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Associations of fractalkine receptor (CX3CR1) and CCR5 gene variants with hypertension, diabetes and atherosclerosis in chronic renal failure patients undergoing hemodialysis

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Abstract
Purpose We aimed to investigate the associations of fractalkine receptor (CX3CR1) V249I, T280M and CCR5-59029 A/G gene polymorphisms in chronic renal failure (CRF) subjects undergoing hemodialysis and to evaluate possible associations of these polymorphisms with hypertension (HT), diabetes mellitus (DM) and atherosclerosis (AS).
Methods A total of 225 CRF subjects undergoing hemodialysis and 201 healthy controls were enrolled in the study. CRF subjects were divided into three major subgroups according to comorbidities including HT ($n = 127$), DM ($n = 65$) and AS ($n = 33$). Genotyping was done using polymerase chain reaction–restriction fragment length polymorphism method.
Results The II genotype and I allele frequencies of CX3CR1 V249I polymorphism were found significantly more frequent in CRF subjects, CRF subjects with DM and CRF subjects with AS compared with controls ($p < 0.05$ for all comparisons). G allele frequency of CCR5 polymorphism was found significantly more prevalent in CRF subjects with DM than that of controls. Further, GG genotype and G allele frequencies of CCR5 polymorphism were significantly more prevalent in CRF subjects with AS compared with controls ($p < 0.05$). We also explored these polymorphisms among CRF subjects with and without following comorbidities: HT, DM, AS. We found significant association between CRF subjects with HT and without HT in terms of genotype and allele frequencies of V249I polymorphism ($p < 0.05$). CX3CR1 T280M polymorphism was not found significantly different in none of the comparisons.
Conclusion These data demonstrate possible associations between CX3CR1 V249I and CCR5-59029 A/G polymorphisms and/or HT, DM and AS in CRF subjects.

Keywords Chronic renal failure · CCR5 · CX3CR1 · Hypertension · Diabetes mellitus · Atherosclerosis · Polymorphism

Introduction

Chronic kidney disease (CKD) is a heterogeneous disease affecting the structure and function of the kidney and an increasing health problem worldwide. CKD leads to end-stage renal disease (ESRD) which is resulted in very severely reduced kidney function and required hemodialysis or kidney transplantation [1, 2]. The prevalence of chronic kidney disease in Turkey has been reported as 15.7 %, and cardiovascular risk factors have been found significantly more frequent in CKD [3].

Due to the existence of acute and chronic pro-inflammatory conditions in CKD, inflammation is an important contributing factor for morbidity and mortality of the disease [4]. Chemokines are small molecular weight molecules

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which promote directed migration of leukocytes, epithelial cells and endothelial cells [5]. Chemokines help activation of inflammatory cells and selective migration to damaged renal tissues. During renal inflammation, intrinsic renal cells and infiltrating cells express chemokines and chemokine receptors [6]. In response to stimulation with pro-inflammatory cytokines, all types of renal cells are able to produce various kinds of chemokines [4–6].

Fractalkine (CX3CL1) acts as both a chemoattractant molecule and an adhesion molecule. Fractalkine is activated by pro-inflammatory cytokines like interferon-γ and TNF-α [7]. During renal inflammatory response, fractalkine derived from proximal tubules plays an important role in the recruitment and retention of leukocytes to interstitium [8, 9]. CX3CR1 is a membrane-bound receptor of fractalkine and is expressed at detectable level on $CD3^+$ T cells (predominantly $CD8^+$ T cells), $CD14^+$ monocytes and $CD16^+$ NK cells [10].

CCR5 is a cell surface receptor of the CC chemokines including RANTES, MCP-2, MIP-1α and MIP-1β. Approximately 10 % of monocytes and 20–30 % of peripheral T cells are $CCR5^+$ cells [11]. $CCR5^+$ lymphocytes accumulate in interstitium, and in human kidney biopsies of various renal diseases, the amount of the interstitial $CCR5^+$ lymphocytes has been found to be correlated with serum creatinine levels [12].

Various polymorphisms have been identified in chemokines and their receptors. These polymorphisms have been suggested to be associated with renal disease and acute and chronic kidney rejection [13]. In addition to renal diseases, these polymorphisms have been studied in glucometabolic-, cardiovascular- and obesity-related diseases [14, 15]. A moderate protective effect of CX3CR1 deficiency on glucose intolerance and insulin resistance has been demonstrated by Shah et al. [16]. CCR5-delta 32 deletion and CCR5-59029 A/G single nucleotide polymorphisms (SNPs) are the most commonly studied polymorphisms in the CCR5 gene. V249I and T280M SNPs are the best defined polymorphisms in CX3CR1 chemokine gene [17].

In the current study, we aimed to show the possible relationships between CCR5-59029 A/G SNP, and CX3CR1 V249I and T280M SNPs and the risk of chronic renal failure (CRF). Moreover, we aimed to investigate whether there is a relationship between these polymorphisms and the comorbidities of CRF including hypertension (HT), diabetes mellitus (DM) and atherosclerosis (AS).

Materials and methods

Subjects

A total of 225 CRF subjects (mean age = 60.7 ± 11.31, 119 male and 106 female) undergoing hemodialysis in different dialysis centers in Sivas city of Turkey were enrolled in the current study. All CRF subjects included in the study were stage 5 CKD (ESRD). As the healthy control group, 201 age-, sex- and ethnicity-matched healthy individuals with the same geographic region and with no history of DM, HT and renal or cardiovascular diseases were included in the current study. All participants of the current study were Caucasians of Turkish origin. Information about age, gender, body mass index, dialysis duration, parental consanguinity and comorbidities of chronic kidney disease and laboratory findings including albumin, creatinine, c-reactive protein, sedimentation, total cholesterol, HDL cholesterol, LDL cholesterol, hemoglobin, platelet count, glucose, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained from medical records of CRF subjects.

In the current study, CRF subjects were divided into three major subgroups according to comorbidities including HT, DM and AS. Diagnosis of DM was done according to the criteria of the American Diabetes Association (ADA) (a fasting plasma glucose level ≥126 mg/dl or postprandial plasma glucose level ≥200 mg/dl) [18]. HT was diagnosed according to the guideline of European Society of Hypertension (ESH) and European Society of Cardiology (ESC) (SBP and DBP higher than 140 and 90 mmHg, respectively) [19]. Doppler ultrasonography was used for the evaluation of carotid AS. We considered carotid AS either the presence of plaques or an intima media thickness >1 mm.

Local ethics committee approval was obtained from Clinical Research Ethics Committee of Cumhuriyet University. To participate in the current study, all CRF and control subjects gave a written informed consent. All procedures performed in the current study have been done in accordance with the ethical standards of the Helsinki Declaration.

Genotyping

A total of 2–3 ml venous blood samples obtained from CRF subjects and healthy controls were collected into the K_3EDTA-containing tubes and stored at −20 °C. The spin-column method (Invisorb spin blood, In vitek, Berlin, Germany) was used for isolation of the total genomic DNA. Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method was used to determine all three polymorphisms. For genotyping of both CX3CR1 SNP and CCR5-59029 A/G SNP, 150–200 ng (1 µl) genomic DNA was added to a final 25-µl mixture containing 12.5 µl 2× PCR Master Mix (Thermo Fisher Scientific, Massachusetts, USA), 9.5 µl dH_2O and 1 µl (10 pmol) of each primers. Primer sequences, PCR conditions, restriction endonucleases and RFLP profiles of the studied gene polymorphisms are demonstrated in Table 1.

Table 1 Primer sequences, PCR conditions, restriction enzymes and RFLP profiles of the studied gene polymorphisms

Polymorphism	Primer sequences	PCR conditions	Endonucleases and conditions	Product size (PCR)	RFLP products
CCR5 -59029 A/G	F: CCCGTGAGCCCATAGTTAAAACTC R: TCACAGGGCTTTTCAACAGTAAGG	94° 4 min 94° 30 sec 55° 45 sec 72° 60 sec 72° 7 min 35 cycles	Bsp1286I, 37 °C for 1 hour	258	AA: 258bp AG:130bp, 258bp GG: 130bp
CX3CR1 V249I	F: CCGAGGTCCTTCAGGAAATCT R: TCAGCATCAGGTTCAGGAACTC	94° 3 min 94° 30 sec 52° 40 sec 72° 55 sec 72° 10 min 35 cycles	AclI, 37 °C for 16 hours	588	VV: 383bp, 205bp VI: 588bp, 383bp, 205bp II: 588bp
CX3CR1 T280M			BsmbI, 55 °C for 16 hours		TT:75bp, 216bp, 297bp TM:372bp, 297bp, 216bp, 75bp MM: 372bp, 216bp

Statistical analysis

All statistical analyses of the current study were performed by Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS IBM, New York, USA). Statistical power and sample size were calculated using PASS software (Power Analysis and Sample-Size package; NCSS Statistical Software, Utah, USA). Sample size was calculated as 225 CRF subjects and 201 controls at the 5 % level of significance and with 80 % power. Descriptive values were expressed as mean ± standard deviation (SD) or frequency and percent as appropriate. All comparisons were made between CRF subjects or CRF subgroups and controls, and CRF subjects with and without HT, DM and AS. For the comparisons of genotype and allele frequencies, Chi-square test or Fisher's exact test was used. p value was considered as significant using two-sided comparisons if less than 0.05. All results were expressed with a 95 % confidence interval.

Table 2 Baseline characteristics of CRF subjects and controls

Characteristics	CRF ($n = 225$)	Controls ($n = 201$)
Age (years)	60.7 ± 11.3	58.7 ± 12.4
Male	119 (52.9)	102 (50.7)
Female	106 (47.1)	99 (49.3)
Body mass index	31.2 ± 6.3	30.7 ± 7.2
Dialysis duration (years)	4.9 ± 4.3	–
Parental consanguinity	27 (12)	Unknown
Hypertension	127 (56.4)	–
Diabetes mellitus	65 (28.9)	–
Atherosclerosis	33 (14.7)	–
Nephrolithiasis	17 (7.6)	–
Polycystic kidney	15 (6.7)	–
Glomerulonephritis	10 (4.4)	–

Data were expressed as mean ± SD or frequency and percent (%) as appropriate

CRF Chronic renal failure

Results

In the current study, we examined the frequencies of CCR5-59029 A/G, CX3CR1 V249I and CX3CR1 T280M polymorphisms in 225 CRF subjects undergoing hemodialysis and 201 control subjects. Baseline characteristics of CRF and control subjects are given in Table 2. Laboratory findings of CRF subjects were as follows: albumin 3.6 ± 1.8 g/dl, creatinine 7.2 ± 2.7 mg/dl, c-reactive protein 25.5 ± 27.5 mg/l, sedimentation 72.3 ± 32.9 mm/h, total cholesterol 177.1 ± 54.9 mg/dl, HDL cholesterol 37.5 ± 13.9 mg/dl, LDL cholesterol 112.2 ± 75.4 mg/dl, hemoglobin 11.3 ± 1.54 g/dl, platelet count 265.7 ± 82.8 10^3/µl, glucose 127.9 ± 65.7 mg/dl, SBP 136.3 ± 21.4 mmHg and DBP 79.5 ± 12.2 mmHg.

Table 3 shows the genotype distribution and allele frequencies of CRF subjects, CRF subgroups and controls. All comparisons in Table 3 were made between CRF subjects or subgroups of CRF subjects and controls. Allele frequencies and genotype distribution of CCR5-59029 A/G SNP was not found as significant in CRF subjects compared with controls ($p > 0.05$). Both genotype and allele frequencies of V249I polymorphism were statistically significantly different in CRF subjects than in controls ($p = 0.005$ for genotype, $p = 0.027$ for allele). CX3CR1 249 II genotype and I allele showed 2.86 and 1.38 times higher risk of CRF susceptibility, respectively (OR 2.86, 95 % CI 1.40–5.84 for II genotype; OR 1.38, 95 % CI 1.03–1.85 for I allele). Statistically significant difference was not observed in terms of genotype and allele frequencies of T280M SNP between CRF subjects and control group (p values > 0.05).

To show possible associations of these chemokine polymorphisms with comorbidities of the CRF, subjects were divided into three major subgroups according to their comorbidities including HT, DM and AS. Genotype and allele frequencies of subgroups of CRF are given in Table 3.

Table 3 Genotype and allele frequencies of CRF subjects, CRF subgroups and controls

Polymorphism	CRF ($n = 225$)	CRF with HT ($n = 127$)	CRF with DM ($n = 65$)	CRF with AS ($n = 33$)	Control ($n = 201$)
CCR5-59029 A/G					
AA	62 (27.6)	35 (27.6)	15 (23.1)	7 (21.2)	67 (33.3)
AG	94 (41.8)	56 (44.1)	27 (41.5)	11 (33.3)	85 (42.3)
GG	69 (30.6)	36 (28.3)	23 (35.4)	15 (45.5)	49 (24.4)
*p value	>0.05	>0.05	>0.05	**0.039****	–
OR (95 % CI)					
GG versus AA + AG	1.37 (0.89–2.1)	1.22 (0.74–2.02)	1.69 (0.93–3.1)	**2.58 (1.21–5.51)**	–
A	0.484	0.496	0.438	0.379	0.545
G	0.516	0.504	0.562	0.621	0.455
p value	>0.05	>0.05	**0.034**	**0.012**	–
OR (95 % CI)					
G versus A	1.27 (0.97–1.66)	1.21 (0.88–1.66)	**1.53 (1.02–2.28)**	**1.96 (1.14–3.35)**	–
CX3CR1 V249I					
VV	90 (40.0)	62 (48.8)	22 (33.8)	12 (36.4)	100 (49.8)
VI	103 (45.8)	52 (41.0)	35 (53.9)	14 (42.4)	90 (44.7)
II	32 (14.2)	13 (10.2)	8 (12.3)	7 (21.2)	11 (5.5)
p value	**0.005**	>0.05	**0.034**	**0.006**	–
OR (95 % CI)					
II versus VV + VI	**2.86 (1.40–5.84)**	1.96 (0.85–4.64)	2.42 (0.93–6.31)	**4.65 (1.65–13.05)**	–
II + VI versus VV	**1.48 (1.01–2.18)**	1.03 (0.66–1.61)	**1.93 (1.07–3.46)**	1.73 (0.80–3.70)	–
V	0.651	0.693	0.608	0.576	0.721
I	0.349	0.307	0.392	0.424	0.279
p value	**0.027**	>0.05	**0.014**	**0.016**	–
OR (95 % CI)					–
I versus V	**1.38 (1.03–1.85)**	1.14 (0.81–1.61)	**1.67 (1.10–2.52)**	**1.90 (1.11–3.25)**	
CX3CR1 T280M					
TT	189 (84.0)	107 (84.2)	54 (83.1)	26 (78.8)	178 (88.6)
TM	34 (15.1)	19 (15.0)	10 (15.4)	7 (21.2)	22 (10.9)
MM	2 (0.9)	1 (0.8)	1 (1.5)	0 (0)	1 (0.5)
p value	>0.05	0.442	0.316	0.236	–
OR (95 % CI)					
MM + TM versus TT	1.47 (0.84–2.58)	1.44 (0.75–2.75)	1.57 (0.72–3.44)	2.08 (0.81–5.33)	–
T	0.915	0.917	0.908	0.894	0.94
M	0.085	0.083	0.092	0.106	0.06
p value	>0.05	>0.05	>0.05	>0.05	–
OR (95 % CI)					
M versus T	1.48 (0.87–2.52)	1.41 (0.77–2.6)	1.6 (0.77–3.3)	1.86 (0.77–4.53)	–

Data were expressed as frequency and percent (%)

CRF chronic renal failure, *HT* hypertension, *DM* diabetes mellitus, *AS* atherosclerosis, *OR* odds ratio, *CI* confidence interval

* All comparisons were made between patients with CRF/CRF subgroups and controls. Chi-square test or Fisher's exact test was used for the comparisons

** Bold values were statistically significant ($p < 0.05$)

For CCR5-59029 A/G polymorphism, genotype and allele frequencies were not statistically different between CRF subjects with HT and controls. Allele frequency of CRF subjects with DM was statistically significantly higher than that of controls ($p = 0.034$; OR 1.53, 95 % CI 1.02–2.28). However, genotype distribution was not statistically different between the two groups ($p > 0.05$). Both genotype and allele frequencies were significantly higher in CRF

subjects with AS ($p = 0.039$ for genotype, $p = 0.012$ for allele).

For CX3CR1 V249I polymorphism, genotype and allele frequencies were not statistically different between CRF subjects with HT and controls (p values > 0.05). Genotype and allele frequencies of both CRF subjects with DM and CRF subjects with AS were statistically significantly different than those of control group ($p < 0.05$ for all comparisons). For CX3CR1 T280M SNP, there was no statistically significant difference between all subgroups and control group ($p > 0.05$ for all comparisons).

We also compared these SNPs among the CRF subjects with and without following comorbidities: HT, DM, AS. Any significant association was not found between subjects with HT and without HT, subjects with DM and without DM and subjects with AS and without AS in terms of genotype and allele frequencies of CCR5-59029 A/G and CX3CR1 T280M SNP. However, a significant association was found between subjects with HT and without HT in terms of genotype and allele frequencies of CX3CR1 V249I SNP ($p = 0.005$ for genotype; $p = 0.0014$ for allele). Individuals having VI + II genotype were found 2.38 (95 % CI 1.36–4.17) times higher in CRF subjects without HT compared with CRF subjects with HT. In addition, individuals having VI genotype were found 1.75 times higher in CRF subjects without HT compared with CRF subjects with HT (VI vs. VV + II; OR 1.75, 95 % CI 1.02–3.00). The V allele of V249I SNP was found higher in CRF subjects without HT compared to CRF subjects with HT (V vs. I; OR 1.87, 95 % CI 1.27–2.76). V249I SNP was not significant among CRF subjects with DM and without DM and subjects with AS and without AS ($p > 0.05$) (Table 4).

Discussion

In the present study, we investigated the role of CCR5-59029 A/G, CX3CR1 V249I and CX3CR1 T280M gene polymorphisms in development of CRF and comorbidities of the disease including HT, DM and AS. We genotyped above-mentioned chemokine receptor SNPs in 225 CRF subjects requiring hemodialysis and 201 healthy controls by PCR–RFLP method.

In the pathogenesis of renal diseases, chemokines and their corresponding receptors mediate leukocyte infiltration and activation during kidney inflammation. Infiltrating leukocytes contribute to renal damage as mediate extracellular matrix production by producing and releasing inflammatory and profibrotic cytokines, such as TNF-α, IL-1β and interferon-γ, and various growth factors [20]. The studies investigating the role of polymorphisms and mutations in genes encoding chemokines and chemokine receptors in renal diseases have increased steadily. In our previous studies, we demonstrated that the polymorphisms in MCP-1 and CCR2 genes have increased the risk of CRF development [21, 22].

CX3CR1 is present on both interstitial and glomerular infiltrating leukocytes [10]. Recently, it has been reported that CX3CR1 decreases fibrosis of kidney by inhibiting proliferation of profibrotic macrophages [23]. Fractalkine and CX3CR1 interaction may increase the monocyte infiltration into the interstitium and interstitial injury [24]. The V249I and T280M SNPs of CX3CR1 gene have been found to be related to a decreased fractalkine signaling and resulted in reduced adhesive function [25]. In Indian population, Borkar et al. [26] determined that the frequency of CX3CR1 V249I II genotype and I allele was significantly higher in ESRD patients. Several previous studies have suggested the significant role of CX3CR1 in the allograft rejection. It has been shown that CX3CR1-positive macrophages were markedly increased in acute kidney allograft rejection [27, 28]. CX3CR1 V249I VV genotype was associated with increased risk of delayed kidney allograft function [29].

Chronic kidney disease is generally associated with old age, obesity, HT, diabetes and cardiovascular disease [1]. Furthermore, in the Mediterranean countries including Turkey, familial Mediterranean fever (FMF) is another risk factor for the development of CKD [30]. Decreased kidney function increases the risk of AS and cardiovascular death. $CD4^+/CX3CR1^+$ T cells contribute to the excess atherosclerotic inflammation in renal failure [31]. In a cohort of 161 ESRD subjects undergoing dialysis, Losito et al. [32] found no statistically significant difference between V249I and T280M polymorphisms of CX3CR1 and ESRD. Yadav et al. [33] have demonstrated a significant increase with regard to $CD4^+/CX3CR1^+$ T cells, plasma fractalkine level and intima media thickness in subjects with CKD, and the authors suggest that fractalkine–CX3CR1 pathway may have a contributory role in the development and progression of AS in CKD subjects. To investigate the role of V249I and T280M SNPs in the development of AS, a plethora of studies have been done by different researchers in different populations. A recent meta-analysis suggests that V249I and T280M polymorphisms of CX3CR1 gene were associated with the susceptibility to AS [34]. In the present study, we found that the II genotype and I allele increased risk of AS in CRF subjects 4.65 and 1.90 times, respectively.

Diabetic nephropathy is the major microvascular complication of DM, and microalbuminuria is considered as one of the earliest clinical signs of diabetic nephropathy [35]. In a recent study, serum level of fractalkine was found to be significantly increased in type 2 diabetic nephropathy compared with type 2 diabetic without nephropathy and the control group [36]. Fractalkine/CX3CR1 pathway may

Table 4 Genotype and allele frequencies among CRF subjects with and without hypertension, diabetes mellitus and atherosclerosis

Polymorphism	CRF with HT ($n = 127$)	CRF without HT ($n = 98$)	CRF with DM ($n = 65$)	CRF without DM ($n = 160$)	CRF with AS ($n = 33$)	CRF without AS ($n = 192$)
CCR5-59029 A/G						
AA	35 (27.6)	27 (27.5)	15 (23.1)	47 (29.3)	7 (21.2)	55 (28.6)
AG	56 (44.1)	38 (38.8)	27 (41.5)	67 (41.9)	11 (33.3)	83 (43.3)
GG	36 (28.3)	33 (33.7)	23 (35.4)	46 (28.8)	15 (45.5)	54 (28.1)
*p value	>0.05		>0.05		>0.05	
A	0.496	0.469	0.438	0.503	0.379	0.503
G	0.504	0.531	0.562	0.497	0.621	0.497
p value	>0.05		–		>0.05	
CX3CR1 V249I						
VV	62 (48.8)	28 (28.6)	22 (33.8)	68 (42.5)	12 (36.4)	78 (40.6)
VI	52 (41)	51 (52)	35 (53.9)	68 (42.5)	14 (42.4)	89 (46.4)
II	13 (10.2)	19 (19.4)	8 (12.3)	24 (15)	7 (21.2)	25 (13)
p value	**0.005****		>0.05		>0.05	
OR (95 % CI)***	**VV versus VI + II = 2.38 (1.36–4.17); VI versus VV + II = 1.75 (1.02–3.00)**		–		–	
V	0.693	0.545	0.608	0.637	0.576	0.638
I	0.307	0.455	0.392	0.367	0.424	0.362
p value	**0.0014**		>0.05		>0.05	
OR (95 % CI)	**V versus I = 1.87 (1.27–2.76)**	–	–			
CX3CR1 T280M						
TT	107 (84.2)	82 (83.7)	54 (83.1)	135 (84.4)	26 (78.8)	163 (84.9)
TM	19 (15.0)	16 (16.3)	10 (15.4)	25 (15.6)	7 (21.2)	28 (14.6)
MM	1 (0.8)	–	1 (1.5)	–	0 (0)	1 (0.5)
p value	>0.05		>0.05		>0.05	
T	0.917	0.918	0.908	0.922	0.894	0.922
M	0.083	0.082	0.092	0.078	0.106	0.078
p value	>0.05	>0.05	>0.05			

Data were expressed as frequency and percent (%)

CRF chronic renal failure, *HT* hypertension, *DM* diabetes mellitus, *AS* atherosclerosis, *OR* odds ratio, *CI* confidence interval

* All comparisons were made between CRF with and without the following comorbidities: hypertension, diabetes, atherosclerosis. Chi-square test or Fisher's exact test was used for the comparisons

** Bold values were statistically significant ($p < 0.05$)

*** Odds ratios were calculated for high-risk allele and genotypes of CX3CR1 V249I polymorphism and only expressed in statistically significant ($p < 0.05$) comparisons

play an important role in diabetic renal injury by upregulating extracellular matrix synthesis. In a recent study, diabetic CX3CR1 knockout mice showed no significant changes in plasma glucose level. On the other hand, compared with diabetic WT mice, collagen, fibronectin and fractional mesangial area, which are the markers of renal inflammation, extracellular matrix and fibrosis, have been found markedly lower in diabetic CX3CR1 knockout mice [37]. Fractalkine expression and $CX3CR1^+$ cell infiltration in diabetic kidneys may play an important role in progression of diabetic nephropathy [38]. In our study, compared with CX3CR1 V249I VV genotype, individuals having II + IV genotype showed 1.93-fold higher risk of DM. Furthermore, in terms of I allele, there was 1.67-fold risk of CRF subjects with DM.

In CKD, HT is one of the most common comorbidities and a major risk factor for cardiovascular disease [1]. Angiotensin II, which is one of the hormones that cause HT, raises blood pressure by a number of actions including vasoconstriction, increased aldosterone synthesis, sympathetic nerve activity and renal actions [39]. In a recent study, Rius et al. [40] showed that angiotensin II

stimulates the expression of CX3CL1 and arteriolar leukocyte adhesion was significantly reduced in CX3CR1$^{(-/-)}$ mice. In vitro studies have been shown that fractalkine is able to induce reactive oxygen species, including superoxide ions, which results in reduced availability of nitric oxide and endothelial dysfunction [41, 42]. Marasini et al. [43] reported that the CX3CR1 249I allele might be associated with systemic sclerosis-associated pulmonary arterial HT.

Studies performed with CCR5$^{(-/-)}$ mouse models have demonstrated that CCR5 deficiency aggravates kidney failure in nephrotoxic serum-induced nephritis [44] and exacerbates lipopolysaccharide-induced acute kidney injury [45]. In many studies performed with kidney transplantation patients, researchers have demonstrated that CCR5-59029 SNP was a risk factor for kidney rejection [17, 46–48]. Borkar et al. [26] have found a significant difference with regard to the genotype frequency of CCR5-59029 A/G polymorphism between ESRD subjects and controls. They also observed that the A allele of CCR5-59029 A/G SNP was significantly more frequent in the chronic glomerulonephritis patients. Yadav et al. [49] found that AA genotype and A allele of CCR5-59029 A/G polymorphism were significantly more frequent in DM subjects compared with healthy controls and also significantly more frequent in the diabetic nephropathy compared with DM. In a study performed with type 1 diabetes patients, male carriers of the 59029 G allele associated with increased risk of diabetic nephropathy [50]. Among Asian Indians, the A allele of CCR5 gene-59029 A/G SNP was found significantly associated with diabetic nephropathy. Furthermore, the A allele was found significantly correlated with ESRD risk in their study [51]. In our study, it has been found that G allele increases diabetic nephropathy risk 1.53-fold.

Relatively small number of subjects could be considered as the major limitation of the current study. Furthermore, because of the etiologic causes, comorbidities of the chronic kidney disease and heterogeneous distribution, it is not easy to homogenize and standardize the disease based on only one comorbidity such as HT, DM or AS. Therefore, we analyzed our subjects based on the observed comorbidity. Thus, several cases had two or more comorbidities in our subgroups. In addition, we did not measure the studied chemokine receptors in the level of mRNA and protein.

In conclusion, results of our study suggest that II genotype and I allele of CX3CR1 V249I SNP could be considered as an independent risk factor for CRF. Furthermore, CCR5-59029 A/G SNP is associated with CRF subjects with DM and CRF subjects with AS, and CX3CR1 V249I polymorphism is associated with three comorbidities including HT, DM and AS. Further studies in larger sample size are required to show possible associations of these chemokine receptor polymorphisms in CRF subjects with HT, DM and AS.

Compliance with ethical standards

Conflict of interest All of the authors of the current study declare that they have no conflict of interest.

Ethical approval Local ethics committee approval was obtained from Clinical Research Ethics Committee of Cumhuriyet University, Sivas, Turkey. All procedures performed in the current study have been done in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent All subjects and control subjects gave a written informed consent to participate in the current study.

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