RANTES Promoter Genotype Is Associated With Diabetic Nephropathy in Type 2 Diabetic Subjects

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OBJECTIVE — To evaluate the effect of RANTES gene promoter polymorphism and RANTES receptor (CCR5 gene) promoter polymorphism on diabetic nephropathy in Japanese type 2 diabetic subjects.

RESEARCH DESIGN AND METHODS — A total 616 Japanese subjects with type 2 diabetes were recruited. Polymorphisms of −28 C/G and −403 G/A in the RANTES gene promoter region, and of 59029 G/A in the CCR5 gene promoter region were detected by PCR-RFLP (restriction fragment length polymorphism). The association of these genotypes with nephropathy was analyzed.

RESULTS — While the RANTES −403 genotype showed no association with nephropathy, the frequency of the –28G allele was significantly higher in the DN2 group (urinary albuminuria-to-creatinine ratio [ACR] ≧300 mg/g creatinine, serum creatinine <2.0 mg/dl) than in the DN0 (ACR <30 mg/g creatinine) and DN1 (ACR ≥30 mg/g creatinine and <300 mg/g creatinine) groups. The frequency of a RANTES –28G-positive genotype (C/G or G/G) was higher in the DN2 group than in the DN0 and DN1 groups (34% vs. 25 and 20%, $P = 0.0268$, $\chi^2 = 4.905$), and the frequency of a CCR5 59029 A-positive genotype (G/A or A/A) was higher in the DN1 and DN2 groups than in the DN0 group (84 and 85% vs. 76%, $P = 0.0123$, $\chi^2 = 6.269$). Discriminant analysis showed that the RANTES –28G-positive genotype and CCR5 59029A-positive genotype were independently associated with nephropathy. The percentage of macroalbuminuria was twofold higher in the subjects having −28G or 59029A and threefold higher in the subjects having −28G and 59029A than in the subjects without −28G and 59029A.

CONCLUSIONS — The RANTES promoter –28G genotype and CCR5 promoter 59029A genotype may be independent risk factors for diabetic nephropathy in patients with type 2 diabetes and may have an additive effect on nephropathy.

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Diabetic nephropathy is a serious complication in individuals with type 2 diabetes because of premature mortality due to coronary heart disease or chronic renal failure (1). It has previously been reported that monocyte/macrophage infiltration was detected in the glomeruli of rats with streptozocin-induced diabetes and in renal biopsy specimens from patients with diabetic nephropathy. These data suggest that chemokine signals are upregulated in diabetes and that monocyte recruitment to the kidneys and differentiation into macrophages may be associated with the development or progression of diabetic nephropathy (2–5). A hyperglycemic state is thought to increase the secretion of cytokines, such as tumor necrosis factor-α (TNF-α) or interleukin-1β (IL-1β), probably through activation of protein kinase C, oxidative stress, and formation of advanced glycation end products (6–11). In turn, TNF-α and IL-1β stimulate the expression of a chemokine, known as regulated upon activation, normal T-cell expressed and secreted (RANTES), by human mesangial cells (12,13). Since the major receptor for RANTES expressed by monocyte/macrophages in renal tissue is chemotactic cytokine receptor 5 (CCR5), RANTES and CCR5-mediated signals may promote monocyte/macrophage infiltration, differentiation, and activation (4,5,14).

We previously reported the effect of CCR5 promoter 59029 G/A polymorphism in patients with type 2 diabetes on the development of diabetic nephropathy (15). An increase of CCR5 expression on 59029 A type had been observed by in vitro reporter gene analysis and actually confirmed in the peripheral blood of individuals with the 59029A genotype (16,17). We previously demonstrated that the 59029 A–positive genotype (G/A and A/A) showed a significantly higher frequency in type 2 diabetic patients with microalbuminuria or macroalbuminuria than in those with normoalbuminuria, and showed that this genotype may be an independent risk factor for diabetic nephropathy by logistic regression analysis. These findings suggest that signaling via CCR5 may play a key role in the development of diabetic nephropathy.

Recently, single nucleotide polymorphisms (SNPs) −28C/G and −403G/A have been identified in the promoter region of the RANTES gene and have shown

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Abbreviations: ACR, albuminuria-to-creatinine ratio; CCR5, chemotactic cytokine receptor 5; IL-1β, interleukin-1β; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; TNF-α, tumor necrosis factor-α;

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

a possible association with RANTES gene expression. Enhancement of RANTES gene expression has been observed in the −28G genotype, and the −28G allele is rare among Caucasions (4.0–4.4%) (18–20). Another SNP (−403G/A) has also been examined, and an increase of RANTES expression has been observed in the −403A genotype, which is associated with the development of atopic dermatitis, asthma, and polymyalgia rheumatica, as well as the progression of AIDS (20–23). RANTES and CCR5 genes are located on chromosome 17q11.2-q12 at a distance of between 36.4 and 53.9 cM and on 3p21 at a distance of between 65.1 and 67.7cM. Interestingly, both sites seem to show an association with diabetic nephropathy in Pima Indians by sib-pair linkage analysis (24). However, effects of RANTES promoter polymorphisms −28C/G and −403G/A had not yet been assessed so far on diabetes. Thus, the aim of this study was to evaluate whether RANTES promoter polymorphisms may associate with diabetic nephropathy independently of CCR5 59029 polymorphism and, if so, to further evaluate whether RANTES and CCR5 polymorphisms may interactively relate to diabetic nephropathy.

RESEARCH DESIGN AND METHODS

— A total 616 Japanese patients with type 2 diabetes (403 men and 213 women aged 60.5 ± 0.4 years, mean ± SEM [the same 401 subjects in our previous report (15) and an additional 215 subjects]) were recruited from the outpatient clinic of Juntendo University Hospital (Tokyo, Japan). The diagnosis of type 2 diabetes was established according to the Report of the Expert Committee on Diagnosis and Classification of Diabetes Mellitus (25). Since patients with chronic renal failure tend to also have severe atherosclerosis, we excluded patients showing a serum creatinine level ≥2.0 mg/dl to minimize any bias due to atherosclerosis. To specifically evaluate association of the genotypes with diabetic nephropathy, we excluded the patients with microscopic or macroscopic hematuria, abnormal urinary sediment, or a past history of glomerulonephritis or nephro-ureterolithiasis, dilated renal pelvis, or severe atrophied kidney from the study. All subjects gave written informed consent before enrollment in the study, which was approved by the Ethics Committee of Juntendo University. Hypertension was defined as a systolic blood pressure >140 mmHg and/or a diastolic blood pressure >90 mmHg or the use of oral antihypertensive agents. The stage of nephropathy was determined from the average of at least two measurements of the urinary albumin-to-creatinine ratio (ACR), and the subjects were classified into the following three groups: a normoalbuminuria group (DN0 group, ACR <30 mg/g creatinine), a microalbuminuria group (DN1 group, ACR ≥30 and <300 mg/g creatinine), and a macroalbuminuria group (DN2 group, ACR ≥300 mg/g creatinine). The presence and grade of retinopathy were determined using stereoscopic color fundus photographs and fluorescein angiography. Grading was performed by an experienced ophthalmologist according to the classification of Davis (26).

Genomic DNA was extracted from peripheral blood cells using a DNA extraction kit (QIAamp DNA Blood Kit; Qiagen, Tokyo, Japan). RANTES promoter −28 was detected by PCR–restriction fragment length polymorphism (RFLP), as described previously (19,20). Briefly, genomic DNA was amplified using a forward primer (5′-ACA GAG ACT CGA ATT TCC GGA-3′) and a reverse primer (5′-CCA CGT GCT GTC TTG ATC CTC-3′). The PCR products were digested overnight at 37°C with *Mnl*I (New England Biolabs, Beverly, MA). After digestion, the products were subjected to electrophoresis on 4%NuSieve gel and stained with ethidium bromide. The wild-type allele was detected as bands of 126, 27, and 20 bp, while the mutant allele was detected as bands of 146 and 27 bp.

RANTES −403 was detected by PCR-RFLP, as described previously (22,27). Briefly, genomic DNA was amplified using a forward primer (5′-GCC TCA ATT TAC AGT GTG-3′) and a reverse primer (5′-TGC TTA TTC ATT ACA GAT gTT-3′), which has a substitution (shown by the small letter "g") that created the digest site. The PCR products were digested overnight at 55°C with *Mae*III (Roche Molecular Biochemicals). Then the products were subjected to electrophoresis on 4%NuSieve 3:1 agarose gel and stained with ethidium bromide. The wild-type allele was detected as bands of 122 and 23 bp, while the mutant allele was detected as a band of 135 bp.

CCR5 G59029A was also detected by PCR-RFLP, as described elsewhere (15,16). Briefly, genomic DNA was amplified using a forward primer (5′-CCC GTG AGC CCA TAG TTA AAA CTC-3′) and a reverse primer (5′-TCA CAG GGC TTT TCA ACA GTA AGG-3′). The PCR products were digested for 5 h at 37°C with *Bsp*1 286I (New England Biolabs). The restriction site existed in the G allele of 59029, but not in the A allele. After digestion, the products were subjected to electrophoresis on 2% agarose gel and stained with ethidium bromide.

Results are expressed as the mean ± SEM. The significance of differences in mean values was analyzed by one-way ANOVA, followed by Scheffe's multiple comparison test. The significance of differences of frequency was determined by the χ^2 test, and in multiple 2×2 comparisons, *P* values were adjusted with Bonferroni correction after whole analysis of the χ^2 test. To assess the relationship of RANTES and CCR5 genotypes with nephropathy, discriminant analysis was performed using the SAS statistical package (SAS Institute, Cary, NC).

RESULTS

— The clinical characteristics of the subjects are summarized in Table 1. The mean ages of the microalbuminuria group (DN1) and the macroalbuminuria group (DN2) were significantly higher than that of the normoalbuminuria group (DN0). The estimated duration of diabetes and the percentage of patients with hypertension showed a significant increase along with the severity of nephropathy. Plasma total cholesterol and triglycerides levels were significantly higher in the DN1 and DN2 groups than in the DN0 group, whereas no significant differences were observed between the DN1 and DN2 groups. HbA_{1c} and HDL cholesterol were significantly higher in the DN2 group than in the DN0 group. The rates of the subjects treated by insulin injection and those with severe retinopathy were higher in the DN1 and DN2 groups than in the DN0 group. BMI did not differ among the three groups.

The combinations of the RANTES promoter genotype −403G/A and −28C/G are shown in Table 2. The allele frequency of RANTES −403A and −28G was 34 and 13%, respectively, and these numbers were consistent with the Hardy-Weinberg equilibrium. The two SNPs were in linkage disequilibrium. RANTES −28G/G was not found in subjects with

Table 1—*Clinical characteristics*

	All subjects	Stage of nephropathy		
		Normoalbuminuria (DN0)	Microalbuminuria (DN1)	Macroalbuminuria (DN2)
n	616	355	166	95
M/F (% male)	403/213 (65)	225/130 (63)	110/56 (66)	68/27 (72)
Age (years)	60.5 ± 0.4	59.0 ± 0.5	61.9 ± 0.9*	63.4 ± 1.2*
Duration (years)	11.9 ± 0.4	10.0 ± 0.4	13.6 ± 0.8*	16.3 ± 1.1*†
BMI (kg/m^2)	23.3 ± 0.2	23.1 ± 0.2	23.4 ± 0.3	23.7 ± 0.4
Hypertension/non-HT (% HT)	254/362 (41)	113/242 (32)	82/84 (49)*	59/36 (62)*†
HbA_{1c} (%)	7.17 ± 0.06	7.06 ± 0.07	7.24 ± 0.12	7.48 ± 0.16*
Total cholesterol (mg/dl)	196.2 ± 1.6	192.5 ± 1.9	199.9 ± 3.5*	203.7 ± 5.2*
HDL cholesterol (mg/dl)	54.9 ± 0.7	56.2 ± 0.9	53.7 ± 1.6	52.0 ± 1.8*
Triglycerides (mg/dl)	165.7 ± 7.4	146.0 ± 6.0	184.6 ± 15.2*	206.6 ± 32.6*
Therapy (diet/OA/insulin)	93/281/242	64/178/113	20/70/76*	9/33/53*
Therapy (%)	(15/46/39)	(18/50/32)	(12/42/46)	(9/35/56)
NDR/SDR/PPDR and PDR	404/116/96	272/55/28	87/41/38*	45/20/30*
Retinopathy (%)	(66/19/15)	(77/15/8)	(52/25/23)	(47/21/32)
Serum creatinine (mg/dl)	0.78 ± 0.01	0.71 ± 0.01	0.79 ± 0.02*	1.03 ± 0.04*†
ACR	268.0 ± 36.8	10.9 ± 0.4	107.2 ± 6.0*	1597.2 ± 188.9*†

Data are the mean ± SE or *n* (%). *$P < 0.05$ vs. DN0; †$P < 0.05$ vs. DN1; HT, hypertensive; NDR, nondiabetic retinopathy; SDR, simple diabetic retinopathy; PPDR, preproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; OA, oral administration

the −403 G allele, while RANTES −403G/G was not observed in subjects with the −28 G allele. Thus, we could only detect six genotype combinations.

The characteristics of subjects with or without the RANTES −28G allele or −403A allele, or the CCR5 59029A allele, are shown in Table 3. Clinical characteristics and stages of retinopathy did not differ among these genotypes.

The frequencies of the RANTES and CCR5 promoter genotypes in three groups of patients classified by the stage of nephropathy are shown in Table 4. The DN2 group showed a significantly higher frequency of the RANTES −28C/G and G/G (G-positive) genotypes and a lower frequency of the C/C (G-negative) genotype than the DN0 + DN1 group ($P = 0.0268$, $\chi^2 = 4.905$; 2×2). The frequency of −28G allele was also significantly higher in the DN2 group than in the DN0 + DN1 group ($P = 0.0163$, $\chi^2 = 5.772$; 2×2; C allele vs. G allele). However, the frequencies of RANTES −403 genotypes were not different among the three groups. Concerning CCR5 genotypes, the DN1 + DN2 group showed a significantly higher frequency of the G/A and A/A (A-positive) genotypes and a lower frequency of the G/G (A-negative) genotype than the DN0 group ($P = 0.0123$, $\chi^2 = 6.356$; 2×2).

Table 2—*RANTES promoter −403 and −28 genotype combinations*

−403 genotype	−28 genotype		
	C/C	C/G	G/G
G/G	273	0	0
G/A	162	101	0
A/A	29	37	14

Discriminant analysis of the factors as listed in Table 1 and the three genotypes, distinguishing DN2 from DN0, was done using the forward selection method to assess their relationship with progression from normoalbuminuria to macroalbuminuria. Odds ratios and 95% CIs adjusted for presence of the other variables included in the analysis, as well as *P* values of factors related to macroalbuminuria, are shown in Table 5. The estimated duration of diabetes, hypertension, HbA_{1c}, plasma triglyceride level, the RANTES −28G-positive genotype, and the CCR5 A–positive genotype, but not the RANTES-403A genotype, were significantly associated with nephropathy. Nephropathy in the groups classified by the combination of RANTES −28G and CCR5 59029A genotypes is shown in Fig. 1. The percentage of subjects with macroalbuminuria was significantly different among the three groups ($P = 0.0192$, $\chi^2 = 7.910$; 3×2). The percentage was twofold higher in the group with −28G or 59029A but not significant and threefold higher in the group with −28G and 59029A than that in the group without −28G and 59029A ($P = 0.1554$, $\chi^2 = 3.783$, and $P = 0.0156$, $\chi^2 = 7.818$).

CONCLUSIONS — We previously reported that the CCR5 59029 A–positive genotype (G/A and A/A) may be an independent risk factor for diabetic nephropathy (15). Although major CCR5 ligand RANTES promoter −28C/G and −403G/A polymorphisms were identified (18–23), the effects of them on diabetic nephropathy were not evaluated. In the present study, we first evaluated whether RANTES promoter −28C/G and −403G/A polymorphisms were associated with diabetic nephropathy independently of CCR5 59029G/A polymorphism. The overall allele frequency of RANTES −28G and −403A in subjects with type 2 diabetes was 13 and 34%, respectively, which did not differ from that previously reported in healthy Japanese subjects (−28G: 17.5%; −403A: 35.7%) (18). The CCR5 59029A frequency has not yet been examined in a healthy Japanese population. While the −403G/A genotype was not associated with nephropathy, the −28 G–positive genotype was significantly more common in the groups with macroalbuminuria than in the groups

Table 3—*Clinical characteristics of the RANTES promoter and CCR5 genotypes*

	RANTES −28C/G genotype		RANTES −403G/A genotype		CCR5 59029G/A genotype	
	G allele(−)	G allele(+)	A allele(−)	A allele(+)	A allele(−)	A allele(+)
n	464	152	273	343	126	490
M/F (% male)	306/158 (66)	97/55 (64)	183/90 (67)	220/123 (64)	83/43 (66)	320/170 (65)
Age (years)	60.2 ± 0.5	61.2 ± 0.9	59.7 ± 0.6	61.1 ± 0.6	59.3 ± 0.9	60.8 ± 0.5
Duration (years)	12.0 ± 0.4	11.7 ± 0.8	11.6 ± 0.6	12.2 ± 0.5	11.2 ± 0.8	12.1 ± 0.4
BMI (kg/m^2)	23.2 ± 0.2	23.3 ± 0.3	23.1 ± 0.2	23.4 ± 0.2	23.4 ± 0.3	23.2 ± 0.2
Hypertension/non-HT (% HT)	278/186 (60)	84/68 (55)	168/105 (62)	194/149 (57)	76/50 (60)	286/204 (58)
HbA_{1c} (%)	7.15 ± 0.07	7.25 ± 0.13	7.07 ± 0.08	7.25 ± 0.08	7.11 ± 0.13	7.19 ± 0.07
Total cholesterol (mg/dl)	194.9 ± 1.7	200.2 ± 4.2	195.2 ± 2.3	197.1 ± 2.3	195.2 ± 4.5	196.5 ± 1.7
HDL cholesterol (mg/dl)	55.3 ± 0.9	53.5 ± 1.3	54.9 ± 1.2	54.9 ± 0.9	53.9 ± 1.7	55.1 ± 0.8
Triglycerides (mg/dl)	163.2 ± 6.7	173.1 ± 21.9	157.3 ± 9.0	172.4 ± 11.1	172.2 ± 24.1	164.0 ± 6.9
DR/SDR/PPDR and PDR	312/83/69	92/33/27	186/48/39	218/68/57	89/19/18	315/97/78
Retinopathy (%)	(67/18/15)	(61/22/17)	(68/18/14)	(64/20/17)	(71/15/14)	(64/20/16)

Data are the mean ± SE or *n* (%). HT, hypertensive; NDR, nondiabetic retinopathy; SDR, simple diabetic retinopathy; PPDR, preproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

with normoalbuminuria or microalbuminuria. Discriminant analysis showed that CCR5 A–positive genotype and RANTES −28G-positive genotype were significantly associated with diabetic nephropathy, suggesting that these genotypes may be independently associated with nephropathy. We omitted the DN1 group from discriminant analysis, since microalbuminuria is clinically not independent of normoalbuminuria or macroalbuminuria and can be reversible to such stages by short-term influence of blood pressure or glycemic control. Similarly, some subjects with short duration of diabetes in the DN0 group can develop to microalbuminuria for a few years; thus, these subjects may not be classified into DN1 group. To avoid such misclassification, we further extracted the subjects keeping normoalbuminuria with more than 10 years' duration of diabetes from the DN0 group ($n = 177$ of 355) and performed discriminant analysis. The result also showed the significant associations of RANTES −28C/G and CCR5 59029G/A with nephropathy (RANTES −28G and CCR5 59029A: adjusted odds ratio 2.24 and 2.14; *P* value 0.041 and 0.036). Generally, results from discriminant analysis are statistically more reliable than those from logistic regression analysis when parameters are in normal distribution. Since all serial variables (duration of diabetes, HbA_{1c}, triglyceride) significantly related to nephropathy in a stepwise forward selection method showed normal distribution, we used the results of discriminant analysis. Adjusted odds ratio of RANTES −28G and CCR5 59029A are 2.04 and 1.96, respectively, as shown in Table 5.

Secondly, we evaluated whether RANTES and CCR5 polymorphisms interactively related to diabetic nephropathy. We performed an additional discriminant analysis for adjusted ratio of having both two genetic risk factors [RANTES −28G(+) and CCR5 59029A(+)] comparing with having no risk factor [RANTES −28G(−) and CCR5 59029A(−)] under the same condition in Table 5. As the result, the adjusted odds ratio for macroalbuminuria came up to 3.63 (95% CI 1.362–9.671, $P = 0.010$). This value was about twofold higher than each of adjusted odds ratio of RANTES −28G(+) alone (2.04) or CCR5 59029A(+) alone (1.96) under the same condition as Table 5. Furthermore, as

Table 4—*Distribution of RANTES promoter −28, −403, and CCR5 promoter 59029 genotypes*

	All subjects	Stage of nephropathy		
		DN0	DN1	DN2
n	616	355 (100)	166 (100)	95 (100)
RANTES −28C/G				
CC	464	268 (75)	133 (80)	63 (66)
CG	138	80 (23)	30 (18)	28 (30)
GG	14	7 (2)	3 (2)	4 (4)
G allele frequency (%)	13	13	11	19
G(+) genotype: $P = 0.0268$ (DN0 and 1 vs. 2)				
RANTES −403G/A				
GG	273	154 (43)	80 (48)	39 (41)
GA	263	154 (43)	69 (42)	40 (42)
AA	80	47 (14)	17 (10)	16 (17)
A allele frequency (%)	34	35	31	38
CCR5 59029G/A				
GG	126	85 (24)	27 (16)	14 (15)
GA	315	173 (49)	86 (52)	56 (59)
AA	175	97 (27)	53 (32)	25 (26)
A allele frequency (%)	54	52	58	56
A(+) genotype: $P = 0.0123$ (DN0 vs. 1 and 2)				

Genotype data are the number (%) of patients.

Table 5—*Discriminant analysis of factors related to macroalbuminuria in diabetic nephropathy (stepwise forward selection method)*

	Adjusted odds ratio	95% CI	*P*
Duration of diabetes	1.09	1.057–1.122	<0.001
Hypertension	2.95	1.689–5.151	<0.001
HbA_{1c}	1.29	1.072–1.545	0.007
Triglycerides	1.00	1.001–1.004	0.003
RANTES promoter −28 G(+) genotype	2.04	1.022–4.053	0.043
CCR5 A(+) genotype	1.96	1.049–3.656	0.035

shown in Fig. 1, the percentage of macroalbuminuria was twofold higher in the subjects having −28G or 59029A and threefold higher in the subjects having −28G and 59029A than in the subjects without −28G and 59029A. These data suggest that the RANTES promoter –28G genotype and CCR5 promoter 59029A genotype may have an additive effect on macroalbuminuria.

We preliminarily performed multiple regression analysis between urinary ACR and the same factors in discriminant analysis. The both RANTES −28G and CCR5 59029A significantly correlated to urinary ACR (RANTES −28G and CCR5 59029A: partial correlation coefficient 0.088 and 0.102, *P* value 0.049 and 0.023). Taken together, further prospective study to evaluate the effects of these genotypes and combinational genotypes on development or progression of nephropathy should be needed.

A previous immunohistological study of renal biopsy samples from type 2 diabetic patients with nephropathy showed that infiltrating monocytes and macrophages were increased in the glomeruli during the mild stage of glomerulosclerosis when compared with nondiabetic control subjects and that a further marked increase occurred during the moderate stage of glomerulosclerosis (2). Infiltrating monocyte/macrophages and enhancement of chemokines are also observed in the glomeruli of rats with streptozotocin-induced diabetes (3). These results suggest that monocyte infiltration and differentiation into macrophages, which may be induced by chemokines and adhesion molecules, may contribute to the development of nephropathy and irreversible glomerular damage. Monocyte/macrophage infiltration has also been detected in the glomeruli of patients with rejection after renal transplantation. CCR5 promoter 59029G/A is reported to be associated with acute renal rejection (28). Met-RANTES, which is known as RANTES receptor antagonist, reduces proteinuria, glomerulosclerosis, vascular damage, and tubular damage in patients with renal transplant rejection by blocking monocyte arrest and recruitment through a suppressive effect on transforming growth factor-β and platelet-derived growth factor (29,30). These data suggest that signaling between RANTES and its receptor may play a key role in the activation of monocyte/macrophages in renal tissue.

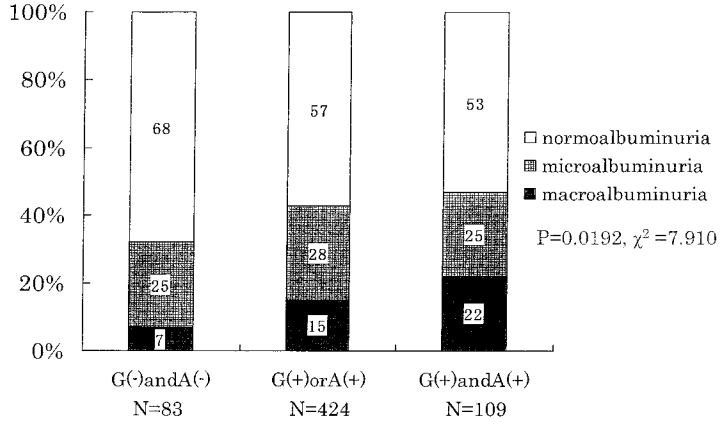

Figure 1— *Frequency of diabetic nephropathy according to RANTES and CCR5 genotype combinations. G(−) and A(−): RANTES −28G allele(−) and CCR5 59029A allele(−); G(+) or A(+): RANTES −28G allele(+) or CCR5 59029A allele(+); G(+) and A(+): RANTES −28G allele(+) and CCR5 59029A allele(+).*

Changes in gene expression in human monocytes after the stimulation of RANTES have been examined by the oligonucleotide array method, showing that RANTES activates the transcription of cytokine genes (MCP-1, pro IL-1β, IL-8, etc.), membrane receptors (oxidized LDL receptor, etc.), regulators of extracellular matrix proteins (MMP-9, etc.), and enzymes regulating intracellular signal transduction (MAPK, etc.) (31). Such signals may lead to the onset and promotion of not only diabetic nephropathy but also of other forms of nephropathy. However, there has been no report concerning the associations of RANTES and CCR5 genotypes with other nephropathy; thus, it may be interesting to clarify this point.

Chemokine signals are also thought to be important in the development of atherosclerosis as well as nephropathy. In the hyperglycemic state, RANTES secretion is increased in platelets, endothelial cells, monocytes, and smooth muscle cells in the vascular wall, which may contribute to the development of atherosclerotic plaque (5,32–37). It is possible that these chemokine and chemokine receptor genotypes may be associated with the carotid artery intima-media thickness (IMT), coronary artery disease, and stroke in type 2 diabetic patients. Thus, further study should be done to clarify the role of chemokine signals in the development of atherosclerotic plaque.

In conclusion, RANTES promoter –28C/G polymorphism is associated with diabetic nephropathy in Japanese patients with type 2 diabetes independently of CCR5 59029 G/A polymorphism. Furthermore, RANTES promoter –28G genotype and CCR5 promoter 59029A genotype may additively relate to diabetic nephropathy. These data suggest that signaling via RANTES and CCR5 may play a key role in the development of diabetic nephropathy. It is important to confirm the effect of these genotypes on diabetic nephropathy by a large-scale cross-sectional and prospective study. Such data will help to clarify the mechanism of

the development and progression of diabetic nephropathy.

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