

SDF1 Gene Variation Is Associated with Circulating *SDF1* α Level and Endothelial Progenitor Cell Number—The Bruneck Study

Qingzhong Xiao¹, Shu Ye², Friedrich Oberhollenzer³, Agnes Mayr³, Marjan Jahangiri⁴, Johann Willeit⁵, Stefan Kiechl⁵, Qingbo Xu^{1*}

1 Cardiovascular Division, King's College London BHF Centre, London, United Kingdom, **2** Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom, **3** Department of Internal and Laboratory Medicine, Bruneck Hospital, Bruneck, Italy, **4** Department of Cardiothoracic Surgery, St. George's Hospital, London, United Kingdom, **5** Department of Neurology, Medical University of Innsbruck, Innsbruck, Austria

Abstract

Background: Stromal cell-derived factor-1 (*SDF1*) and its receptor CXC chemokine receptor 4 (*CXCR4*) play a critical role in progenitor cell homing, mobilization and differentiation. It would be interesting to assess the predictive value of *SDF1* α level for EPC number, and to ascertain whether there is a relationship between *SDF1* gene variation, plasma *SDF1* α level, and the number and function of circulating EPCs. We also tested whether EPC number and function was related to *CXCR4* gene variation.

Methodology and Principal Findings: We genotyped a cohort of individuals who participated in the Bruneck Study for single nucleotide polymorphisms (SNPs) in the *SDF1* and *CXCR4* genes, and measured blood *SDF1* α level as well as EPC number and function. *SDF1* α levels were correlated with age, gender, alcohol consumption, circulating reticulocyte numbers, and concentrations of matrix metalloproteinase-9, C-reactive protein, cystatin C, fibrinogen and homocysteine. In blood samples taken in 2005, EPC number was inversely associated with *SDF1* α level ($p < 0.001$). EPC number in 2005 was also inversely associated with *SDF1* α level in 2000 ($p = 0.009$), suggesting a predictive value of plasma *SDF1* α level for EPC number. There was an association between the *SDF1* gene rs2297630 SNP A/A genotype, increased *SDF1* α level ($p = 0.002$) and lower EPC number ($p = 0.006$).

Conclusions: Our data indicate that a *SDF1* gene variation (rs2297630) has an influence on *SDF1* α level and circulating EPC number, and that plasma *SDF1* α level is a predictor of EPC number.

Citation: Xiao Q, Ye S, Oberhollenzer F, Mayr A, Jahangiri M, et al. (2008) *SDF1* Gene Variation Is Associated with Circulating *SDF1* α Level and Endothelial Progenitor Cell Number—The Bruneck Study. PLoS ONE 3(12): e4061. doi:10.1371/journal.pone.0004061

Editor: Pieter H. Reitsma, Leiden University Medical Center, Netherlands

Received: September 2, 2008; **Accepted:** December 1, 2008; **Published:** December 30, 2008

Copyright: © 2008 Xiao et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: QX thanks support from the British Heart Foundation and Oak Foundation, and SY thanks support from the British Heart Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: qingbo.xu@kcl.ac.uk

Introduction

Asahara and colleagues in 1997 demonstrated for the first time that purified CD34 positive haematopoietic progenitor cells from peripheral blood could differentiate, ex-vivo, into an endothelial phenotype and were named Endothelial Progenitor Cells (EPCs)[1]. Growing evidence indicates that EPCs play a major role in angiogenesis and vasculogenesis[2,3]. Indeed, mobilized EPCs can promote new blood vessel formation in ischaemic tissues, enhancing perfusion and recovery[2,3]. Ex vivo expanded EPCs isolated from peripheral blood can also incorporate into the site of myocardial neovascularization[4], and intracoronary infusion of peripheral blood or bone marrow-derived progenitors in patients with acute myocardial infarction was shown to significantly enhance post-infarction remodelling[5,6]. Moreover, EPC numbers have a prognostic value and can be used as a predictive biomarker in the cardiovascular diseases[7–9]. Other investigators have also shown reduced EPC number in patients

with risk factors for cardiovascular diseases such as diabetes[10,11] and smoking[12], with cessation of smoking resulting in a return of EPC numbers to normal. EPC number is also reduced in groups of patients known to be at higher risk of cardiovascular disease such as those with rheumatoid arthritis[13] or chronic renal failure[14]. EPC number is also known to be reduced in established non-coronary cardiovascular disorders such as in patients with strokes[15], peripheral vascular disease[10] and patients with erectile dysfunction[16]. However, our recent data from a population-based, longitudinal study refuted the traditional view that the EPC number is negatively related to cardiovascular risk factors. We showed that changes of EPC numbers are loosely associated with certain risk factors for the cardiovascular disease and not directly associate with the disease development[17].

Animal studies have indicated that *SDF1* (also known as CXC chemokine ligand 12) and its receptor CXC chemokine receptor 4 (*CXCR4*) plays a critical role in progenitor cell homing, mobilization and differentiation. Inactivation of the *SDF1* or

CXCR4 gene in mice led to early embryonic lethality due to abnormality in the cerebellar and gastrointestinal vasculature and in hematopoiesis development [18–20]. The number of circulating hematopoietic stem cells (HSCs) or EPCs were increased by *SDF1* gene transfer using the adenovirus infection technique [21–23]. Overexpression of SDF1 in ischemic tissues has been found to enhance EPC recruitment from peripheral blood and to induce neoangiogenesis in ischemic tissues [24,25]. Recent evidence also suggests that SDF1 α is a driving force for EPC differentiation [26]. All these findings from animal studies strongly suggest that SDF1 α has a crucial role in stem/progenitor cell mobilization, differentiation, and injured tissues-specific homing. However, it remained unknown whether SDF1 α played such a role in humans. Therefore, we recently examined the levels of SDF1 α and several angiogenic cytokines in relation to circulating EPC numbers in a population-based study. We found that plasma levels of SDF1 α , but not VEGF or G-CSF, were strongly associated with EPC number and function, suggesting a role of SDF1 α in EPC mobilization and differentiation in humans [17]. In that study, the association of SDF1 α levels with EPC numbers was detected using blood samples taken at the same time point. In the present study, we investigated whether SDF1 α levels had a long-term predictive value for EPC numbers.

There is emerging evidence indicating that variation in the human *SDF1* gene can have an influence on SDF1 α levels [27,28], which was demonstrated clearly to be involved in progenitor cell mobilization and differentiation in human and animal studies. Moreover, previous study showed that EPC number is, at least in part, genetically regulated [29], and the presence of SDF1-3'A allele was a predictive factor of CD34⁺ cell mobilization [30], which prompt us to hypothesize that the SDF-1 gene single-nucleotide polymorphism (SNP) might involve in their gene transcription and progenitor cell mobilization, differentiation and homing. In the present study, we examined whether there was a relationship between *SDF1* gene variation, SDF1 α level and circulating EPC number. In addition, we tested whether there was also a relationship between variation in the gene encoding the SDF1 α receptor CXCR4 and EPC number.

Methods

Study Population and clinical variables

The subjects of this study were residents of the Bruneck area in the Bolzano Province of Italy, who participated in the Bruneck Study [31]. Details of the Bruneck study have been described previously [31]. DNA samples were available for 826 subjects, and these were genotyped single nucleotide polymorphisms (SNPs) in the *SDF1* and *CXCR4* genes. Blood samples taken in 2000 (n = 684) and 2005 (n = 574) respectively were used for measurements of SDF1 α levels. EPC number and function were assessed in blood samples collected in 2005 (n = 571 for EPC number and n = 542 for EPC colonies number respectively). Subjects with and those without EPC number and EPC function assessments did not differ in age, sex and cardiovascular risk factors. The appropriate ethics committees (Autonome Provinz Bozen-Sanitätsbetrieb Bozen Ethikkomitee) approved the study protocol and all study subjects gave their written informed consent before entering the study.

Systolic and diastolic blood pressure was taken with a standard mercury sphygmomanometer after at least 10 min of rest (mean of three independent measurements). Hypertension was defined as blood pressure \geq 140/90 mm Hg or the use of anti-hypertensive drugs. The average number of cigarettes smoked per day was noted for each smoker and ex-smoker. Diabetes was diagnosed according to ADA criteria. Assessment of regular alcohol

consumption was performed with a standardized questionnaire and quantified in terms of grams per day (g/day).

EPC culture assay

EPC numbers in the blood samples were determined as described previously [17]. Briefly, positive stained cells for DiI-Ac-LDL and Lectin were considered to be EPCs on day 5 of culture. The total numbers of EPC per well were counted by two trained independent senior investigators blinded to the clinical details of the subjects. The EPC in a minimum of two wells were counted and the average was then recorded.

EPC-colony formation unit (CFU) assay

The EPC-CFU assay performed as described previously [17]. Briefly, PBMNC were resuspended in EPC culture medium (M199 with 20% FCS and antibiotics) and then plated on fibronectin coated 6 well plates at a concentration of 5 million cells per well. The endothelial colonies were counted manually on day 7. Strict guidelines were followed to ensure consistent counting of EPC colonies. Two senior investigators who were blinded to the subjects' clinical status counted the colonies.

Enzyme-linked immunosorbent assay (ELISA)

Commercially available SDF1 α and matrix metalloproteinase-9 (MMP9) ELISA kits (Quantikine, R&D Systems, UK) were used to determine plasma SDF1 α and MMP9 levels. All ELISA tests were carried out at room temperature on freshly thawed plasma samples. The concentration was determined by comparison with a standard curve, following manufacturer's instruction. Other laboratory parameters and vascular risk factors were all examined by standard methods [32].

Genotype analyses

We selected tagging SNPs in the *SDF1* and *CXCR4* genes from the HapMap database (data release in June 2005). Three SNPs in *SDF1* (rs2297630, rs266087 and rs1413519) and two in *CXCR4* (rs16832740 and rs12691874) to capture SNPs with a minor allele frequency >0.2 at these loci and $R^2 > 0.8$ were typed in this study. In addition, we studied the rs266085 SNP which had been reported to influence SDF1 expression [27] and the rs1801157 SNP which had suggested to be associated with plasma SDF1 level [28]. Genomic DNA was extracted from blood samples of the Bruneck study subjects, and genotypes were determined using the TaqMan genotyping method.

Statistical Analysis

The data were analyzed using the SPSS 15.0 and BMDP software packages. Continuous variables were presented as means \pm SD or medians (interquartile range), and dichotomous variables as percentages. Correlations between EPC number and EPC-CFU, SDF1 α level and other parameters were estimated by calculation of crude and partial (age/sex-corrected) Pearson correlation coefficients. Variables with a skewed distribution were log_{B-B}-transformed. Non-parametric tests yielded very similar findings (data not presented). Associations of EPC number, EPC-CFU and SDF1 α level with SDF1 and CXCR4 genotypes were assessed using generalized linear models adjusted for age and sex. Multivariate models additionally included standard cardiovascular risk factors and the factors associated with SDF1 α levels (LDL and HDL cholesterol concentrations, smoking, hypertension, diabetes, alcohol consumption, and levels of CRP, MMP9, cystatin C, fibrinogen and homocystein). A Bonferroni correction was

performed to account for the multiple comparisons (5 SNPs in SDF1 and 2 in CXCR4) performed.

Results

Relationship of plasma SDF1 α level with inflammatory markers and other variables

Near normal distribution of SDF1 α was observed in our study (**Figure 1A**, Kolmogorov-Smirnov $p > 0.05$). Mean SDF1 α levels in 2000 and 2005 were 2652 pg/ml and 2565 pg/ml, with a range of 880 pg/ml to 5595 pg/ml and 1322 pg/ml to 4174 pg/ml, respectively. SDF1 α levels in 2000 and 2005 were highly correlated (intraclass correlation = 0.495; $P < 0.001$), indicating that the SDF1 α level is relatively consistent over time. As expected, both plasma SDF1 α level measured in 2000 ($r = 0.207$, $p < 0.001$) and 2005 ($r = 0.270$, $p < 0.001$) increased with age (**Figure 1B**) and was higher in women (2619 in women vs. 2497 in men, $p = 0.002$). Moreover, plasma SDF1 α level was correlated with alcohol consumption (age/sex-adjusted correlation coefficient $r = -0.087$, $p = 0.042$), reticulocyte numbers ($r = -0.132$, $p = 0.002$), plasma levels of matrix metalloproteinase-9 (MMP9) ($r = 0.146$, $p = 0.001$), C-reactive protein (hsCRP) ($r = 0.090$, $p = 0.037$), cystatin C ($r = 0.277$, $p < 0.001$), fibrinogen ($r = 0.159$, $p < 0.001$), and homocystein ($r = 0.174$, $p = 0.001$). (**Table 1**)

Plasma SDF1 α level predicts EPC number

EPC number and EPC-CFU number (per 1 ml blood) displayed a non-normal distribution, with the majority of subjects (more than 75%) having 0 to 1000 EPC and 0 to 340 EPC-CFU, respectively. SDF1 α levels were significantly and inversely associated with EPC number ($p < 0.001$) in samples collected in 2005 (**Figure 1C**), indicating that SDF1 α has an influence on EPC number in the general population. Importantly, EPC number in 2005 was also associated with SDF1 α level in 2000 ($p = 0.009$), indicating a long-term predictive value of SDF1 α level for circulating EPC number (**Figure 1D**). No association between SDF1 α level and EPC function measured by EPC-CFU was detected in this study.

Association of SDF1 genotyping, plasma SDF1 α level and circulating EPC number

Allele and genotype frequencies of the SDF1 and CXCR4 SNPs examined in this study are shown in **Table 2**. The genotype distributions were consistent with Hardy-Weinberg equilibrium.

The SDF1 rs2297630 SNP was associated with plasma SDF1 α level ($p = 0.002$) and circulating EPC number ($p = 0.006$), with the A/A genotype associating with higher SDF1 α level and lower EPC number (**Figure 2 & Table 3**). These associations remained significant after Bonferroni correction for the number of SNPs tested (Bonferroni corrected $p = 0.014$ and $p = 0.042$, respectively). The associations also remained significant after adjusting for cardiovascular risk factors and the factors that were associated with SDF1 α levels, including LDL and HDL cholesterol concentrations, smoking, hypertension, diabetes, alcohol consumption, and levels of hsCRP, MMP9, cystatin C, fibrinogen and homocystein (**Table 3**).

Plasma SDF1 α level and circulating EPC number was not associated with the other SDF1 or CXCR4 SNPs tested. No association was observed between any of the SNPs tested and EPC-CFU.

Discussion

The population-based, longitudinal design of the Bruneck study allowed us to investigate the relationships between SDF1 α levels at two different time points (2000 and 2005) and the relationships of

the SDF1 α levels at these different time points with circulating EPC numbers. A key finding from our study is the high degree of correlation the two SDF1 α measurements in blood samples taken in 2000 and 2005 respectively, suggesting that although SDF1 α levels are correlated with cardiovascular risk factors, the levels in an individual are relatively consistent over time. In addition, the study revealed an association between SDF1 α level in 2000 and EPC number in 2005, suggesting a long-term predictive value of SDF1 α level for EPC number. Another important, novel finding of this study is that there is an association between variation in the SDF1 gene, SDF1 α level and EPC number, indicating that the inter-individual variability in SDF1 α level and EPC number is influenced by variation in the SDF1 gene.

SDF1 is a member of the chemokine CXCL subfamily originally isolated from murine bone marrow stromal cells and is expressed also by stromal cells of various tissues[33]. Animal studies have shown that increasing SDF expression by adenovirus gene transfer can induce EPC mobilization from bone marrow into the peripheral blood, thereby increasing circulating EPC numbers[21–23], enhancing angiogenesis and blood perfusion in ischemic tissues[25]. These studies have led to the hypothesis that SDF1 plays an important role in EPC mobilization, differentiation and homing. However, data from our study showed that SDF-1 α levels were inversely, rather than positively, associated with circulating EPC numbers. There are several possible explanations. Almost all of the existing data that suggest that SDF1 α can induce circulating EPCs are from studies in mouse, and the situations in humans may be different and more complex. Indeed, a number of studies including the present study have shown that EPC numbers in humans are related to a number of factors including age, gender, smoking, lipid levels, hypertension, diabetes, etc. Secondly, it is possible that the relationship of SDF1 with EPC mobilization, differentiation and homing in the acute phase is different from that in the normal situation. Studies of mouse ischemia models showed that the number of c-Kit⁺ cells in peripheral blood was lower but the level of SDF1 α was much higher at 14 days after ischemia, as compared with control mice[26], suggesting that EPCs are mobilized into peripheral blood from bone marrow after the onset of ischemia, but at a later stage, the numbers of mobilized EPC in peripheral blood decrease due to their homing to the ischemic site. Thirdly, because SDF1 α functions as a chemoattractant for stem/progenitor cells, the SDF1 α gradient between bone marrow, peripheral blood and injured tissue/sites is crucial for stem/progenitor cell mobilization, differentiation and homing[26,34–36]. It has been reported that stem/progenitor cells in bone marrow and peripheral blood are mobilized and homed to the ischemic or injured tissue due to high SDF1 α levels in the ischemic or injured tissue[26] and lower levels of SDF-1 α in the bone marrow[37]. Measurement of plasma SDF1 α level does not represent the gradient mentioned above, and therefore, the inverse relationship between plasma SDF1 α level and circulating EPC number should not be interpreted as in conflict with the notion that SDF1 plays an important role in EPC mobilization, differentiation and homing.

A recent study showed that there is significant correlation in the number of circulating EPCs between parents and their offsprings, leading to the hypothesis that EPC number is, at least in part, genetically regulated[29]. The results of our study support this hypothesis and indicate that such a genetic influence is likely in part due to variation in the SDF1 gene. In particular, we found that SDF1 α level and circulating EPC number are associated with the SDF1 gene rs2297630 SNP in the Bruneck study cohort. A potential interpretation for this novel finding is that variation in the SDF1 gene can influence EPC number via an effect on the

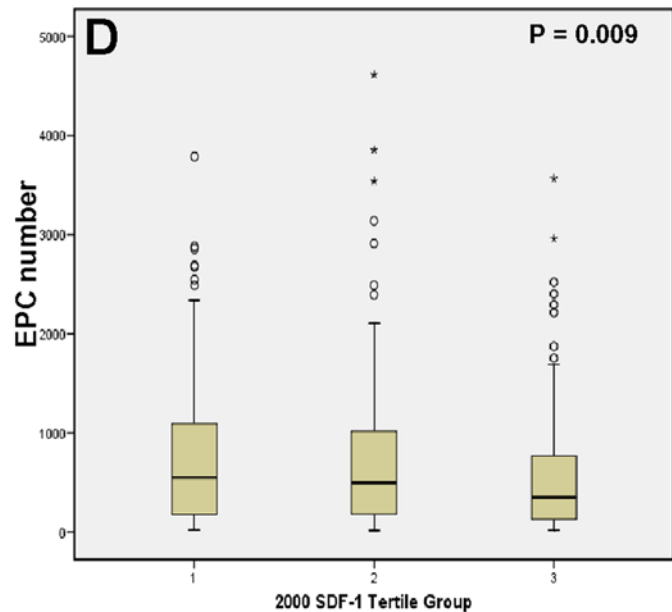
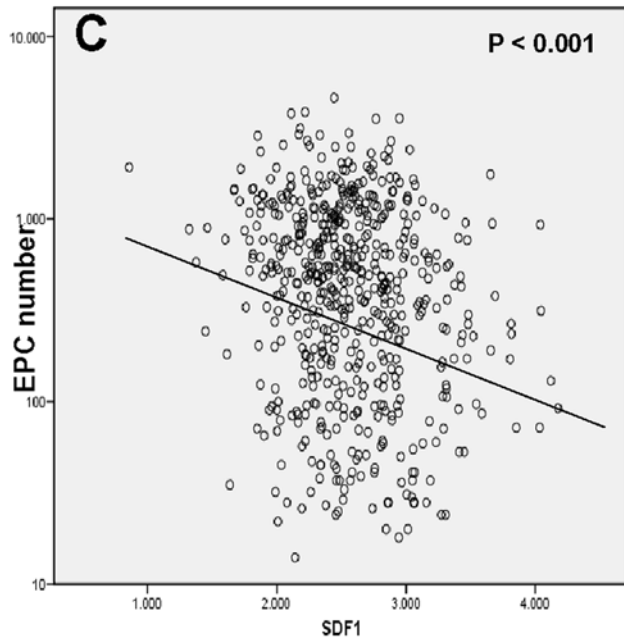
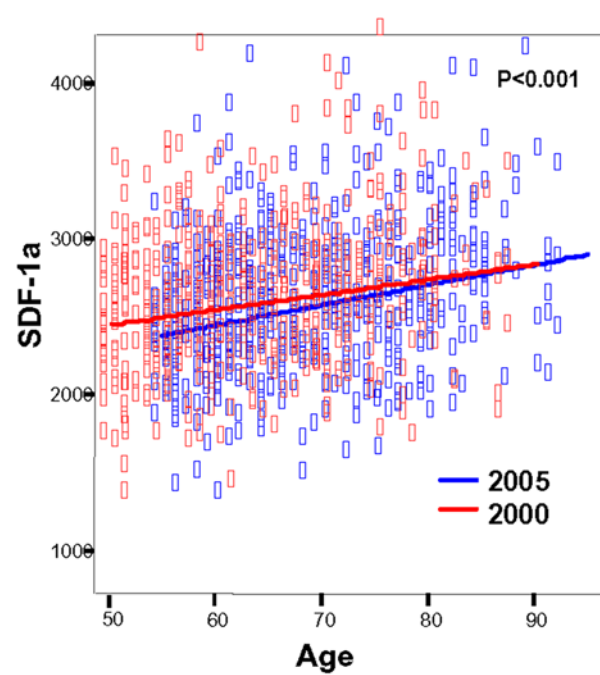
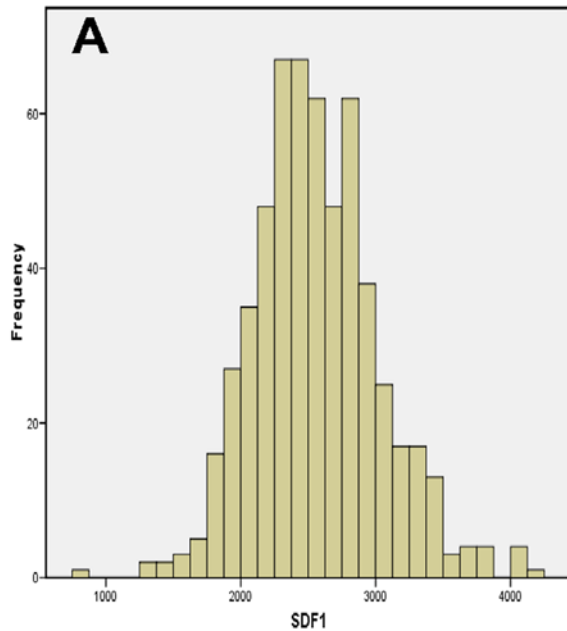
B

Figure 1. Panel **A** shows near normal distribution of SDF1 α in the Bruncek study population. Panel **B** displays the association between SDF-1 α measured in 2000 and in 2005 and age ($r=0.270$, $p<0.001$). Panel **C** illustrates the correlation between SDF1 α levels and EPC number in 2005. The regression line demonstrates clearly that SDF1 α levels are inverse association with EPC number. Panel **D** illustrates the association between EPC numbers in 2005 and SDF1 α level in 2000 (tertile groups). SDF-1 tertile groups are defined as follows: T1<2409, T2 2409–2753 and T3>2753. The box plots indicating EPC number median and IQRs. Notably, EPC numbers are significant associated inversely with SDF-1 α levels, especially much low EPC number were likely observed in the top tertile group of SDF-1 levels.
doi:10.1371/journal.pone.0004061.g001

Table 1. Association between SDF-1 α level and selected vascular risk factors and laboratory parameters (2005).

Variable	SDF-1 α tertile group (pg/ml)			P value for trend
	Low Tertile (lower than 2350)	Medium Tertile (2350–2743)	High Tertile (higher than 2743)	
Alcohol consumption (g/day)	21.9 \pm 27.4	17.9 \pm 23.9	15.1 \pm 24.5	0.042
Reticulocytes (%)	12.7 \pm 3.42	12.2 \pm 3.30	11.7 \pm 3.15	0.002
MMP9 (ng/mL)	69.2 \pm 60.6	80.5 \pm 59.0	87.3 \pm 60.2	0.001
High-sensitivity CRP (mg/L)	3.5 \pm 4.7	3.1 \pm 3.6	5.1 \pm 9.9	0.037
Fibrinogen (mg/dL)	285.4 \pm 53.8	296.4 \pm 54.2	311.4 \pm 64.2	0.009
HOMOCYSTEIN (MMOL/L)	11.6 \pm 5.9	11.4 \pm 5.0	13.7 \pm 7.4	0.017
Cystatin C (mg/L)	0.92 \pm 0.16	0.98 \pm 0.23	1.12 \pm 0.31	<0.001

Values are means \pm SD. P values for trend are from age- and sex-adjusted analyses.
doi:10.1371/journal.pone.0004061.t001

level of SDF1 α . The rs2297630 SNP is located in intron 3 of the *SDF1* gene. It is possible that the association of this SNP with SDF1 α level and circulating EPC number has arisen from a direct functional effect of this SNP on SDF1 expression or mRNA splicing. Alternatively, the rs2297630 SNP might be a functionally neutral marker that is in linkage disequilibrium with a functional polymorphism located elsewhere at the *SDF1* locus.

Two other *SDF1* SNPs, rs266085 and rs1801157, have been reported to be associated with SDF1 levels[27,28] and the latter SNP has also been reported to be associated with CD34⁺ cell mobilization[30]. Some, but not all, studies have suggested that the rs1801157 SNP is associated with HIV infection and AIDS[38–40] and there is also evidence suggesting that this SNP is associated

Table 2. Genotype and allele frequencies of *SDF1* and *CXCR4* SNPs studied

Gene	SNP	Genotype	N (%)	Allele	Frequency
<i>SDF1</i>	rs2297630	GG	463 (60.05)	G	0.78
		AG	271 (35.15)	A	0.22
		AA	37 (4.80)		
	rs266085	GG	307 (42.05)	G	0.63
		AG	311 (42.60)	A	0.37
		AA	112 (15.34)		
	rs1801157	GG	445 (62.94)	G	0.79
		AG	231 (32.67)	A	0.21
		AA	31 (4.38)		
rs1413519	GG	462 (63.20)	G	0.79	
	CG	238 (32.56)	C	0.21	
	CC	31 (4.24)			
<i>CXCR4</i>	rs16832740	AA	447 (62.34)	A	0.79
		AG	237 (33.05)	G	0.21
		GG	33 (4.60)		
	rs12691874	AA	189 (26.21)	A	0.52
		AG	371 (51.46)	G	0.48
		GG	161 (22.33)		

doi:10.1371/journal.pone.0004061.t002

Table 3. Plasma SDF1 α levels and circulating EPC numbers according to *SDF1* and *CXCR4* SNP genotypes.

SNP	Genotype	SDF1 α level	P value	EPC number	P value
SDF1 rs2297630	GG	2562 (2515–2609)	0.002	382 (336–434)	0.006
	AG	2524 (2461–2586)	(0.002)	409 (344–486)	(0.015)
	AA	2863 (2684–3043)		175 (107–286)	
SDF1 rs266085	GG	2572 (2512–2631)	0.661	354 (300–417)	0.793
	AG	2539 (2480–2597)	(0.328)	383 (326–449)	(0.866)
	AA	2529 (2431–2626)		362 (277–473)	
SDF1 rs266087	GG	2570 (2510–2630)	0.931	355 (302–418)	0.702
	AG	2574 (2519–2630)	(0.742)	391 (336–455)	(0.803)
	AA	2552 (2450–2654)		378 (286–499)	
SDF1 rs1801157	GG	2592 (2544–2641)	0.260	370 (323–425)	0.998
	AG	2547 (2483–2612)	(0.208)	368 (307–441)	(0.931)
	AA	2467 (2295–2638)		369 (227–598)	
SDF1 rs1413519	GG	2562 (2513–2611)	0.225	338 (295–386)	0.300
	CG	2588 (2522–2655)	(0.222)	401 (335–482)	(0.229)
	CC	2725 (2541–2909)		397 (240–658)	
CXCR4 rs16832740	AA	2557 (2509–2606)	0.424	374 (328–426)	0.878
	AG	2556 (2487–2624)	(0.747)	395 (329–475)	(0.783)
	GG	2863 (2501–2854)		370 (230–595)	
CXCR4 rs12691874	AA	2514 (2437–2591)	0.306	414 (336–512)	0.272
	AG	2588 (2534–2641)	(0.600)	362 (313–419)	(0.169)
	GG	2571 (2488–2654)		322 (256–404)	

SDF-1 levels and EPC numbers (per 1 ml blood) are age- and sex-adjusted arithmetic (95%CI) and geometric means (95%CI), respectively. P values are from general linear models adjusted for age and sex and in brackets from models additionally adjusted for candidate vascular risk factors and determinants of SDF-1 α levels (LDL and HDL cholesterol [mg/dL], smoking [0,1], hypertension [0,1], diabetes [0,1], alcohol consumption [gram/day], homocystein [μ mol/L], hsCRP [mg/L], MMP9 [ng/mL], cystatin C [mg/L], fibrinogen [mg/dL]).

doi:10.1371/journal.pone.0004061.t003

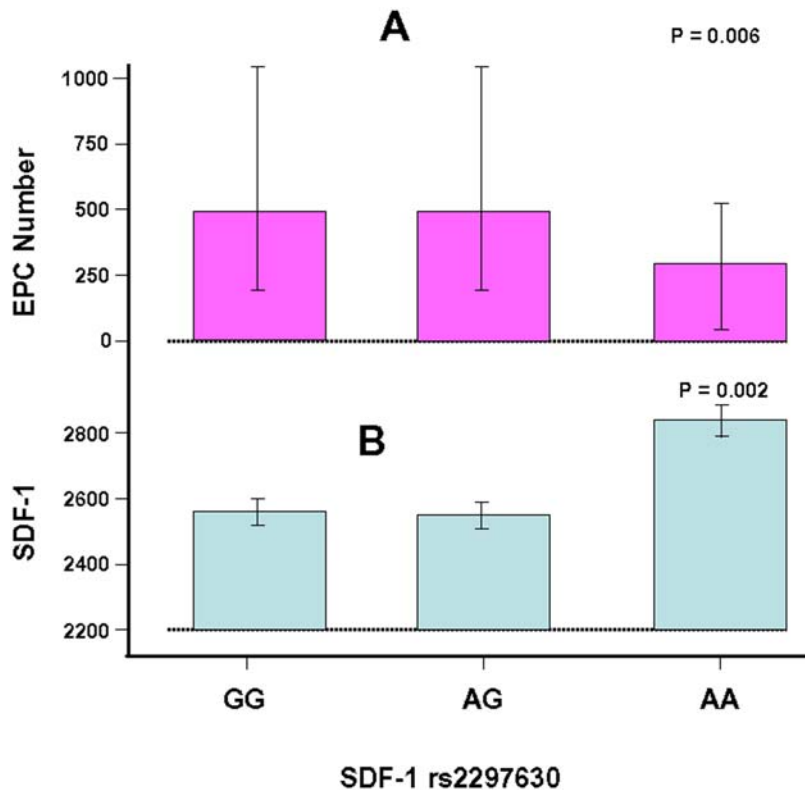


Figure 2. Associations of the SDF1 rs2297630 SNP (2000) with EPC number (median and IQR, A) and blood SDF1 α level (arithmetic means and SD, B) assessed in the 2005 evaluation of the Bruneck Study.

doi:10.1371/journal.pone.0004061.g002

with acute myeloid leukaemia[41], chronic myelogenous leukaemia[42], colorectal cancer[43], sporadic breast cancer[44], carotid artery stenosis[45], type I diabetes[46] and systemic lupus erythematosus[47]. However, in vitro experiments show that this SNP (rs1801157) does not have a direct functional effect on SDF1 expression[48], raising the possibility that it may be a marker for a functional SNP at the *SDF1* locus due to linkage disequilibrium. This possibility is consistent with the fact that the SDF1 gene is transcribed into two isoforms, SDF1 α and SDF1 β , and that the rs1801157 SNP is located in the 3' untranslated region of the SDF1 β transcript but not in the SDF1 α transcript[49,50]. In the Bruneck study cohort, we found that the rs2297630 SNP, but not the rs266085 or rs1801157 SNP, was associated with SDF1 α level and EPC number. Taken together, the studies mentioned above and the present study suggest that variation in SDF1 gene has an influence on SDF1 levels. The populations examined in these different studies may have different genetic structure and linkage disequilibrium patterns, which may explain why different SDF1 SNPs were associated with SDF1 levels in the different studies.

Some particular attentions should be drawn regarding our findings. Firstly, the consideration of the nature of EPC is important, since there is no a unique criteria for identification of EPC, yet. Immense attention regarding the definition of EPC has recently been drawn due to the fact that the characterization and function of EPC isolated with different methodologies were quite different from each other[51]. Myeloid PBMNC derived spindle like EPC, but not circulating CD34+ cells was used to quantify the circulating EPC number in our study. Although a positive relationship between them was reported in the literature, the number of cultured EPC may not always reflect the number of circulating CD34+ and/or KDR+

EPC. Secondly, many diseases have influence on the role of EPC number, such as diabetes[10,11], smoking[12], rheumatoid arthritis[13] or chronic renal failure[14], among them much reduced EPC number have been reported. Extensive adjustments for cardiovascular risk factors and diseases have been conducted in our study to exclude any potential influence on our findings. Finally, no relationship or association between EPC-CFU and plasma SDF1 α levels and SDF-1 gene SNPs were observed in our study, which indicating that neither plasma SDF1 α levels nor SDF-1 SNP could affect the proliferation and migration of EPC reflected by their ability to form EPC colonies in the culture.

In summary, our data indicate that although the SDF1 level and EPC number may both increase in response to acute ischaemic events, the relationship between blood SDF1 level and EPC number is complex and they are inversely correlated in the normal situation as observed in the Bruneck study cohort which was recruited from the general population. Our data also indicate that circulating EPC number is influenced by variation in the SDF1 gene likely via an effect of the genetic variation on SDF1 α expression. These findings help understand the mechanisms underlying the inter-individual variability in EPC number which has an implication in the pathogenesis of atherosclerosis and other cardiovascular diseases.

Author Contributions

Conceived and designed the experiments: QX MJ QX. Performed the experiments: QX SY FO AM JW SK. Analyzed the data: QX SY SK. Contributed reagents/materials/analysis tools: QX MJ QX. Wrote the paper: QX SY SK QX.

References

- Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, et al. (1997) Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275: 964–967.
- Kalka C, Masuda H, Takahashi T, Kalka-Moll WM, Silver M, et al. (2000) Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. *Proc Natl Acad Sci U S A* 97: 3422–3427.
- Takahashi T, Kalka C, Masuda H, Chen D, Silver M, et al. (1999) Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med* 5: 434–438.
- Kawamoto A, Gwon HC, Iwaguro H, Yamaguchi JI, Uchida S, et al. (2001) Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. *Circulation* 103: 634–637.
- Britten MB, Abolmaali ND, Assmus B, Lehmann R, Honold J, et al. (2003) Infarct remodeling after intracoronary progenitor cell treatment in patients with acute myocardial infarction (TOPCARE-AMI): mechanistic insights from serial contrast-enhanced magnetic resonance imaging. *Circulation* 108: 2212–2218.
- Schachinger V, Assmus B, Britten MB, Honold J, Lehmann R, et al. (2004) Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. *J Am Coll Cardiol* 44: 1690–1699.
- Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, et al. (2001) Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 89: E1–7.
- Vasa M, Fichtlscherer S, Adler K, Aicher A, Martin H, et al. (2001) Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. *Circulation* 103: 2885–2890.
- Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, et al. (2003) Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 348: 593–600.
- Fadini GP, Miorin M, Facco M, Bonamico S, Baesso I, et al. (2005) Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus. *J Am Coll Cardiol* 45: 1449–1457.
- Tepper OM, Galiano RD, Capla JM, Kalka C, Gagne PJ, et al. (2002) Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* 106: 2781–2786.
- Kondo T, Hayashi M, Takeshita K, Numaguchi Y, Kobayashi K, et al. (2004) Smoking cessation rapidly increases circulating progenitor cells in peripheral blood in chronic smokers. *Arterioscler Thromb Vasc Biol* 24: 1442–1447.
- Grisar J, Aletaha D, Steiner CW, Kapral T, Steiner S, et al. (2005) Depletion of endothelial progenitor cells in the peripheral blood of patients with rheumatoid arthritis. *Circulation* 111: 204–211.
- Choi JH, Kim KL, Huh W, Kim B, Byun J, et al. (2004) Decreased number and impaired angiogenic function of endothelial progenitor cells in patients with chronic renal failure. *Arterioscler Thromb Vasc Biol* 24: 1246–1252.
- Ghani U, Shuaib A, Salam A, Nasir A, Shuaib U, et al. (2005) Endothelial progenitor cells during cerebrovascular disease. *Stroke* 36: 151–153.
- Foresta C, Caretta N, Lana A, Cabrelle A, Palu G, et al. (2005) Circulating endothelial progenitor cells in subjects with erectile dysfunction. *Int J Impot Res* 17: 288–290.
- Xiao Q, Kiechl S, Patel S, Oberhollenzer F, Weger S, et al. (2007) Endothelial progenitor cells, cardiovascular risk factors, cytokine levels and atherosclerosis—results from a large population-based study. *PLoS ONE* 2: e975.
- Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, et al. (1996) Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXCL12 chemokine PBSF/SDF-1. *Nature* 382: 635–638.
- Tachibana K, Hirota S, Iizasa H, Yoshida H, Kawabata K, et al. (1998) The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. *Nature* 393: 591–594.
- Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR (1998) Function of the chemokine receptor CXCR4 in hematopoiesis and in cerebellar development. *Nature* 393: 595–599.
- Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, et al. (2002) Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell* 109: 625–637.
- Rafii S, Heissig B, Hattori K (2002) Efficient mobilization and recruitment of marrow-derived endothelial and hematopoietic stem cells by adenoviral vectors expressing angiogenic factors. *Gene Ther* 9: 631–641.
- Moore MA, Hattori K, Heissig B, Shieh JH, Dias S, et al. (2001) Mobilization of endothelial and hematopoietic stem and progenitor cells by adenovector-mediated elevation of serum levels of SDF-1, VEGF, and angiopoietin-1. *Ann N Y Acad Sci* 938: 36–45; discussion 45–37.
- Yamaguchi J, Kusano KF, Masuo O, Kawamoto A, Silver M, et al. (2003) Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. *Circulation* 107: 1322–1328.
- Hiasa K, Ishibashi M, Ohtani K, Inoue S, Zhao Q, et al. (2004) Gene transfer of stromal cell-derived factor-1alpha enhances ischemic vasculogenesis and angiogenesis via vascular endothelial growth factor/endothelial nitric oxide synthase-related pathway: next-generation chemokine therapy for therapeutic neovascularization. *Circulation* 109: 2454–2461.
- De Falco E, Porcelli D, Torella AR, Straino S, Iachinoto MG, et al. (2004) SDF-1 involvement in endothelial phenotype and ischemia-induced recruitment of bone marrow progenitor cells. *Blood* 104: 3472–3482.
- Kimura R, Nishioka T, Soemantri A, Ishida T (2005) Allele-specific transcript quantification detects haplotypic variation in the levels of the SDF-1 transcripts. *Hum Mol Genet* 14: 1579–1585.
- Soriano A, Martinez C, Garcia F, Plana M, Palou E, et al. (2002) Plasma stromal cell-derived factor (SDF)-1 levels, SDF-1-3'A genotype, and expression of CXCR4 on T lymphocytes: their impact on resistance to human immunodeficiency virus type 1 infection and its progression. *J Infect Dis* 186: 922–931.
- Whittaker A, Moore JS, Vasa-Nicotera M, Stevens S, Samani NJ (2008) Evidence for genetic regulation of endothelial progenitor cells and their role as biological markers of atherosclerotic susceptibility. *Eur Heart J* 29: 332–338.
- Benboubker L, Watier H, Carion A, Georget MT, Desbois I, et al. (2001) Association between the SDF1-3'A allele and high levels of CD34(+) progenitor cells mobilized to peripheral blood in humans. *Br J Haematol* 113: 247–250.
- Xu Q, Willeit J, Marosi M, Kleindienst R, Oberhollenzer F, et al. (1993) Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis. *Lancet* 341: 255–259.
- Xu Q, Schett G, Perschinka H, Mayr M, Egger G, et al. (2000) Serum soluble heat shock protein 60 is elevated in subjects with atherosclerosis in a general population. *Circulation* 102: 14–20.
- Nagasawa T, Nakajima T, Tachibana K, Iizasa H, Bleul CC, et al. (1996) Molecular cloning and characterization of a murine pre-B-cell growth-stimulating factor/stromal cell-derived factor 1 receptor, a murine homolog of the human immunodeficiency virus 1 entry coreceptor fusin. *Proc Natl Acad Sci U S A* 93: 14726–14729.
- Aiuti A, Webb IJ, Bleul C, Springer T, Gutierrez-Ramos JC (1997) The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. *J Exp Med* 185: 111–120.
- Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, et al. (1999) Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science* 283: 845–848.
- Hattori K, Heissig B, Tashiro K, Honjo T, Tateno M, et al. (2001) Plasma elevation of stromal cell-derived factor-1 induces mobilization of mature and immature hematopoietic progenitor and stem cells. *Blood* 97: 3354–3360.
- Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, et al. (2002) G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat Immunol* 3: 687–694.
- Winkler C, Modi W, Smith MW, Nelson GW, Wu X, et al. (1998) 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* 279: 389–393.
- Mummidi S, Ahuja SS, Gonzalez E, Anderson SA, Santiago EN, et al. (1998) Genealogy of the CCR5 locus and chemokine system gene variants associated with altered rates of HIV-1 disease progression. *Nat Med* 4: 786–793.
- Brambilla A, Villa C, Rizzardi G, Veglia F, Ghezzi S, et al. (2000) Shorter survival of SDF1-3'A/3'A homozygotes linked to CD4+ T cell decrease in advanced human immunodeficiency virus type 1 infection. *J Infect Dis* 182: 311–315.
- Dommenge F, Cartron G, Espanel C, Gally J, Domenech J, et al. (2006) CXCL12 polymorphism and malignant cell dissemination/tissue infiltration in acute myeloid leukemia. *FASEB J* 20: 1913–1915.
- de Oliveira CE, Cavassin GG, Perim Ade L, Nasser TF, de Oliveira KB, et al. (2007) Stromal cell-derived factor-1 chemokine gene variant in blood donors and chronic myelogenous leukemia patients. *J Clin Lab Anal* 21: 49–54.
- Dimberg J, Hugander A, Lofgren S, Wagsater D (2007) Polymorphism and circulating levels of the chemokine CXCL12 in colorectal cancer patients. *Int J Mol Med* 19: 11–15.
- Zafiroopoulos A, Crikas N, Passam AM, Spandidos DA (2004) Significant involvement of CCR2-64I and CXCL12-3a in the development of sporadic breast cancer. *J Med Genet* 41: e59.
- Ghilardi G, Biondi ML, Turri O, Pateri F, d'Eril GM, et al. (2008) Genetic control of chemokines in severe human internal carotid artery stenosis. *Cytokine* 41: 24–28.
- Ide A, Kawasaki E, Abiru N, Sun F, Fukushima T, et al. (2003) Stromal-cell derived factor-1 chemokine gene variant is associated with type 1 diabetes age at onset in Japanese population. *Hum Immunol* 64: 973–978.
- Lima G, Soto-Vega E, Atisha-Fregoso Y, Sanchez-Guerrero J, Vallejo M, et al. (2007) MCP-1, RANTES, and SDF-1 polymorphisms in Mexican patients with systemic lupus erythematosus. *Hum Immunol* 68: 980–985.
- Arya SK, Ginsberg CC, Davis-Warren A, D'Costa J (1999) In vitro phenotype of SDF1 gene mutant that delays the onset of human immunodeficiency virus disease in vivo. *J Hum Virol* 2: 133–138.
- Nagasawa T, Kikutani H, Kishimoto T (1994) Molecular cloning and structure of a pre-B-cell growth-stimulating factor. *Proc Natl Acad Sci U S A* 91: 2305–2309.
- Shirozu M, Nakano T, Inazawa J, Tashiro K, Tada H, et al. (1995) Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene. *Genomics* 28: 495–500.
- Prater DN, Case J, Ingram DA, Yoder MC (2007) Working hypothesis to redefine endothelial progenitor cells. *Leukemia* 21: 1141–1149.