CSF administration, *VCAM1* expression in BM decreases, which facilitates the egress of CD34+ cells into the PB.[15] We observed a complete disappearance of *VCAM1* expression in PB after G-CSF administration. This reduced expression in BM and in PB could lead to continuous circulation of CD34+ cells. In contrast to our results, Ulyanova *et al*. detected increased *VCAM1* expression in PB after G-CSF. However, their results were obtained in mice, not in humans, and mechanisms mediating hematopoietic progenitor cell release may differ between species.[9] Interestingly, the VCAM1-1591C allele has been associated with a decrease in both total white blood cells and CFU-GEMM progenitor cells among benzene-exposed workers,[16] suggesting that it may be a factor in the regulation of hematopoiesis.

CD44 is a ubiquitously expressed transmembrane glycoprotein that regulates cell adhesion, movement and activation of cells. Depending on the molecules to which it binds, different responses are generated.[17,18] Different variant isoforms are generated by alternative RNA splicing[19] and a variety of microRNA affects its expression in cancer.[20] After G-CSF mobilization, CD44 levels are diminished on CD34+ cells relative to BM-resident CD34+ cells.[21] We observed an overall decrease of CD44 mRNA expression after G-CSF; however, this effect was not detected in individuals carrying the recessive TT genotype, who had the lowest mobilization yields. This scarce reduction of *CD44* expression could be influential in retaining CD34+ cells in BM and lead to a lower mobilization yield. The mechanism of this effect in donors carrying the CD44-2392C SNP is unknown, but the position of the SNP in the 3'UTR region makes it sus-

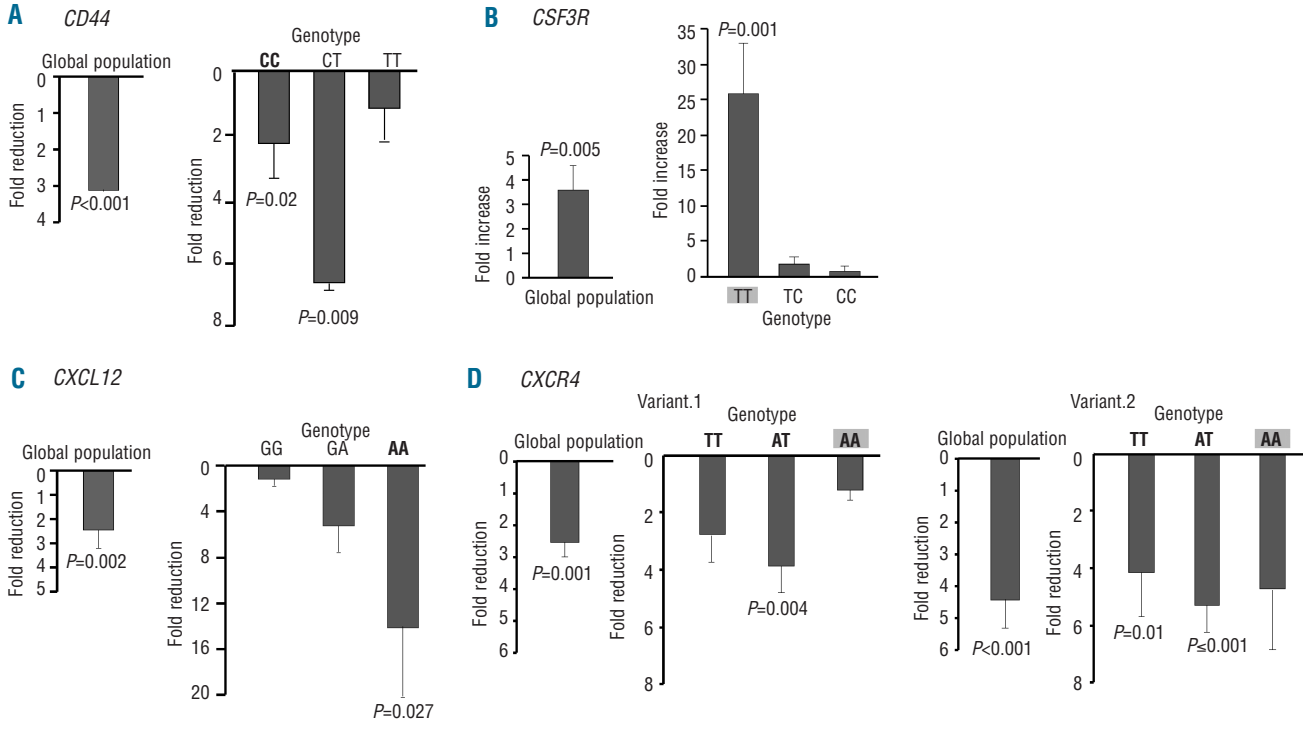

Figure 2. Effect of G-CSF on mRNA levels of CD44, CSF3R, CXCL12 and CXCR4 in PB. (A) Reduction of *CD44* expression in the global population and in the different genotype groups of rs13347 in *CD44* after G-CSF. (C) Increase of *CSF3R* expression after G-CSF in the global population and in the different genotype groups of rs3917924 in *CSF3R*. (D) Decrease of *CXCL12* expression after G-CSF in the global population and in the different genotype groups of rs1801157 in *CXCL12*. (E) Reduction of expression after G-CSF of *CXCR4* variant.1 and variant.2 in the global population and in the different genotype groups of rs2680880 in *CXCR4*. Shaded and bold genotypes are associated with lower and higher amounts of CD34+ cells in PB after G-CSF administration, respectively. In all the cases, genotype groups at steady sate were used as calibrators. In the case of the global population, the mean of all steady state values was used as a calibrator. For the different genotypes, each genotype after G-CSF was calibrated with the value of the corresponding genotype at steady state. All data were normalized with β-actin. Data are given as mean ± S.E.M. All expression values are linear values relative to the calibrator group. Calibrator groups have a value equal to one; fold reduction or fold increase of the gene expression obtained is relative to that value.

ceptible to a different degree of mRNA degradation among the genetic variants and deserves further study. Thus, we show for first time that genetic variants in *VCAM1* and *CD44* affect the number of CD34+ cells collected by apheresis after G-CSF administration.

G-CSF induces CD34+ cell mobilization via stimulation of CSF3R, which is present in both CD34+ cells and neutrophil granulocytes, and has been found to have important biological functions. The currently accepted explanation for G-CSF-induced CD34+ cell mobilization is that this drug enriches the BM microenvironment with proteolytic enzymes released by neutrophils, which cleave the adhesion of CD34+ cells to the BM microenvironment.[22] However, mice deficient in neutrophil proteases exhibit normal CD34+ cell mobilization in response to G-CSF,[23] suggesting other possible mechanisms for CD34+ cell mobilization. G-CSF may act as a powerful chemotactic agent for human CD34+ cells, a function mediated through CSF3R.[24] Moreover, the relatively minor importance of blood G-CSF level for effective CD34+ cell mobilization suggests that its ligand, CSF3R, may play a more significant role.[7] In addition to CD34+ cell mobilization and myeloid development, G-CSF and CSF3R are involved in differentiation of adult neural stem cells, an effect which appears to be specifically mediated through CSF3R.[25,26] These substantial functions of CSFR3 would explain the biological impact of a genetic polymorphism in *CSF3R*. We detected increased CSF3R mRNA expression after G-CSF administration in those individuals with the TT variant in *CSF3R*. However, whether this increase in mRNA CSF3R expression is the reason for the poor hematopoietic stem cell mobilization is presently unclear. The biological impact of the CSF3R SNP could also be due to its capacity to modify the functional properties or the sensitivity of CSF3R to G-CSF.

The association of CXCL12-801A with a higher number of G-CSF-mobilized CD34+ cells has been reported in healthy donors[27] and in patients.[28] The CXCL12/CXCR4 interaction plays an important role in the homing of hematopoietic progenitor cells and their egress to PB during situations of stress.[29] The mechanisms leading to CD34+ cell mobilization involve decreased CXCL12 expression in both BM and PB.[1,30] CXCL12-801A has been linked to both higher[31] and lower[32] CXCL12 protein plasma levels. We found that under steady state conditions, CXCL12-801A was not associated with different mRNA expression levels. However, after G-CSF, the CXCL12-801A allele was related to much lower mRNA *CXCL12* expression than the other genetic variants. The CXCL12-801A allelic variant is a result of the rs1801157 SNP, which is located in a highly demethylated area of the 3'UTR region. This SNP confers a G to A transition in nucleotide position 801, resulting in a loss of a methylation site which could affect the methylating effect of G-CSF,[33] and leading to a greater decrease in *CXCL12* expression in the individuals carrying the polymorphism. Our results showing this reduced *CXCL12* expression only in individuals carrying the CXCL12-801A allele and only after G-CSF would support this hypothesis. Of note, the CXCL12-801A allelic variant has been associated with a higher incidence of breast and lung cancer, acute myeloid leukemia, lymphoma and chronic myeloproliferative disease, and with a slower progression to AIDS.[31,34-38]

An interaction between CXCL12 and CXCR4 is critical for the trafficking of hematopoietic progenitor cells in the BM, and an interruption of this interaction can affect CD34+ cell mobilization by G-CSF.[1] New mechanisms of mobilization using AMD3100 as a CXCR4 antagonist have shown efficacy in healthy volunteers and in patients.[39,40] CXCR4 has two isoforms due to alternative splicing. Variant 2 contains a shorter N-terminus compared to variant 1. The rs2680880 SNP in *CXCR4* is located in an intron at the 5'UTR region close to the region where the alternative splicing of the two variants takes place. High-affinity binding to CXCL12 requires the extracellular N-terminal domain of CXCR4,[41] this binding is mediated through the first 38 amino acids at the N-terminus of CXCR4 (p38) present in both variants, where three Tyr residues play important roles.[42] Previous studies of the structure responsible for the binding of CXCR4 to CXCL12[41,42] have considered only variant 2. We reasoned that G-CSF may have a differential effect on expression levels of the two *CXCR4* variants and found that while the G-CSF effect was found for both variants, it was much more pronounced for variant 2, indicating a more important role for this variant in G-CSF mobilization. Due to the longer N-terminus of variant 1 and the involvement of the N-terminus of CXCR4 in the binding of CXCL12 to CXCR4,[42] we suggest that the longer N-terminus could cause a stronger interaction of CXCR4 variant 1 with CXCL12 leading to a lower mobilization. We also reasoned that the three *CXCR4* genotypes could have a differential effect on the expression of the two isoforms. The lower levels of variants 1 and 2 in the AA genotype indicate a lesser effect of G-CSF, leading to lower mobilization. The TT genotype, with higher levels of variant 2, mobilizes less than AT genotype. This could be due to an additive effect of the reduction of the expression of the two variants in the AT genotype, which has the same levels as the two variants at steady state; this would, in turn, lead to greater mobilization in the AT genotype than in the TT genotype, where only the effect of variant 2 is observed. We, therefore, hypothesize that the rs2680880 SNP in *CXCR4* could modulate the alternative splicing in *CXCR4* to produce lower expression of *CXCR4* variant 2 in the AA genotype.

In summary, the present study has shown that SNP in genes involved in homing and migration of CD34+ cells are strongly related to the degree of CD34+ cell mobilization after G-CSF. Moreover, some of these SNP are associated with a differential level of mRNA expression. We have shown for the first time that genetic variants in *VCAM1* and *CD44* are related to the number of CD34+ cells collected after G-CSF administration. We also report the effect of G-CSF on a genetic variant in *CD44* influencing the number of CD34+ cells collected. We note the influence of a genetic variant in *CSFR3* on the number of CD34+ cells mobilized by G-CSF, and that the previously reported association of the CXCL12-801A allele with the number of CD34+ cells after G-CSF administration[27] might be due to a dramatic G-CSF-induced decrease in gene expression in donors with this genetic variant.

Authorship and Disclosures

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