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New mechanisms of *CCR5*-Δ32 carriers' advantage – Impact on progenitor cells and renal function

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ABSTRACT

Background: CCR5 is a chemokine receptor expressed by various populations including leukocytes, smooth muscle cells and endothelium. Δ32 polymorphism of CCR5 gene has been connected with, inter alia, cardiovascular disease development. The aim of our study was to evaluate impact of *CCR5* variant on CD34+ and CD34+VEGFR2+ cells - populations involved in cardiovascular system homeostasis and regeneration.
Methods and results: We have examined 170 Polish subjects from Pomeranian region. The analysis concerned *CCR5* polymorphism and flow cytometry evaluation of whole blood cells. Our results indicate that individuals with at least one *CCR5*-Δ32 allele are characterized by greater number of CD34+CXCR4+, CD34+VEGFR2+ and CD34+VEGFR2+c-Kit + cells than their wild type counterparts. This group also exhibits more beneficial values of renal function parameters.
Conclusion: Maintaining greater size of CD34+ and CD34+VEGFR2+ populations as well as proper kidney function may constitute mechanisms that connect chemokine receptor polymorphism with cardiovascular system health.

1. Introduction

Chemokines are small proteins with a primary function of inducing migration processes. They are secreted by various populations in either inducible or constitutive fashion (Ishida et al., 2012). Chemokine receptor 5 (CCR5), characteristic for resting T-lymphocytes, monocytes/macrophages, dendritic cells, vascular smooth muscle cells and endothelium (Hyde et al., 2010) belongs to β-chemokines and G-protein coupled receptors. It binds various ligands but three of them, namely CCL3 (MIP-1α), CCL4 (MIP-1β) and CCL5 (RANTES) are recognized as the most physiologically relevant agonists. This chemokine receptor has first attracted attention as a co-receptor for macrophage-tropic HIV-1 infections. Nonetheless, growing evidence for chemokines' impact on cardiovascular disease (CVD) has brought interest in receptors' role during pathogenesis of other disorders as well (Jones et al., 2011).

CCR5 encoding gene is polymorphic with one of the most intensely studied alterations being the 32-base pair deletion (*CCR5*-Δ32) (Jones et al., 2011). Removal of the nucleotides changes the reading frame leaving the protein without second extracellular loop. As severely shortened receptor retains in the endoplasmic reticulum (Liu et al., 1996) individuals with Δ32/Δ32 genotype are lacking CCR5 on their cells, whereas in heterozygotes its expression is reduced (Benkirane et al., 1997). Macrophage-tropic HIV-1 is unable to enter macrophages and other CCR5-expressing cells in the absence of functional receptor. Impact of the polymorphism on a CVD development and progression has been subsequently investigated, but the results seem ambiguous (Jones et al., 2011). Hyde et al. have recognized deletion allele as linked with more advantageous lipid profile (Hyde et al., 2010). Others have described Δ32/Δ32 genotype as being protective against severe coronary artery disease (Szalai et al., 2001) or early myocardial infarction episodes (González et al., 2001). On the other hand, lack of connections between *CCR5*-Δ32 polymorphism and coronary artery disease has also been described (Simeoni et al., 2004).

Blood circulating endothelial progenitor cells (EPCs) have been described for the first time by Asahara et al. in 1997. The population is able to differentiate into adult endothelial cells and as such participates in new blood vessel formation (Asahara et al., 1997) and regeneration of impaired endothelium monolayer (Urbich and Dimmeler, 2004). EPCs are usually distinguished via the analyses of CD34, VEGFR2 and/or CD133 surface markers expression (Timmermans et al., 2009). Alterations of their number and function have been connected with various disorders and risk factors (Fadini et al., 2007; Huang et al., 2014).

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These cells are presently considered useful indicators of endothelium regeneration (Urbich and Dimmeler, 2004) as well as comprehensive vascular health and homeostasis. CD34 + VEGFR2 + population has been described as a predictor of cardiovascular events incidence (Werner et al., 2005) (Schmidt-Lucke et al., 2005) and cardiovascular death (Werner et al., 2005). Great prognostic values have been also recognized for CD34 + and CD34 + CD133 + populations (Patel et al., 2015).

c-Kit as a receptor for stem cell factor (SCF) regulates: proliferation, migration and survival of haematopoietic, progenitor and germ cells (Lennartsson and Ronnstrand, 2012). It has been suggested that EPCs are characterised by expression of this receptor (Kocher et al., 2001).

CXCR4 is a G protein-coupled receptor for chemoattractant stromal derived factor 1 (SDF-1). Mobilisation of progenitor cells from a bone marrow and their subsequent homing are regulated by the CXCR4 (Caiado and Dias, 2012). CXCR4-expressing cells have been recognized as more effective in improving perfusion and capillary density in ischemic regions (Seeger et al., 2009).

Cardiovascular disease development have been also connected with compromised kidney function. Drastic decline in eGFR (estimated glomerular filtration rate) leads to greater risk of CVD as well as all-cause mortality (Matsushita et al., 2009). Although the exact mechanisms of this contribution are not fully described, few valid hypotheses have been proposed. Patients suffering from chronic kidney disease (CKD) are likely to be also burdened with classical CVD risk factors, e.g. age, hypertension, type 2 diabetes mellitus. Activation of RAAS (renin–angiotensin–aldosterone system), increased oxidative stress and inflammatory response or abnormal mineral metabolism are further alterations characteristic for cardiovascular disorders as well as kidney disease (Liu et al., 2014). The explanation concerning vasculature being affected by uraemic toxins has also been proposed (Lekawanvijit and Krum, 2014). Therefore, CKD is accepted as an independent cardiovascular risk factor. Simultaneously, haemodynamic alterations that accompany CVD are likely to initiate renal inflammation and subsequent renal dysfunction (Liu et al., 2014). Even initial stages of renal insufficiency are accompanied by adverse changes in endothelium. Hsu et al. have demonstrated that the hypertension-related decline in eGFR correlates with number of endothelial cells-derived apoptotic microparticles and microparticles to endothelial progenitors ratio. One may speculate that impaired renal function is a consequence of, inter alia, compromised number and/or regenerative capacity of endothelial progenitor cells (Hsu et al., 2013).

Presented study was designed to evaluate the influence of *CCR5*-Δ32 polymorphism on the quantity of various CD34 + and CD34 + VEGFR2 + populations and indicate parameters that may be responsible for potential differences with special consideration for parameters illustrating renal function.

2. Materials and methods

2.1. Subjects

170 volunteers from Pomerania region were recruited by the Department of Family Medicine of Medical University of Gdansk and 7th Navy Hospital in Gdańsk. 68% of enrolled subjects have been suffering from hypertension (disease duration 7.0 ± 6.1 years). Hypertension was diagnosed according to the European Society of Hypertension and European Society of Cardiology guidelines (Mancia et al., 2013). All of the patients have been treated with hypotensive drugs. Subjects suffering from hypertension-related complications: left ventricular hypertrophy, coronary artery disease, stroke and hypertensive retinopathy were excluded from the project. In the study we have also included patients suffering from: atherosclerosis, osteoporosis, hiperlipidemia, hypothyroidism and hyperthyroidism, whereas individuals with the history of cancer, diabetes, autoimmune disorders and ongoing infectious diseases were excluded.

Table 1
Clinical characteristics of subjects according to CCR5-Δ32 genotype.

Characteristics	wt/wt	wt/Δ32 and Δ32/Δ32	*p*
N	129	41	
Age (years)	64.7 ± 9.7	62.8 ± 13.0	0.31
Sex (m/f)	46/83	19/22	0.22
BMI (kg/m^2)	28 ± 3	27 ± 3	0.16
SBP (mmHg)	146 ± 15	140 ± 19	0.07
DBP (mmHg)	84 ± 7	86 ± 8	0.24
Glucose (mg/dl)	108 ± 16	103 ± 14	0.09
CRP (mg/dl)	4.9 ± 4.6	4.8 ± 3.7	0.67
TC (mg/dl)	113 ± 29	113 ± 21	0.92
HDL-C (mg/dl)	58 ± 9	57 ± 9	0.53
LDL-C (mg/dl)	120 ± 19	122 ± 15	0.62
Fibrinogen (g/l)	3.72 ± 0.44	3.49 ± 0.57	0.007*
Creatinine (mg/dl)	0.86 ± 0.16	0.80 ± 0.12	0.02*
eGFR (ml/min/1.73 m^2)	85 ± 15	93 ± 13	0.001*
BUN (mg/dl)	16.1 ± 4.3	15.2 ± 2.6	0.18
Atherosclerosis (%)	6	0	0.18
Osteoporosis (%)	6	1	0.46
Hyperlipidemia (%)	9	3	0.58
Hypothyroidism (%)	14	4	0.39
Hyperthyroidism (%)	5	0	0.24

Variables are presented as mean ± SD; abbreviations: N: number of subjects, BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; eGFR: estimated glomerular filtration rate; BUN: blood urea nitrogen; p indicates differences between analysed genotypes; statistically significant differences ($p < 0.05$) have been marked with "*".

Standard clinical parameters including: complete blood count, glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, fibrinogen, creatinine and blood urea nitrogen levels have been quantified (Table 1) using an automatic biochemical analyser. Blood pressure was measured twice after 20 min rest in sitting position.

Estimated glomerular filtration rate (eGFR) was calculated in accordance with MDRD (Modification of Diet in Renal Disease) study (Mula-Abed et al., 2012). The rate calculations were based on the formula: GFR (ml/min/1.73m2) = 186.3 x [(creatinine concentration (mg/dL)] -1.154 x age – 0.203 121 x C; C for men = 1.0; C for women = 0.742.

This study was approved by the Ethics Committee of Medical University of Gdańsk (NKEBN/68/2011) and our investigation was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments on human subjects. Written informed consent was obtained from all the participants.

2.2. Flow cytometry analyses

Samples preparation and analyses were performed as described previously by (Skrzypkowska et al. (2017)).

2.3. Genotyping of the CCR5Δ32 polymorphism

Genomic DNA was isolated from EDTA-treated blood using the EXTRACTME DNA BLOOD kit (Blirt, Poland) according to the manufacturer protocol. The genotyping of rs333 polymorphism was performed as described by (Słomiński et al. (2017)).

2.4. Statistics

The results were analysed with the use of Statistica 12.0 (StatSoft, Inc USA). The $\chi 2$ test was used to determine the conformation of the allelic frequencies according to Hardy–Weinberg equilibrium proportions. Due to non-parametric distribution differences in the cell proportions and most of clinical features were evaluated using the Mann-Whitney U test. Normally distributed variables were analysed by ANOVA test. The $\chi 2$ Pearson test was applied for dichotomous

variables. The correlations between the variables were evaluated by the Spearman's test. Multiparametric regression analyses with forward selection approach were carried out to distinguish factors affecting analysed cell populations. Logistic models were used to evaluate the effect of genotypes on the risk of hypertension and reduced eGFR development. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. CCR5-Δ32 polymorphism and clinical characteristics of subjects

Participants have been divided according to their *CCR5*-Δ32 genotype. Genotype frequencies of analysed group were as follows: wt/wt – 76%, wt/Δ32 – 21%, Δ32 /Δ32 – 3%, whereas allele distributions were: wt – 86.5% and Δ32 – 13.5%. The assumption of Hardy-Weinberg equilibrium was satisfied ($p = 0.21$).

Clinical parameters are presented in Table 1. Due to low frequency of the Δ32 homozygous we have decided to analyze them together with heterozygous volunteers. Analysis of clinical parameters revealed lack of differences in values of age, body mass index, blood pressure, glucose, C-reactive protein, total cholesterol (TC), blood urea nitrogen as well as sex distribution between common homozygous and subjects with at least one Δ32 allele. We also haven't observed significant differences neither in high-density lipoprotein cholesterol nor low-density lipoprotein cholesterol. Simultaneously, subjects with wt/Δ32 or Δ32/Δ32 genotypes had more favorable, lower levels of fibrinogen ($p = 0.007$) and creatinine ($p = 0.02$) and elevated values of estimated glomerular filtration ($p = 0.001$). Occurrence of chosen disorders was comparable between both groups (Table 1).

3.2. Distribution of CCR5-Δ32 genotypes and allele frequencies in healthy and hypertensive subjects

Almost 68% (115 subjects) of the group comprised of hypertensive patients. The treatment scheme was as follows: 37% (43 subjects) received β-blockers, 24% (28 subjects) received Ca-blokers, 8% (9 subjects) received α-blockers, 37% (43 subjects) received angiotensin-converting-enzyme inhibitors, 12% (14 subjects) received angiotensin II receptor blockers, 21% (24 subjects) received diuretics and 22% (25 subjects) received statins. No differences in therapy according to genotype distribution were observed. Genotype distributions in hypertensive group as well as in normotensive subjects were in Hardy-Weinberg equilibrium ($p = 0.32$ and 0.47 respectively). In the healthy control group frequencies of alleles were as follows: 0.846 for wt and 0.154 for Δ32. For hypertensive individuals the respective values were 0.874 and 0.126. Genotype distributions in groups were comparable ($p = 0.50$). The risk of hypertension development was similar between wt and carriers of Δ32 allele (OR = 1.28; 95% CI = 0.614–2.682, $p > 0.05$) (Table 2).

Table 2
Distribution of CCR5-Δ32 genotypes and allele frequencies in normotensive subjects and hypertensive patients.

CCR5 genotypes	Normotensive ($N = 55$)		Hypertensive ($N = 115$)		Fisher's exact test
	N	%	*N*	%	
wt/wt	40	72.7	89	77.4	$p = 0.31$
wt/Δ32	13	23.7	23	20.0	
Δ32/Δ32	2	3.6	3	2.6	
wt frequency	0.846		0.874		
Δ32 frequency	0.154		0.126		

N: number of patients; *p* indicates significance between normotensive and hypertensive groups.

3.3. Distribution of CCR5-Δ32 genotypes and allele frequencies according to eGFR

Among analysed cases 108 subjects (63.5%) had estimated glomerular filtration rate below 90 mL/min/1.73m^2. The genotypes distribution were in Hardy-Weinberg equilibrium ($p = 0.30$), with allele frequency of 0.935 for wt and 0.065 for Δ32. Genotype distributions characteristic for subjects with eGFR above 90 mL/min/1.73m^2 were also confronted to Hardy-Weinberg equilibrium ($p = 0.45$). In this group allele frequencies were 0.742 for wt and 0.258 for Δ32. The frequency of Δ32 allele was higher in individuals with eGFR > 90 mL/min/1.73m^2 when compared to subjects with lower values of eGFR ($p < 0.00001$). The risk of eGFR below normal range was seven times higher in wt subjects than in Δ32 carriers (OR = 7.03; 95% CI = 3.221–15.342, $p < 0.00001$) (Table 3).

3.4. Number of CD34+ and CD34+VEGFR2+ cells among CCR5-Δ32 genotypes

Numbers of CD34+ ($p = 0.34$) and CD34+c-Kit+ ($p = 0.74$) cells were comparable between distinguished groups. The wt/Δ32 and Δ32/Δ32 carriers exhibited higher number of CD34+CXCR4+ cells ($p = 0.04$) than wild type participants. This group was also characterized by greater population of CD34+VEGFR2+ ($p = 0.002$) and CD34+VEGFR2+c-Kit+ ($p = 0.006$). Quantities of CD34+VEGFR2+CXCR4+ were similar in both groups ($p = 0.38$) (Table 4).

3.5. Relationship between selected parameters and number of CD34+CXCR4+ cells

Spearman's rank correlations analyses revealed that the number of CD34+CXCR4+ cells correlated with age (R = -0.20, $p = 0.01$), total cholesterol (R = 0.19, $p = 0.01$) and glucose levels (R = -0.20, $p = 0.007$). A significant correlation was not however observed between cells and body mass index (R = -0.05, $p = 0.46$), values of systolic blood pressure (R = -0.11, $p = 0.15$), diastolic blood pressure (R = 0.08, $p = 0.28$) as well as glomerular filtration rate (R = 0.12, $p = 0.10$). Multiparametric regression analyses with forward selection approach revealed that systolic blood pressure (β = -0.27, $p = 0.01$) and concentration of glucose (β = -0.17, $p = 0.02$) significantly decrease number of CD34+CXCR4+ cells. Correlations were calculated for both - wild type and Δ32 allele carriers together (Table 5).

3.6. Relationship between selected parameters and number of CD34+VEGFR2+ cells

Univariate analysis proven correlations between number of CD34+VEGFR2+ cells and age (R = -0.20, $p = 0.01$), systolic blood pressure (R = -0.18, $p = 0.02$) as well as cholesterol concentration (R = 0.21, $p = 0.004$). Other clinical characteristics, namely: body mass index (R = -0.10, $p = 0.24$), diastolic blood pressure (R = 0.10, $p = 0.39$), glucose (R = -0.13, $p = 0.10$) and glomerular filtration rate (R = 0.12, $p = 0.11$) were not correlated with the quantities of CD34+VEGFR2+ cells. Multivariable analyses indicated that systolic blood pressure significantly affects this population (β = -0.25, $p = 0.03$). Correlations were calculated for both - wild type and Δ32 allele carriers together (Table 5).

4. Discussion

The *CCR5* polymorphism has been indicated as affecting vasculature and as such involved in the development of cardiovascular disorders. We propose mechanisms by which *CCR5* variants may contribute to vascular homeostasis. We have found that Δ32 allele bearing individuals are characterized by higher number of: CD34+CXCR4+,

Table 3
Distribution of CCR5-Δ32 genotypes and allele frequencies in subjects with eGFR above and below 90 mL/min/1.73m2.

CCR5 genotypes	> 90 mL/min/1.73m2 (*N* = 62)		< 90 mL/min/1.73m2 (*N* = 108)		Fisher's exact test
	N	%	*N*	%	
wt/wt	33	53.2	96	88.9	$p < 0.00001$*
wt/Δ32	26	41.9	11	10.2	
Δ32/Δ32	3	4.9	1	0.9	
wt frequency	0.742		0.935		
Δ32 frequency	0.258		0.065		

N: number of patients; p indicates significance between analysed groups; statistically significant differences ($p < 0.05$) have been marked with "*".

Table 4
Number of CD34+ and CD34+VEGFR2+ cells in subjects according to the CCR5-Δ32 genotype.

Cell phenotype	wt/wt	wt/Δ32 and Δ32/Δ32	*p*
CD34+ (cells/ml)	2002 ± 747	2144 ± 1043	0.34
CD34+c-Kit+ (cells/ml)	683 ± 399	706 ± 353	0.74
CD34+CXCR4+ (cells/ml)	721 ± 304	840 ± 385	0.04*
CD34+VEGFR2+ (cells/ml)	97 ± 55	131 ± 82	0.002*
CD34+VEGFR2+c-Kit+ (cells/ml)	31 ± 27	50 ± 37	0.006*
CD34+VEGFR2+CXCR4+ (cells/ml)	32 ± 29	37 ± 22	0.38

Variables are presented as mean ± SD; p indicates differences between analysed genotypes; statistically significant differences ($p < 0.05$) have been marked with "*".

Table 5
Correlates of CD34+CXCR4+ and CD34+VEGFR2+ cells.

CD34+CXCR4+	R	p[a]	β	p[b]
Age (years)	−0.20	0.01*	−0.14	0.07
BMI (kg/m^2)	−0.05	0.46		
SBP (mmHg)	−0.11	0.15	−0.27	0.01*
DBP (mmHg)	0.08	0.28		
TC (mg/dl)	0.19	0.01*		
Glucose (mg/dl)	−0.20	0.007*	−0.17	0.02*
eGFR (ml/min/1.73 m^2)	0.12	0.10		
CD34+VEGFR2+	**R**	**p[a]**	**β**	**p[b]**
Age (years)	−0.20	0.01*	−0.14	0.06
BMI (kg/m^2)	−0.10	0.24	−0.08	0.29
SBP (mmHg)	−0.18	0.02*	−0.25	0.03*
DBP (mmHg)	0.10	0.39	0.19	0.09
TC (mg/dl)	0.21	0.004*		
Glucose (mg/dl)	−0.13	0.10		
eGFR (ml/min/1.73 m^2)	0.12	0.11		

Abbreviations: BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; eGFR: estimated glomerular filtration rate; pa indicates significance of Spearman's rank correlations; pb indicates significance of multiparametric analyses; statistically significant associations ($p < 0.05$) have been marked with "*".

CD34+VEGFR2+ and CD34+VEGFR2+c-Kit+ cells than wild type controls. Moreover, this group also exhibits more beneficial values of kidney function parameters.

The *CCR5*-Δ32 allele that is found approximately in 10% of the European population (Martinson et al., 1997), has been connected with CVD. Its protective role is usually connected with compromised inflammatory responses (Balistreri et al., 2008) as a total or partial loss of the receptor attenuates the progression of CCR5 ligands-based inflammation (González et al., 2001; Asahara et al., 1997).

It has been acknowledged that CCR5 takes part in overall atherosclerotic plaques formation and progression of the disease as it affects inflammatory cell mobilization and infiltration. The CCL5 has been reported as probably most important ligand in these processes (Jones et al., 2011).

One may speculate about protective role of the polymorphism against hypertension as analyses conducted on a murine model have revealed that hypertension-related angiotensin II upregulates the expression of CCR5 in T cells and CCL5 in vasculature. Upregulated expression of both receptor and ligand probably favors accumulation of lymphocytes within blood vessels (Guzik et al., 2007).

Majority of our subjects suffered from primary hypertension. Unfortunately, we have not recognized differences in the allele frequencies among normotensive and hypertensive volunteers. Wild type subjects had higher systolic blood pressure than heterozygous and rare homozygous but the results did not reach statistical significance. Likewise, Zhang et al. in a large study on American and Polish citizens have not disclosed connection between *CCR5* alleles and hypertension occurrence (Zhang et al., 2006).

The impact of attenuated receptor expression on inflammation is not the only explanation of frequently portrait differences in various disease incidences according to *CCR5* genotype. Hyde et al. have recognized advantageous effect of Δ32 allele on a lipid profile of individuals with established cardiovascular disease (Hyde et al., 2010). We have not observed similar results in volunteers without diagnosed CVD. Nonetheless, we have noted different preponderance of group with Δ32/wt or Δ32/Δ32 genotypes - higher quantities of CD34+CXCR4+, CD34+VEGFR2+ and CD34+VEGFR2+c-Kit+ cells.

CCR5 has been detected on endothelial cells as they show chemotactic activity towards its ligands (Berger et al., 1999). Presence of the receptor has been also confirmed for EPCs (Spring et al., 2005). Homing and incorporation of progenitors into angiogenic sites is based on multiple processes - mobilization, chemoattraction, adhesion, transmigration, tissue invasion and differentiation into mature endothelial cells (Urbich and Dimmeler, 2004). Ishida et al. have demonstrated that CCR5-deficient mice are characterized by attenuated c-Kit+Tie2+ EPCs homing and neovascularisation during wounds repair. This group has not observed alterations in the number of cells in wild type and CCR5-deficient mice indicating that CCR5-mediated signals may not regulate mobilization of EPCs from a bone marrow. Authors hypothesized that the receptor is rather responsible for progenitors homing control. Group concluded that it is the CCL5 that induces EPCs migration in CCR5-dependent manner (Ishida et al., 2012). Analyses on a murine model conducted by Zhang et al. confirmed that receptor is likely to take part in EPCs homing and migration as its overexpression improved recruitment of progenitors into atherosclerotic plaques. CCR5-overexpressing cells were further more efficient in maintaining plaques stability and improving endothelial dysfunction (Zhang et al., 2015). The reason why people bearing Δ32 allele have higher number of circulating EPCs could be connected with lesser vasculature damage leading to fewer progenitors being recruited from peripheral blood. On the other hand, our results could be explained by impaired cells homing due to reduced expression/absence of homing receptor.

Studies by Quinones et al. on a murine model have shown that loss of CCR5 has a protective effect during latter stages of atherosclerosis. This has been connected with, inter alia, reduced macrophage

accumulation and decreased concentrations of IL-6 and MCP-5. Animals with *CCR5* knockout had higher number of bone marrow Tie2 + Flk1 + c-Kit + EPCs. In humans, rare homozygous had increased proportions of circulating CD34 + Tie2 + progenitors when compared to common homozygous, but the results were not statistically significant (Quinones et al., 2007). Distinct way of EPCs identification and comparison of subjects according to their *CCR5* genotype may explain differences from our study.

The frequency of Δ32 allele was higher in individuals with normal eGFR and the risk of eGFR declining below normal range was several times higher for wildtype subjects. As wt/Δ32 and Δ32/Δ32 participants had simultaneously higher number of progenitor cells and more advantageous values of eGFR - key indicator of kidney function, we were hoping to find connections between these features, specially due to the fact that groups showed similarity in non-renal parameters. Such associations seemed more even likely as EPCs participate in vasculature regeneration during renal artery stenosis and restore renal function of ischemic kidneys as portrayed by improved eGFR (Chade et al., 2009).

We have noted correlations between CD34 + or CD34 + VEGFR2 + populations and few of the clinical parameters (age, glucose, total cholesterol, blood pressure), but unfortunately, we have not observed comparable significant connections when analysing filtration rate.

EPCs are affected by impaired renal function - the alterations concern progenitors' number along with their activity. This relationship could be grounded in the fact that individuals with CKD are also more likely to suffer from conditions that attenuate EPCs quantity and function, e.g. high blood pressure, dyslipidaemia, diabetes or increased inflammatory reaction and oxidative stress (Bahlmann et al., 2010). Furthermore, diminished number of CD34 + cells has been recognized as an independent predictor of cardiovascular events and all-cause mortality in haemodialysis-receiving CKD patients (Maruyama et al., 2008).

As CCL5 participates in formation of inflammatory infiltrate during glomerulonephritis, the CCL5 receptors blockage leads to decreased inflammation. Simultaneously, chemokine is involved in repair of glomerular vasculature through promoting EPCs recruitment and adhesion of CD34 + cells to activated endothelium. Overall, one may speculate that ligand regulates homing and incorporation of progenitors into damaged renal endothelium. On the other hand, it has been suggested that EPCs may stimulate glomerular microvascular repair mainly in a paracrine manner. As the analyses on a rat model focused on a CCL5, one must bear in mind that the ligand binds to additional receptors: CCR1, CCR3 and CCR9 as well (Rookmaaker et al., 2007). These results once more suggest that the higher number of CD34 + VEGFR2 + cells in a circulation of individuals with at least one copy of Δ32 allele could result from less intense homing.

Influence of chemokine receptor polymorphisms on indicators of renal function, such as eGFR, are not often described. They are more frequently linked with allograft rejection/function after renal transplantation (Segerer and Nelson, 2005). Recently, polymorphisms of chemokine fractalkine receptor CX3CR1 have been *associated* with CKD occurrence, but values of renal parameters did not differ among patients (Yadav et al., 2016). Sezgin et al. have described an altered distribution of CCR2-V64I polymorphism genotypes in the population of chronic renal failure sufferers when compared to healthy population, but provided very little clinical data (Sezgin et al., 2011). Extensive analyses have also revealed associations of various chemokine polymorphisms, including: CCL2, IL8, CCR5 and MMP9 with increased risk of diabetic nephropathy [000], but very little is known about impact of chemokine-related polymorphisms in population free from severe conditions like diabetes, heart or kidney disease.

We have found that the presence of *CCR5*-Δ32 allele is connected with increased number of cells that could be recognized as EPCs. At the same time, heterozygous and rare homozygous for the allele are characterized by improved kidney function. We have failed to find connections between these features, which may suggest that the mechanisms of CCR5-Δ32 carriers' advantage may be independent of each other.

Conflict of interest

The authors declare that they have no conflict of interest.

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