

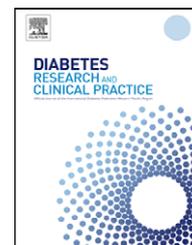


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CCR5 Δ 32 genotype is associated with outcome in type 2 diabetes mellitus

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ABSTRACT

Aims: To test whether the genetic variant CCR5 Δ 32 in the CC-chemokine receptor 5, which is known to lead to CCR5 deficiency, is associated with mortality in type 2 diabetes patients.

Methods: We examined the effect of presence or absence of the CCR5 Δ 32 on overall and cardiovascular mortality risk in the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) cohort, a type 2 diabetes patient cohort.

Results: We studied 756 patients with a mean duration of follow-up of 5.4 (\pm 1.4) years. 194 patients died during follow up of which 83 were cardiovascular deaths. 144 subjects (19%) carried the CCR5 Δ 32 deletion. CCR5 Δ 32 carriers had an adjusted hazard ratio of 0.62 (95%CI: 0.40–0.96; p = 0.03) for all-cause mortality and 0.63 (95%CI: 0.33–1.19; p = 0.16) for cardiovascular mortality.

Conclusions: The presence of CCR5 Δ 32 is associated with better survival in type 2 diabetes patients. These data suggest that it is worthwhile to explore the protective potential of pharmacological blockade of CCR5 in type 2 diabetic patients.

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1. Introduction

Chemokines and their receptors have a central role in leucocyte trafficking, and are involved in the pathophysiology of various inflammatory disorders [1,2]. Genetic variability in the chemokine cascades could therefore potentially modify inflammatory processes. For the CC-chemokine receptor 5 (CCR5) several polymorphisms have been described. Among these, the CCR5 Δ 32 genetic variant, consisting of a 32-basepair deletion in the open reading frame effectively results in functional CCR5 deficiency by absence of CCR5 membrane expression. Heterozygous subjects express a lower amount of functional receptors compared to wild-type homozygotes [3]. The pathophysiological significance of the CCR5 Δ 32 genetic

variant is demonstrated by its association with resistance to HIV infection, where CCR5 modulates virus entry [4,5].

CCR5 is expressed on T cells, macrophages, smooth muscle cells and endothelial cells [6,7]. These cells are involved in the chronic inflammatory state present in insulin resistance, type 2 diabetes, atherosclerosis and uremia [2,8–10]. In line with its protective effect in HIV, CCR5 Δ 32 has also been shown to be associated with better outcome in patients with a high risk for atherosclerotic cardiovascular disease, dialysis patients and renal transplant recipients, probably by modulation of the inflammatory response in these conditions [11–15].

Type 2 diabetes is characterized by a particularly elevated overall and cardiovascular mortality, attributed at least partly to a generalized inflammatory condition [8,16]. This elicits the

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hypothesis that CCR5 Δ 32 could be associated with mortality risk in type 2 diabetes as well. To test this hypothesis, in the current study we investigated whether the presence or absence of CCR5 Δ 32 is associated with overall and/or cardiovascular mortality in a longitudinal follow-up cohort of type 2 diabetic patients.

2. Patients and methods

2.1. Patients

This study is part of the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC). In this project, general practitioners (GP) receive support by diabetic specialists for the implementation of the Dutch national guidelines in care of type 2 diabetic patients. Patients were recruited from the eastern part of the Netherlands. In a part of this project all patients with type 2 diabetes, exclusively treated by their GP are followed annually. These patients ($n = 1149$) are part of the present study. Eligibility criteria were: type 2 diabetes, as defined by the national guidelines of the Dutch college of general practitioners (based on the 1997 American Diabetes Association criteria) treated by a general practitioner. All patients gave informed consent before being included. The study was approved by the local medical ethics committee. Patients were included between January 1998 and December 1999. Details were described previously [17]. For the current analyses data were used from patients who gave permission for DNA analyses ($n = 798$). Patients were followed until date of death or date of censoring, i.e. withdrawal from the study or end of the follow-up period (December 2004).

2.2. Demographic and clinical data

The following data were collected: age, gender, smoking habit, medication use, systolic and diastolic standing, office blood pressure after 5 min rest (Tycos sphygmomanometer; Welch Allyn B.V., Delft, The Netherlands), medical history and comorbidity, body mass index (BMI) and diabetes duration. A blood sample and a urine sample were obtained. Serum lipids, serum creatinine and urine albumine/kreatinine ratio were determined by routine assays (Roche/ Hitachi modular analyzer; Roche diagnostics, Laval, QC, Canada). HbA1C was determined by high performance liquid chromatography (Primus CLC-385; Primus Corp., Kansas City, MO, USA). Creatinine clearance was calculated using the MDRD formula [18]. Patients with a history of angina pectoris, myocardial infarction, heart failure, stroke or claudication at time of inclusion were defined as having cardiovascular disease. Dates of death were determined by reviewing patient records or were reported by the general practitioner and were checked at the Central Bureau of Statistics. The cause of death was obtained by linking the number of the death certificate to the primary cause of death as coded by a physician from the Central Bureau of Statistics. Cardiovascular causes of death were coded according to the International Classification of Diseases (ICD), 9th revision. Death due to ischemic coronary heart disease, heart failure, and cerebrovascular disease were coded as cardiovascular death.

2.3. DNA preparation and polymerase chain reaction analysis

The CCR5 gene is located on chromosome 3p21. In the assay, genotypes were determined by discrimination during the polymerase chain reaction (PCR) with two allele-specific probes (PE Biosystems, Foster city, CA, USA). Each assay requires two unlabeled primers (Life Technologies, Foster city, CA, USA). The PCR was accomplished by using Taqman universal master mix (PE Biosystems, Foster city, CA, USA). A detailed description was published previously [19]. Patients were divided in two groups according to their CCR5 genotype namely those homozygous for the major allele (non-carriers) and those with 1 or 2 deletion alleles (carriers). Patients with one or two deletion alleles were grouped together, as it has been demonstrated that presence of one minor allele is sufficient to compromise CCR5 function [3]. Moreover, the number of individuals homozygous for the minor allele was too low to provide adequate statistical power to analyze as a separate group.

2.4. Statistics

Hardy–Weinberg equilibrium was calculated using the gene-counting method. Differences between groups were tested with the chi-square test for dichotomous and categorical variables and one-way ANOVA for continuous variables. Survival of overall and cardiovascular mortality was investigated using univariate and multivariate Cox's proportional-hazard analyses. A primary multivariate analysis included age and sex as possible confounders. In further multivariate analyses, additional adjustment was performed for variables with significant difference between the two genotype groups. Finally, additional adjustment was performed for variables with more than 10% difference between the two groups. Cumulative hazards were calculated to display the survival model graphically. All statistical analyses were performed with SPSS statistical software (version 14.0; SPSS, Chicago, IL, USA). A p -value of 0.05 was assumed to indicate statistical significance for all analyses.

3. Results

A total of 798 patients were included. In 42 patients (5.3%) the CCR5 genotype could not be determined. These patients showed similar baseline characteristics to the genotyped patients (data not shown). Further statistical analyses were performed on the 756 patients who were genotyped for CCR5.

The CCR5 ins32 (+)/del32 (Δ) genotype was distributed as follows: 613 +/+ (81.1%), 137 +/ Δ (18.1%) and 6 Δ / Δ (0.8%). The genotype frequency did not deviate significantly from Hardy–Weinberg equilibrium ($P = 0.58$). As stated in the methods section we combined carriers of the CCR5 Δ 32 genetic variant into a single carrier genotype group of 143 individuals (18.9%).

Table 1 lists the baseline characteristics of the population stratified by CCR5 genotype. The patient characteristics for the different genotype groups were largely similar, except systolic and diastolic blood pressure and HDL cholesterol level. Carriers of the Δ 32 polymorphism had a higher systolic and

Table 1 – Baseline characteristics of the ZODIAC cohort.

	CCR5 +/+ (n = 613)	CCR5 +/- and Δ/Δ (n = 143)	p
Male sex (%)	42.4	38.5	0.39
Age (years)	68 ± 11	68 ± 10	0.44
Smoking (%)			
Current	18.8	14.0	0.41
Never	51.3	54.4	
Previously	29.8	31.6	
CVD (%)	36.2	28.7	0.10
DM duration (years)	7.2 ± 8.0	6.2 ± 6.1	0.17
SBP (mmHg)	153 ± 25	159 ± 27	0.01
DBP (mmHg)	84 ± 12	86 ± 11	0.04
BMI (kg/m ²)	28.8	28.7	0.80
TC (mmol/l)	5.6 ± 1.1	5.8 ± 1.1	0.10
HDL-c (mmol/l)	1.16 ± 0.33	1.23 ± 0.34	0.03
HbA1C (mmol/l)	7.4 ± 1.2	7.3 ± 1.3	0.19
eGFR (ml/min)	75.9 ± 17.7	76.2 ± 16.8	0.89
Alb/crea ratio >2.5 males, >3.5 females	36.8	40.2	0.48
ADT (%)			
No medication (only diet)	12.2	12.4	0.76
Only oral drugs	73.1	69.9	
Only insulin	12.8	16.2	
Insulin and oral drugs	1.9	1.5	
AHT (%)	43.4	39.7	0.43
LLD (%)	11.3	5.1	0.03
Aspirin (%)	12.0	9.6	0.43

CVD, history of cardiovascular disease; ADT, anti-diabetic treatment; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; TC, total cholesterol; HDL-c, HDL-cholesterol; eGFR, estimated GFR by MDRD formula; AHT, anti-hypertensive treatment; LLD, lipid-lowering drugs. Data are presented as percentage or mean (SD).

diastolic blood pressure and a higher HDL cholesterol level. Also the use of lipid lowering drugs was different between both groups, with less use of lipid lowering drugs in carriers.

The mean follow-up duration was 5.4 ± 1.4 with a maximum of 6.8 years. In 46 (38 (6.2%) non-carriers and 8 (5.6%) carriers) of the 756 patients no follow-up data were available.

A total of 194 (25.7%) patients died during the follow-up period at an average of 4.8% per year 167 (27.2%) in the non-carrier group and 27 (18.8%) in the carrier group. None of the six patients homozygous for the deletion allele died during follow-up. From the total number of deaths 83 (42.7%) were of cardiovascular cause (72 (11.7%) in the non-carrier and 11 (7.7%) in the carrier group). In 44 patients the cause of death was classified as due to malignancy, in 15 patients cause of death was classified as due to respiratory causes, in 5 patients cause of death was classified as trauma, and in 47 patients

death was due to other causes. These causes of death were distributed equally between the two genotype groups.

In multivariate Cox regression analyses (adjusted for age and sex), the hazard ratios of CCR5Δ32 carriers compared to non-carriers were 0.62 (95% CI: 0.40–0.96, *p* = 0.03) for all-cause mortality and 0.63 (95% CI: 0.33–1.19, *p* = 0.16) for cardiovascular mortality (Table 2). For mortality due to ischemic heart disease (*n* = 31) alone, the adjusted hazard ratio was 0.29 (95% CI: 0.07–1.21; *p* = 0.09). The adjusted hazard ratio for non-cardiovascular mortality was 0.61 (95% CI: 0.34–1.10, *p* = 0.10). Further adjustment for factors that were significantly different at baseline between the two genotype groups (blood pressure, HDL cholesterol and lipid lowering medication) did not alter the results. In an additional multivariate model, adjustment for factors that were not significantly different but had a more than 10% difference between the two genotype groups at

Table 2 – Hazard ratios, 95%CI and *p*-values for all-cause and cardiovascular mortality by CCR5Δ32 genotype.

	Crude overall mortality	<i>p</i> -value	Adjusted overall mortality	<i>p</i> -value
CCR5 +/+	1	0.04	1	0.03
CCR5 +/- and Δ/Δ	0.64 (0.41–0.98)		0.62 (0.40–0.96)	
	Crude cardiovascular mortality	<i>p</i> -value	Adjusted cardiovascular mortality	<i>p</i> -value
CCR5 +/+	1	0.17	1	0.16
CCR5 +/- and Δ/Δ	0.64 (0.34–1.21)		0.63 (0.33–1.19)	

Non-carriers (CCR5 +/+) were used as reference in Cox regression analysis. In the crude model no further adjustments were made. In the adjusted model, age and sex were included as potential confounders.

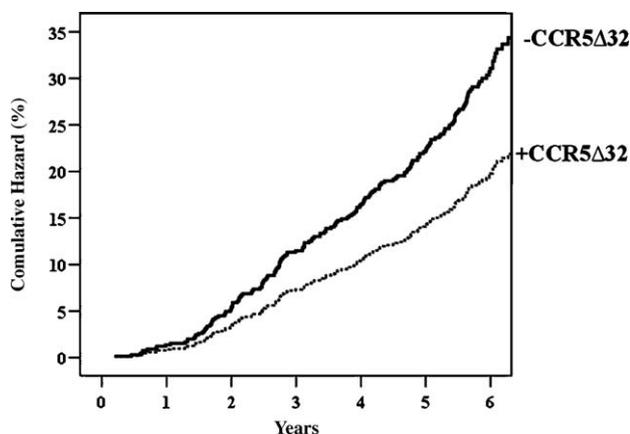


Fig. 1 – Cumulative hazard (%) for all-cause mortality in DM II patients stratified by presence of the CCR5 Δ 32 minor allele. Non-carriers (–CCR5 Δ 32) show a higher ($p = 0.038$) hazard for mortality compared to carriers (+CCR5 Δ 32).

baseline (CVD, diabetic duration and anti-diabetic treatment) did not materially affect the results (data not shown). Fig. 1 illustrates the survival model for all-cause mortality depending on CCR5 Δ 32 genotype.

4. Discussion

In this longitudinal follow-up in type 2 diabetes patients the presence of the CCR5 Δ 32, leading to CCR5 deficiency, was associated with lower all-cause mortality. Hazard ratios for cardiovascular and non-cardiovascular mortality and especially mortality due to ischemic heart disease were also lower in carriers of the CCR5 Δ 32 variant but these differences did not reach statistical significance. These data are the first to demonstrate the association of CCR5 Δ 32 with mortality in patients with diabetes type 2.

These data are in line with the impact of the CCR5 Δ 32 in several other populations. Data in HIV infection, where CCR5 modulates virus entry, provided the first evidence for pathophysiological impact of the CCR5 deficiency that is conferred by CCR5 Δ 32 as carriers showed resistance against HIV infection [5]. Causality was supported by a recent case report on a patient with acute myeloid leukaemia and HIV infection, who remained without viral rebound after transplantation with stem cells from a donor homozygous for CCR5 Δ 32 [4]. Case-control studies in cardiovascular disease demonstrated that CCR5 Δ 32 is associated with a reduced incidence of myocardial infarction at younger age in men and with protection against coronary heart disease [12,14]. In a nested case-control study within the Nurses' Health Study a possible association was found between reduced incidence of early onset coronary heart disease in women [13]. Indeed, in animal studies, it has been suggested that CCR5 and its ligands play a role in the pathogenesis and progression of vascular disease [20–22]. From the function of CCR5 it would be logical to assume that CCR5 Δ 32 modulates inflammatory responses. In line with this assumption, CCR5 Δ 32 is associated with protection against rheumatoid arthritis and with better outcome in renal transplantation, a condition characterized

by persistent inflammation [11,23]. Recently we demonstrated gene-environment interaction between CCR5 Δ 32 and inflammatory status in two independent cohorts of dialysis patients, where CCR5 Δ 32 abolishes the well-established association between elevated CRP and mortality [15]. Taken together these studies suggest that CCR5 Δ 32 modulates outcome in various inflammatory-driven disease processes. Our current data extend these findings to type 2 diabetes.

Our study was not designed to address the mechanisms underlying the impact of CCR5 Δ 32 on mortality, but several inferences can be made. Most likely a dysfunctional CCR5 could be related to lower over-all mortality by modulating inflammatory responses. Interestingly, a Polish study reported over expression of CCR5 on circulating blood mononuclear cells in type 2 diabetic patients compared to healthy controls [24]. In addition, high plasma levels of the CCR5 ligand CCL5 were associated with increased cardiac mortality [25]. Another study by Boger et al. suggested that up regulation of CCR5 could lead to accelerated atherosclerosis in type 2 diabetes mellitus patients on hemodialysis [26]. Also other CCR5 polymorphisms have been implicated in the development of diabetic complications [27,28]. Together these findings support involvement of CCR5-mediated inflammatory processes in the outcome of diabetes. Thus, the CCR5 deficiency resulting from the CCR5 Δ 32 variant could explain why carriers had a better survival in our study.

What could be the implications of our findings? First, they could contribute to risk stratification. Moreover, they support the rationale for pharmacological blockade of the CCR5 as a preventive strategy. This idea is supported by animal data showing that the CCR5 antagonist Met-RANTES reduced progression of atherosclerosis in hypercholesterolemic mice and with reduced neo-intimal plaque area and macrophage infiltration in apoE deficient mice [21,29]. Finally, treatment with TAK-799, a CCR5 chemokine receptor antagonist, reduced lesion development in a collar-induced carotid artery atherosclerosis model [30]. In humans, pharmacological blockade of the CCR5 is also feasible, as recently this strategy has been introduced for treatment of HIV infection, but so far no experience is available in other conditions [31].

Our study has several limitations. We excluded a number of patients for which CCR5 genotype was not determined that could potentially introduce a selection-bias. However, it is highly unlikely that this sporadic technical failure would distribute unequally among patients, as patient characteristics were similar in genotyped versus non-genotyped subjects. Another limitation is that causes of death could have been misdocumented and hereby may have biased the result concerning cardiovascular and non-cardiovascular mortality. However, this could not have influenced our main outcome, i.e. all-cause mortality. The incidence of mortality in our study population is comparable to that reported in literature, suggesting that in terms of mortality, our study population resembles a ordinarily type 2 diabetes population [32]. Population stratification is a form of confounding that may occur in genetic association studies when a distinct population comprises subgroups with different genetic background. Unfortunately, data on ethnicity were not recorded. However in the region of the Netherlands where the study was

performed the vast majority of inhabitants is of Caucasian origin. Besides this the genotype did not deviate from Hardy–Weinberg equilibrium. So, this form of confounding is not likely to play an important role. To overcome bias through selection and population stratification replication of our findings in an independent population would have been helpful. Whereas this is a single center study, and no formal replication is provided, our data are in line with those in other populations, thus supporting its credibility.

In genetic association studies adjustment for other factors than age and sex could potentially introduce interference in the causal pathway and thereby bias through overadjustment [33]. For this reason we reported unadjusted hazard ratios and hazard ratios adjusted for age and sex in the manuscript. Even adjustment for the factors that were at baseline significantly different or showed a more than 10% difference between the two genotype groups did not materially affect our conclusions. Moreover, as CCR5Δ32 carriers survived longer despite higher blood pressure it could be hypothesized that carriers are more resistant to adverse events related to elevated blood pressure. Similarly, less lipid lowering treatment in CCR5Δ32 carriers apparently did not adversely affect their survival. Finally, we only studied a single polymorphism. The observed effect does not necessarily causally implicate this particular polymorphism, but could be due to another variant in linkage disequilibrium with the studied deletion. This is a point that deserves further investigation. However, our efforts as reported in the present study were not toward in-depth characterization of the gene locus, but rather to investigate whether the reported impact in the literature of CCR5Δ32, leading to CCR5 deficiency, was also present in a diabetic population.

CCR5 deficiency due to the presence of the CCR5Δ32 genotype, is associated with improved survival in type 2 diabetes. These data are in line with previous data and support the pathophysiological impact of the CCR5. They suggest that pharmacological blockade of the CCR5, that has recently become feasible in humans, could have the potential to improve prognosis in type 2 diabetes.

Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

- [1] M. Baggiolini, Chemokines and leukocyte traffic, *Nature* 392 (6676) (1998) 565–568.
- [2] I.F. Charo, R.M. Ransohoff, The many roles of chemokines and chemokine receptors in inflammation, *N. Engl. J. Med.* 354 (6) (2006) 610–621.
- [3] M. Benkirane, D.Y. Jin, R.F. Chun, R.A. Koup, K.T. Jeang, Mechanism of transdominant inhibition of CCR5-mediated HIV-1 infection by ccr5delta32, *J. Biol. Chem.* 272 (49) (1997) 30603–30606.
- [4] G. Hutter, D. Nowak, M. Mossner, S. Ganepola, A. Mussig, K. Allers, et al., Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation, *N. Engl. J. Med.* 360 (7) (2009) 692–698.
- [5] R. Liu, W.A. Paxton, S. Choe, D. Ceradini, S.R. Martin, R. Horuk, et al., Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection, *Cell* 86 (3) (1996) 367–377.
- [6] C.J. Raport, J. Gosling, V.L. Schweickart, P.W. Gray, I.F. Charo, Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1beta, and MIP-1alpha, *J. Biol. Chem.* 271 (29) (1996) 17161–17166.
- [7] J.N. Willcox, N.A. Nelken, S.R. Coughlin, D. Gordon, T.J. Schall, Local expression of inflammatory cytokines in human atherosclerotic plaques, *J. Atheroscler. Thromb.* 1 (Suppl 1) (1994) S10–S13.
- [8] P. Libby, Inflammation in atherosclerosis, *Nature* 420 (6917) (2002) 868–874.
- [9] S.E. Shoelson, J. Lee, A.B. Goldfine, Inflammation and insulin resistance, *J. Clin. Invest.* 116 (7) (2006) 1793–1801.
- [10] P. Stenvinkel, Inflammation in end-stage renal disease: the hidden enemy, *Nephrology (Carlton)* 11 (1) (2006) 36–41.
- [11] M. Fischeder, B. Luckow, B. Hocher, R.P. Wuthrich, U. Rothenpieler, H. Schneeberger, et al., CC chemokine receptor 5 and renal-transplant survival, *Lancet* 357 (9270) (2001) 1758–1761.
- [12] P. Gonzalez, R. Alvarez, A. Batalla, J.R. Reguero, V. Alvarez, A. Astudillo, et al., Genetic variation at the chemokine receptors CCR5/CCR2 in myocardial infarction, *Genes Immun.* 2 (4) (2001) 191–195.
- [13] J.K. Pai, P. Kraft, C.C. Cannuscio, J.E. Manson, K.M. Rexrode, C.M. Albert, et al., Polymorphisms in the CC-chemokine receptor-2 (CCR2) and -5 (CCR5) genes and risk of coronary heart disease among US women, *Atherosclerosis* 186 (1) (2006) 132–139.
- [14] C. Szalai, J. Duba, Z. Prohaszka, A. Kalina, T. Szabo, B. Nagy, et al., Involvement of polymorphisms in the chemokine system in the susceptibility for coronary artery disease (CAD). Coincidence of elevated Lp(a) and MCP-1-2518 G/G genotype in CAD patients, *Atherosclerosis* 158 (1) (2001) 233–239.
- [15] F. Muntinghe, M. Verduijn, M. Zuurman, D. Grootendorst, J.J. Carrero, A.R. Quershi, et al., CCR5 deletion protects against inflammation-associated mortality in dialysis patients, *J. Am. Soc. Nephrol.* 20 (2009) 1641–1649.
- [16] S.M. Haffner, S. Lehto, T. Ronnema, K. Pyorala, M. Laakso, Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction, *N. Engl. J. Med.* 339 (4) (1998) 229–234.
- [17] N. Kleefstra, L.J. Ubink-Veltmaat, S.T. Houweling, K.H. Groenier, B. Meyboom-de Jong, H.J. Bilo, Cross-sectional relationship between glycaemic control, hyperglycaemic symptoms and quality of life in type 2 diabetes (ZODIAC-2), *Neth. J. Med.* 63 (6) (2005) 215–221.
- [18] G. Manjunath, M.J. Sarnak, A.S. Levey, Prediction equations to estimate glomerular filtration rate: an update, *Curr. Opin. Nephrol. Hypertens.* 10 (6) (2001) 785–792.
- [19] V.J. Clark, N. Metheny, M. Dean, R.J. Peterson, Statistical estimation and pedigree analysis of CCR2-CCR5 haplotypes, *Hum. Genet.* 108 (6) (2001) 484–493.
- [20] S. Potteaux, C. Combadiere, B. Esposito, C. Lecureuil, H. it-Oufella, R. Merval, et al., Role of bone marrow-derived CC-chemokine receptor 5 in the development of atherosclerosis of low-density lipoprotein receptor knockout mice, *Arterioscler. Thromb. Vasc. Biol.* 26 (8) (2006) 1858–1863.
- [21] N.R. Veillard, B. Kwak, G. Pelli, F. Mulhaupt, R.W. James, A.E. Proudfoot, et al., Antagonism of RANTES receptors reduces atherosclerotic plaque formation in mice, *Circ. Res.* 94 (2) (2004) 253–261.

- [22] A. Zerneck, E.A. Liehn, J.L. Gao, W.A. Kuziel, P.M. Murphy, C. Weber, Deficiency in CCR5 but not CCR1 protects against neointima formation in atherosclerosis-prone mice: involvement of IL-10, *Blood* 107 (11) (2006) 4240–4243.
- [23] V. Pokorny, F. McQueen, S. Yeoman, M. Merriman, A. Merriman, A. Harrison, et al., Evidence for negative association of the chemokine receptor CCR5 d32 polymorphism with rheumatoid arthritis, *Ann. Rheum. Dis.* 64 (3) (2005) 487–490.
- [24] J. Dytfeld, P. Bogdanski, D. Pupek-Musialik, P.P. Jagodzinski, W. Bryl, A. Kujawa, [Expression of chemokine receptor CCR5 in patients with type 2 diabetes], *Pol. Merkur. Lekarski.* 20 (116) (2006) 195–198.
- [25] E. Cavusoglu, C. Eng, V. Chopra, L.T. Clark, D.J. Pinsky, J.D. Marmor, Low plasma RANTES levels are an independent predictor of cardiac mortality in patients referred for coronary angiography, *Arterioscler. Thromb. Vasc. Biol.* 27 (4) (2007) 929–935.
- [26] C.A. Boger, M. Fischereider, M. Deinzer, C. Aslanidis, G. Schmitz, M. Stubanus, et al., RANTES gene polymorphisms predict all-cause and cardiac mortality in type 2 diabetes mellitus hemodialysis patients, *Atherosclerosis* 183 (1) (2005) 121–129.
- [27] K. Nakajima, Y. Tanaka, T. Nomiyama, T. Ogihara, L. Piao, K. Sakai, et al., Chemokine receptor genotype is associated with diabetic nephropathy in Japanese with type 2 diabetes, *Diabetes* 51 (1) (2002) 238–242.
- [28] P. Prasad, A.K. Tiwari, K.M. Kumar, A.C. Ammini, A. Gupta, R. Gupta, et al., Association of TGFbeta1, TNFalpha, CCR2 and CCR5 gene polymorphisms in type-2 diabetes and renal insufficiency among Asian Indians, *BMC Med. Genet.* 8 (2007) 20.
- [29] A. Schober, D. Manka, P. von Hundelshausen, Y. Huo, P. Hanrath, I.J. Sarembock, et al., Deposition of platelet RANTES triggering monocyte recruitment requires P-selectin and is involved in neointima formation after arterial injury, *Circulation* 106 (12) (2002) 1523–1529.
- [30] E.J. van Wanrooij, H. Happe, A.D. Hauer, P. de Vos, T. Imanishi, H. Fujiwara, et al., HIV entry inhibitor TAK-779 attenuates atherogenesis in low-density lipoprotein receptor-deficient mice, *Arterioscler. Thromb. Vasc. Biol.* 25 (12) (2005) 2642–2647.
- [31] G. Fatkenheuer, A.L. Poznaniak, M.A. Johnson, A. Plettenberg, S. Staszewski, A.I. Hoepelman, et al., Efficacy of short-term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1, *Nat. Med.* 11 (11) (2005) 1170–1172.
- [32] S.D. Kanters, J.D. Banga, R.P. Stolk, A. Algra, Incidence and determinants of mortality and cardiovascular events in diabetes mellitus: a meta-analysis, *Vasc. Med.* 4 (2) (1999) 67–75.
- [33] K.J. Jager, C. Zoccali, A. Macleod, F.W. Dekker, Confounding: what it is and how to deal with it, *Kidney Int.* 73 (3) (2008) 256–260.