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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Supplementary Material

Design and Methods

Genomic DNA isolation and allelic discrimination polymerase chain reaction

DNA was isolated from 100 μL of apheresis product or 200 μL of peripheral blood (PB) isolated by centrifugation on Ficoll-Hypaque (Lymphoprep Axis-Shield, PoC AS, Oslo, Norway) from 3 mL of PB collected into EDTA. A QIAamp DNA Blood Mini Kit (QIAgen, Germany) was used according to the manufacturer's instructions. The concentration and purity of DNA were determined by absorbance readings at a wavelength of 260 nm and a ratio of 260/280 nm, respectively, with Nanodrop, ND-1000 (Wilmington, DE, USA). Allelic variants were genotyped by allelic discrimination polymerase chain reaction (PCR) assays using TaqMan® Genotyping Assays (PE Applied Biosystems, Foster City, CA, USA). Specific primers and FAM™ and VIC™ dye-labeled probes were ordered by either Custom Taqman® SNP Genotyping Assays using File Builder Software v3.0 or Taqman® SNP Assays Applied Biosystems service. PCR reactions were carried out in 96-well plates at a final volume of 12.5 μL containing 50 ng of genomic DNA, 6.25 μL 1 x Genotyping Master Mix and 0.25 μL 1 x SNP Assay. Negative controls were also run. PCR reactions were performed on an ABI Prism 7500 FAST Real Time PCR System (Applied Biosystems, Foster City, CA, USA). Conditions were as follows: initial denaturation at 95°C for 10 min, 45 cycles of denaturation (93°C for 15 sec) followed by annealing and elongation (60°C for 1 min). The software determined the fluorescent signal from the FAM™ and/or VIC™ labeled probe.

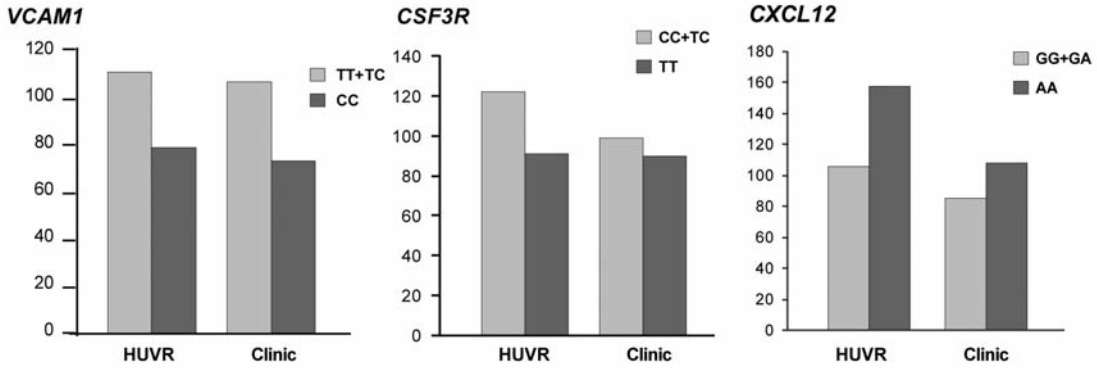
RNA isolation and gene expression analysis

Total RNA was isolated from 100 μL of apheresis product using a RNeasy Blood Mini Kit (Qiagen, Valencia, CA), and from 800 μL of a leukocyte concentrate obtained by a density gradient separation on Ficoll-Hypaque from 3 mL of PB collected into EDTA using TRIzol (Invitrogen Ltd, Paisley, UK), both according to the manufacturers' protocol. RNA isolated was treated twice with *DNase I* using the RNase-Free DNase kit (QIAgen, Germany) for 20 min. RNA integrity was checked on a 2% agarose gel. The concentration, purity and quality of total RNA were determined by spectrophotometry using Nanodrop ND-1000. Total RNA (1 μg) from each sample was reverse-transcribed using the iScript™ cDNA Synthesis kit (Bio-Rad). Real-time PCR analysis was carried out using an iCycler (Strategene Mx 3005P™). Reactions were done in a 25 μL volume containing cDNA generated from 10 ng of original RNA template, 12.5 μL of iQTM SYBR Green Supermix (Bio-Rad) and 300 nM each of specific forward and reverse primers. The primer sequences were as follows: ACTBFw/ACTBRv: 5'ggacttcgagcaagagatgg3'/5'agcactgtgttggcgtacag3', VCAM1Fw/VCAM1Rv: 5'aaaagcggagacaggagaca/3'agcacgagaagctcaggaga, CD44Fw/CD44Rv: 5'gggtctgccagaggcccaga3'/5'cagcctctacctgtcgcccca3', CSF3RFw/CSF3RRv: 5'ggaagacagcggacggat3'/5'tctgagaagaccaccggagtg3', CXCL12Fw/CXCL12Rv: 5'actctccgtcagccgcattg3'/5'aatcggcatgggcatctgtag3', CXCR4Fw.1/CXCR4Rv.1: 5'tgtccattcctttgcctctttt3'/5'agaggaggtcggccactgaca3', CXCR4Fw.2/CXCR4Rv.2: 5'ggaggggatcagtatatacac3'/5'acaatgccagttaagaa3'; for *β-actin*, *VCAM1*, *CD44*, *CSF3R*, *CXCL12* and *CXCR4* variant1 and variant 2, respectively. Matching oligonucleotide primers were designed using Oligo *v* 6.89 software (Medprobe). The amplification protocol used was as follows: initial denaturation and enzyme activation for 3 min 30 sec at 95 °C, followed by 40 cycles of 95 °C for 15 sec, optimal annealing temperature of 68°C for 15 sec and elongation at 72°C for 30 sec. Each assay was done in duplicate. For normalization of cDNA loading, all samples were run in parallel with the housekeeping gene *β-actin*. Relative mRNA expression was determined using the $2^{(-\Delta\Delta Ct)}$ method and calibrating each SNP with the genotype at steady state.[22,23]

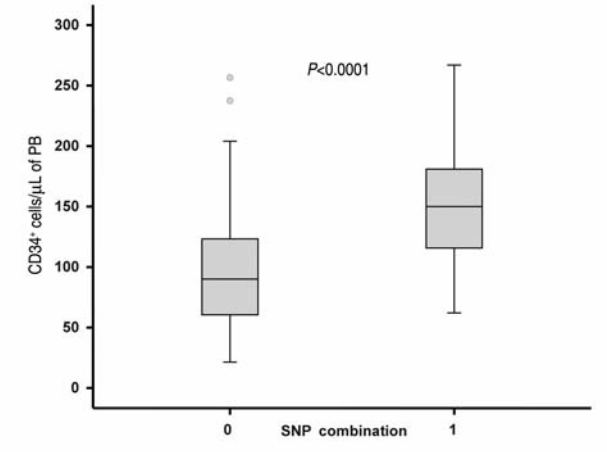
References

1. Gerli G, Vanelli C, Turri O, Erario M, Gardellini A, Pugliano M, et al. SDF1-3'A gene polymorphism is associated with chronic myeloproliferative disease and thrombotic events. Clin Chem. 2005;51(12):2411-4.
2. Winkler C, Modi W, Smith MW, Nelson GW, Wu X, Carrington M, et al. Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant. ALIVE Study, Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC). Science. 1998;279(5349):389-93.
3. Razmkhah M, Doroudchi M, Ghayumi SM, Erfani N, Ghaderi A. Stromal cell-derived factor-1 (SDF-1) gene and susceptibility of Iranian patients with lung cancer. Lung Cancer. 2005;49(3):311-5.
4. Zafiropoulos A, Crikas N, Passam AM, Spandidos DA. Significant involvement of CCR2-64I and CXCL12-3a in the development of sporadic breast cancer. J Med Genet. 2004;41(5):e59.

5. de Oliveira CE, Cavassin GG, Perim AL, Nasser TF, de Oliveira KB, Fungaro MH, et al. Stromal cell-derived factor-1 chemokine gene variant in blood donors and chronic myelogenous leukemia patients. J Clin Lab Anal. 2007;21(1):49-54.
6. Dommange F, Cartron G, Espanel C, Gallay N, Domenech J, Benboubker L, et al. CXCL12 polymorphism and malignant cell dissemination/tissue infiltration in acute myeloid leukemia. FASEB J. 2006;20(11):1913-5.
7. Maley SN, Schwartz SM, Johnson LG, Malkki M, Du Q, Daling JR, et al. Genetic variation in CXCL12 and risk of cervical carcinoma: a population-based case-control study. Int J Immunogenet. 2009;36(6):367-75.
8. Lan Q, Zhang L, Shen M, Smith MT, Li G, Vermeulen R, et al. Polymorphisms in cytokine and cellular adhesion molecule genes and susceptibility to hematotoxicity among workers exposed to benzene. Cancer Res. 2005;65(20):9574-81.
9. Wang SS, Carreon JD, Hanchard B, Chanock S, Hisada M. Common genetic variants and risk for non-Hodgkin lymphoma and adult T-cell lymphoma/leukemia in Jamaica. Int J Cancer. 2009;125(6):1479-82.
10. Cruz M, Valladares-Salgado A, Garcia-Mena J, Ross K, Edwards M, Angeles-Martinez J, et al. Candidate gene association study conditioning on individual ancestry in patients with type 2 diabetes and metabolic syndrome from Mexico City. Diabetes Metab Res Rev. 2010;26(4):261-70.
11. Fan AZ, Yesupriya A, Chang MH, House M, Fang J, Ned R, et al. Gene polymorphisms in association with emerging cardiovascular risk markers in adult women. BMC Med Genet. 2010;11:6.
12. Gómez-Gallego F, Ruiz JR, Buxens A, Altmäe S, Artieda M, Santiago C, et al. Are elite endurance athletes genetically predisposed to lower disease risk? Physiol Genomics. 2010;41(1):82-90.
13. Santiago C, Ruiz JR, Buxens A, Artieda M, Arteta D, González-Freire M, et al. Trp64Arg polymorphism in ADRB3 gene is associated with elite endurance performance. Br J Sports Med. 2010 Jun 11. [Epub ahead of print]
14. Correia C, Coutinho AM, Almeida J, Lontro R, Lobo C, Miguel TS, et al. Association of the alpha4 integrin subunit gene (ITGA4) with autism. Am J Med Genet B Neuropsychiatr Genet. 2009;150B(8):1147-51.
15. O'Doherty C, Roos IM, Antiguedad A, Aransay AM, Hillert J, Vandenbroeck K. ITGA4 polymorphisms and susceptibility to multiple sclerosis. J Neuroimmunol. 2007;189(1-2):151-7.
16. Hsing AW, Sakoda LC, Rashid A, Andreotti G, Chen J, Wang BS, et al. Variants in inflammation genes and the risk of biliary tract cancers and stones: a population-based study in China. Cancer Res. 2008;68(15):6442-52.
17. Hirose Y, Chiba K, Karasugi T, Nakajima M, Kawaguchi Y, Mikami Y, et al. A functional polymorphism in THBS2 that affects alternative splicing and MMP binding is associated with lumbar-disc herniation. Am J Hum Genet. 2008;82(5):1122-9.
18. Chen HY, Lin WY, Chen YH, Chen WC, T. FJ, Tsai CH. Matrix metalloproteinase-9 polymorphism and risk of pelvic organ prolapse in Taiwanese women. Eur J Obstet Gynecol Reprod Biol. 2010;149(2):222-4.
19. Ahluwalia TS, Khullar M, Ahuja M, Kohli HS, Bhansali A, Mohan V, et al. Common variants of inflammatory cytokine genes are associated with risk of nephropathy in type 2 diabetes among Asian Indians. PLoS One. 2009;4(4):e5168.
20. Yang W, White B, Spicer EK, Weinstein BL, Hildebrandt JD. Complex haplotype structure of the human GNAS gene identifies a recombination hotspot centred on a single nucleotide polymorphism widely used in association studies. Pharmacogenetics. 2004;14(11):741-7.
21. Jia H, Hingorani AD, Sharma P, Hopper R, Dickerson C, Trutwein D, et al. Association of the G(s)alpha gene with essential hypertension and response to beta-blockade. Hypertension. 1999;34(1):8-14.
22. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods. 2001;25(4):402-8.
23. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc. 2008;3(6):1101-8.



Online Supplementary Figure S1. Association of SNP rs1041163 in *VCAM1*, rs3917924 in *CSF3R* and rs1800157 in *CXCL12* with $CD34^+$ cell count in PB after G-CSF administration. Comparative mean values of $CD34^+$ cells/μL of PB at fifth day of G-CSF between the two populations: Hospital Universitario Virgen del Rocío, Seville (HUVR) and Hospital Clinic, Barcelona (Clinic).



Online Supplementary Figure S2. Allelic combination of SNP associated with higher values of $CD34^+$ cells in PB after G-CSF. 1 indicates donors carrying the allelic combination of T allele in *VCAM1*, C allele in CD44, C allele in *CSF3R*, A allele in *CXCL12* and T allele in *CXCR4*. 0 indicates the rest of the donors.

Online Supplementary Table S1. Main characteristics of group 1 donors (the group receiving G-CSF) and of group 2 donors (steady state).

	Donors' characteristics	Median, Range
Group 1, Caucasian Spanish donors	Number	112
	Male: Female	58:54
	Age	38 [12-64]
	Weight	73 [42-121]
	G-CSF	Filgrastim s.c. (10 μg/kg; 5 days)
Group 2, Caucasian Spanish donors	Number	107
	Male:Female	33:74
	Age	34 [18-68]
	Weight	69 [40-110]
	G-CSF	No

Online Suppplementary Table S2. Candidate genes and SNP selected for the study. Name, rs code, and position are denoted according to the NCBI database. Association studies described for each SNP are indicated.

Gene	SNP (rs code)	Location	Amino acid change	Associations previously described
CXCL12	rs1801157	3'UTR Ex4 A>G		Higher incidence of breast and lung cancer, acute myeloid leukemia, lymphoma and chronic myeloproliferative disease, and slower progression to AIDS [1-6]
	rs1029153	3'UTR Ex4 C>T		No association described
	rs754618	5'UTR A>G		Increased risk of infection in HIV patients[6]
	rs266093	3'UTR Ex 4C>G		With two other different SNP defines an haplotype inversely associated with cervical cancer risk[7]
CXCR4	rs2680880	5'UTR Int 1 A>T		No association described
VCAM1	rs1041163	5'UTR C>T		Decrease in WBC and CFU-GEMM progenitor cells among benzene-exposed workers.[8] Decreased non-Hodgkin lymphoma and adult T-cell lymphoma/leukemia risk[9]
G-CSF	rs1042658	3'UTR Ex 5 C>T		No association described
	rs2827	3'UTR Ex 5 C>T		No association described
	rs2227316	5'UTR C>T		No association described
	rs2227319	5'UTR A>G		No association described
ADRB3	rs4994	Ex 1 C>T	W>R	R variant associated with type 2 diabetes and metabolic syndrome.[10] Higher concentration variations in serum C-reactive protein in adult women.[11] Different allele distribution among non-athletic healthy control subjects and elite endurance athletes[12] and with elite endurance performance[13]
	rs9694197	3'UTR A>G		No association described
CD34	rs658347	3'UTR C>T		No association described
	rs7572	3'UTR Ex 8 C>T		No association described
VLA4	rs1449263	Int 1 A>G		C allele higher levels of a serum autoantibody directed to brain tissue, which is more frequent in autistic patients[14] and weakly increased in multiple sclerosis patients[15]
CSF3R	rs3917924	Int 2 C>T		No association described
	rs3917971	Int 6 G>T		No association described
CXCL2	rs3806792	5'UTR C>T		No association described
CXCR2	rs4674259	5'UTR Ex1 A>G		No association described
	rs1126579	3'UTR Ex3 C>T		This SNP is a haplotype associated with risk of bile duct and billiary stones[16]
CD44	rs9666607	Ex10 A>G	K>R	No association described
	rs13347	3'UTR Ex18 C>T		No association described
KL	rs2046971	Int1 C>G		No association described
c-Kit	rs6554198	5'UTR A>G		No association described
	rs6554199	5'UTR G>T		No association described
MMP-9	rs17576	Ex6 A>G	Q>R	Associated with lumbar-disc herniation;[17] AG and GG genotypes associated with pelvic organ prolapse.[18] Q variant associated with increased risk of type 2 diabetes with nephropathy[19]
CTSG	rs8019787	3'UTR C>G		No association described
GNAS	rs7121	Ex 5 C>T	Synonymous	This SNP is in linkage disequilibrium[20] and the T allele is associated with hypertension[21]

Online Supplementary Table S3. Cell counts in PB in group 1 and in group 2. In group 1, values both before G-CSF and at the fifth day of G-CSF are shown. In group 2, values shown correspond to steady state levels.

Population	Cell count	Median [range]
Group 1		
	WBC count $\times 10^9$/L before G-CSF	6.7 [4-18]
	Platelet count $\times 10^9$/L before G-CSF	235 [84-535]
	WBC count $\times 10^9$/L at 5th day of G-CSF	50.4 [18-94]
	Platelet count $\times 10^9$/L at 5th day of G-CSF	207 [101-421]
	CD34$^+$ cells/μL at 5th day of G-CSF	99.4 [21-267]
	CD34$^+$ cells $\times 10^6$/kg of donor after G-CSF	6.3 [1-24]
	Total CD34$^+$ cells $\times 10^6$ after the 1st apheresis	477 [84-2006]
Group 2		
	WBC count $\times 10^9$/L	5.9 [3-14]
	Platelet count $\times 10^9$/L	196 [24-366]
	CD34$^+$ cells/μL	5.7 [1-51]

Online Supplementary Table S4. SNP not associated with CD34$^+$ cell count/μL of PB and with CD34$^+$ cells/kg of donor after the first apheresis. Genotype frequencies show numerical values for each genotype

SNP (gene)	Genotype frequencies	CD34$^+$ cells /μL of PB Median [range]	*P*	CD34$^+$ cells /kg of donor Median [range]	*P*
rs1029153 (*CXCL12*)					
	CC (63)	102 [30-267]		6.1 [1.6-23.6]	
	CT (37)	95 [21-204]	0.435	6.5 [1.4-23.1]	0.82
	TT (12)	89 [38-238]		6.2 [2.7-15.7]	
rs754618 (*CXCL12*)					
	GG (63)	105 [30-267]		6 [1.6-23.6]	
	GA (38)	96 [21-204]	0.519	6.9 [1.4-23.1]	0.636
	AA (11)	79 [38-238]		6.1 [2.8-15.7]	
rs266093 (*CXCL12*)					
	GG (47)	100 [36-265]		6.2 [1.6-23.6]	
	GC (54)	105 [21-267]	0.407	6.6 [1.4-23.1]	0.31
	CC (11)	69 [30-162]		5 [2-11.8]	
rs1042658 (*G-CSF*)					
	CC (44)	104 [38-204]		6.3 [2.5-23.1]	
	CT (52)	96 [21-267]	0.7	5.8 [1.4-23.6]	0.26
	TT (16)	102 [55-238]		7.1 [3.7-17.9]	
rs2827 (*G-CSF*)					
	CC (73)	97 [30-267]		6.1 [2-23.6]	
	CT (35)	99 [21-252]	0.739	6.3 [1.4-17.3]	0.81
	TT (4)	120 [92-162]		7.8 [5.8-11.8]	
rs2227316 (*G-CSF*)					
	CC (73)	97 [30-267]		6.1 [2-23.6]	
	CT (35)	99 [21-252]	0.739	6.3 [1.4-17.3]	0.81
	TT (4)	120 [92-162]		7.8 [5.8-11.8]	
rs2227319 (*G-CSF*)					
	GG (52)	101 [21-252]		6.3 [1.4-17.9]	
	GA (43)	94 [30-267]	0.988	6 [2-23.6]	0.62
	AA (17)	106 [38-204]		6.2 [3-23.1]	
rs4994 (*ADRB3*)					
	TT (82)	96 [21-267]		6.3 [1.4-23.6]	
	TC (29)	103 [38-257]	0.372	6.1 [2.2-16]	0.585
	CC (1)	102		10.5	
rs9694197 (*ADRB3*)					
	AA (100)	96 [21-267]		6.2 [1.4-23.6]	
	AG (12)	123 [62-204]	0.295	7.6 [2.2-16]	0.78
rs658347 (*CD34*)					
	CC (30)	106 [30-226]		6.2 [2-23.1]	
	CT (53)	102 [21-267]	0.432	6.6 [1.4-23.6]	0.46
	TT (29)	87 [48-252]		6 [2.1-13.2]	

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rs7572 (*CD34*)					
	CC (69)	94 [31-267]		6 [1.6-16]	
	CT (40)	115 [21-265]	0.229	7.2 [1.4-23.6]	0.113
	TT (3)	94 [69-197]		7.1 [5.2-17.3]	
rs1449263 (*VLA4*)					
	AA (38)	105 [43-267]		6.2 [1.6-17.9]	
	AG (40)	101 [21-265]	0.742	7.1 [1.4-23.6]	0.91
	GG (26)	92 [30-227]		5.4 [2-17.3]	
rs3917971 (*CSF3R*)					
	GG (51)	94 [21-265]		6.5 [1.4-23.6]	
	GT (53)	100 [31-267]	0.327	6.2 [1.6-23.1]	0.83
	TT (8)	115 [62-252]		6.3 [2.2-12.4]	
rs3806792 (*CXCL2*)					
	CC (37)	103 [36-267]		5.9 [2.2-12.9]	
	CT (60)	92 [21-265]	0.495	6.3 [1.4-23.6]	0.42
	TT (15)	106 [38-227]		7 [1.6-17.3]	
rs4674259 (*CXCR2*)					
	GG (35)	99 [21-267]		6.9 [1.4-23.1]	
	GA (57)	94 [31-265]	0.673	6 [1.6-23.6]	0.8
	AA (20)	104 [48-252]		6.6 [2.1-17.3]	
rs1126579 (*CXCR2*)					
	CC (34)	100 [21-267]		7 [1.4-23.1]	
	CT (58)	94 [31-265]	0.703	6 [1.6-23.6]	0.92
	TT (20)	103 [48-252]		6.1 [2.1-17.3]	
rs9666607 (*CD44*)					
	GG (57)	99 [30-267]		6.3 [1.6-23.6]	
	GA (48)	99 [21-204]	0.976	5.7 [1.4-21.7]	0.72
	AA (7)	108 [69-176]		8.2 [6.1-12.4]	
rs2046971 (*KL*)					
	GG (69)	99 [30-257]		5.9 [2-17.9]	
	GC (36)	89 [21-267]	0.266	6.6 [1.4-23.6]	0.35
	CC (7)	135 [88-192]		8.4 [6.3-13.8]	
rs6554198 (*c-Kit*)					
	AA (52)	97 [30-267]		6.3 [1.6-23.1]	
	AG (47)	103 [21-267]	0.656	6.5 [1.4-23.6]	0.67
	GG (13)	87 [43-238]		5.1 [2.1-17.9]	
rs6554199 (*c-Kit*)					
	TT (45)	95 [30-267]		6.2 [1.6-23.1]	
	TG (53)	103 [21-265]	0.807	6.7 [1.4-22.2]	0.46
	GG (14)	93 [43-238]		5.2 [2.1-17.9]	
rs17576 (*MMP-9*)					
	AA (48)	98 [21-267]		6.3 [1.4-23.6]	
	AG (51)	105 [30-252]	0.834	6 [2-16]	0.7
	GG (12)	98 [40-184]		7 [1.6-23.1]	
rs8019787 (*CTSG*)					
	GG(28)	104 [21-170]		5.3[1.4-13.3]	
	GC (56)	102 [31-267]	0.12	6.3 [2-23.6]	0.15
	CC (26)	83 [38-227]		7.1 [2.2-17.3]	
rs7121 (*GNAS*)					
	CC (35)	94[21-104]		6.6 [1.4-16]	
	CT (56)	100 [31-267]	0.784	5.8 [1.6-23.1]	0.5
	TT (21)	100 [30-265]		6.7 [2-23.6]	

Online Supplementary Table S4. Effect of the various SNP associated with degree of mobilization.

SNP (gene), position	Effect after G-CSF
rs1041163 (*VCAM1*), 5'UTR	TT genotype: lower $CD34^+$ cells in PB and lower mobilization yield
rs13347 (*CD44*), 3'UTR	CC genotype: higher $CD34^+$ cells in PB and higher mobilization yield
rs3917924 (*CSF3R*), Intron 2	TT genotype: lower $CD34^+$ cells in PB and increased *CSF3R* expression
rs1801157 (*CXCL12*), 3'UTR	AA genotype: higher $CD34^+$ cells in PB and reduction of *CXCL12* expression
rs2680880 (*CXCR4*), 5'UTR	AA genotype: lower mobilization yield

Online Supplementary Table S6. Characteristics and apheresis results of the validation population. *after 1st apheresis.

Parameter	N, median [range]
Number	88
Male:female	49:38
Age, years	48 [20-70]
Weight, kg	74 [53-118]
G-CSF dose	Filgrastim s.c. (16 μg/kg; 5 days)
WBC count $\times 10^9$/L before G-CSF	6.7 [4-11]
Platelet count $\times 10^9$/L before G-CSF	257 [158-388]
WBC count $\times 10^9$/L at 5th day of G-CSF	52 [23-99]
Platelet count $\times 10^9$/L at 5th day of G-CSF	202 [33-555]
$CD34^+$ cells/μL at 5th day of G-CSF*	95 [19-364]
$CD34^+$ cells $\times 10^6$/kg of donor*	4 [1-9]
$CD34^+$ cells $\times 10^6$/kg of recipient*	4 [1-13]
Total $CD34^+$ cells $\times 10^6$*	254 [46-724]

WBC: white blood cell.