Replication Study for the Association Between Four Loci Identified by a Genome-Wide Association Study on European American Subjects With Type 1 Diabetes and Susceptibility to Diabetic Nephropathy in Japanese Subjects With Type 2 Diabetes

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OBJECTIVE—Genetic factors are believed to contribute to the development and progression of diabetic nephropathy. Recently, a genome-wide association study for diabetic nephropathy revealed four novel candidate loci in European American subjects with type 1 diabetes. In this study, we determined the association of the four loci with diabetic nephropathy in Japanese subjects with type 2 diabetes.

RESEARCH DESIGN AND METHODS—We genotyped 11 singlenucleotide polymorphisms (SNPs) in four distinct loci (rs39059 and rs39075 in the *CPVL/CHN2*, rs1888747 and rs10868025 in *FRMD3*, rs739401 and rs451041 in *CARS*, and rs1041466, rs1411766, rs6492208, rs7989848, and rs9521445 in a chromosome 13q locus) in four independent Japanese populations.

RESULTS—Six SNPs were nominally associated with diabetic nephropathy in one of the four Japanese populations ($P < 0.05$; rs451041 in study 1; rs39059 and rs1888747 in study 3; rs1411766 in studies 1 and 4; and rs7989848 and rs9521445 in study 4); however, no significant association was observed for any SNP after correction for multiple testing errors in the individual populations. Nevertheless, a meta-analysis performed for the data obtained from all four populations revealed that one SNP (rs1411766) in chromosome 13q was significantly associated with diabetic nephropathy in the Japanese populations (nominal $P = 0.004$, corrected $P = 0.04$, odds ratio 1.26 [95% CI = 1.07–1.47]).

CONCLUSIONS—Our results suggest that the rs1411766 locus may be commonly involved in conferring susceptibility to diabetic nephropathy among subjects with type 1 or type 2 diabetes across different ethnic groups. ***Diabetes*** **59:2075–2079, 2010**

Diabetic nephropathy is a leading cause of end-stage renal disease in Western countries (1) and in Japan (2). Several genetic and environmental factors are likely to contribute to its development and progression (3,4), but the precise mechanism for this contribution is unknown.

Both candidate gene approaches and genome-wide linkage analyses have suggested several candidate genes with potential impact on diabetic nephropathy. However, these findings have not been robustly replicated (5,6), and many genes responsible for susceptibility to diabetic nephropathy remain to be identified.

To identify loci involved in susceptibility to common diseases, we initiated a large-scale association study using single nucleotide polymorphisms (SNPs) from a Japanese SNP database (http://snp.ims.u-tokyo.ac.jp/index_ja.html) (7,8). Through this project, we have previously identified genes encoding solute carrier family 12 (sodium/chloride) member 3 (*SLC12A3*, MIM 600968, http://www.ncbi.nlm.nih.gov/omim]) (9); engulfment and cell motility 1 (*ELMO1*, MIM 606420) (10); neurocalcin δ (*NCALD*, MIM 606722) (11); and acetyl-CoA carboxylase beta gene (*ACACB*, MIM 601557) (12) as being associated with susceptibility to diabetic nephropathy. The association of *ELMO1* with diabetic nephropathy has been confirmed in African Americans (13) and European Americans (14).

The recent genome-wide association studies (GWASs) using populations in the Genetics of Kidneys in Diabetes (GoKinD) collection led to the identification of four distinct loci as novel candidate loci for susceptibility to diabetic nephropathy in European American subjects with type 1 diabetes (15): the *CPVL/CHN2* locus on chromosome 7, the *FRMD3* locus on chromosome 9, the *CARS* locus on chromosome 11, and a locus near *IRS2* on chromosome 13. Because the frequencies of some genetic variations are known to significantly differ among ethnic groups, it is now necessary to evaluate the role of these loci in conferring susceptibility to diabetic nephropathy in other ethnic populations.

To determine whether the genetic variations identified through the GWASs on European Americans with type 1

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diabetes are associated with susceptibility to diabetic nephropathy in Japanese individuals with type 2 diabetes, we studied the association between the SNPs in the above four loci and diabetic nephropathy in Japanese subjects with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects, DNA preparation, and SNP genotyping

Study 1. DNA samples were obtained from the peripheral blood of patients with type 2 diabetes who regularly visited the outpatient clinics at Shiga University of Medical Science, Tokyo Women's Medical University, Juntendo University, Kawasaki Medical School, Iwate Medical University, Toride Kyodo Hospital, Kawai Clinic, Osaka City General Hospital, Chiba Tokushukai Hospital, or Osaka Rosai Hospital. Diabetes was diagnosed according to the World Health Organization criteria. Type 2 diabetes was clinically defined as a disease with gradual adult onset. Subjects who tested positive for anti-GAD antibodies and those diagnosed with mitochondrial disease (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes [MELAS]) or maturity onset diabetes of the young (MODY) were not included. The patients were divided into two groups: *1*) the nephropathy group (n = 754, mean ± SE age 60.1 ± 0.4 years, diabetes duration 19.3 ± 0.4 years, BMI 23.7 ± 0.2 kg/m^2) comprised patients with diabetic retinopathy and overt nephropathy indicated by a urinary albumin excretion rate (AER) ≥200 μg/min or a urinary albumin-to-creatinine ratio (ACR) ≥300 mg/g creatinine (Cr) and *2*) the control group (n = 558, age 62.4 ± 0.5 years, diabetes duration 15.3 ± 0.4 years, BMI 23.6 ± 0.2 kg/m^2) comprised patients who had diabetic retinopathy but no evidence of renal dysfunction (i.e., AER <20 μg/min or ACR<30 mg/g Cr). The AER or ACR were measured at least twice for each patient.

Study 2. We selected diabetic nephropathy patients and control patients among the subjects enrolled in BioBank Japan. Nephropathy cases were defined as patients with type 2 diabetes having both overt diabetic nephropathy and diabetic retinopathy (n = 449, age 64.7 ± 0.4 years, BMI 23.5 ± 0.2 kg/m^2). The control subjects were patients with type 2 diabetes who had diabetic retinopathy and normoalbuminuria (n = 965, age 64.8 ± 0.3 years, BMI 23.8 ± 0.1 kg/m^2).

Study 3. Patients with type 2 diabetes were recruited from the participants of the Shiga Prospective Observational Follow-up Study for Diabetic Complications (16). Patients classified as having microalbuminuria (200 μg/min > AER ≥ 20 μg/min) on the basis of at least two measurements of AER in 24-h urine collections were followed-up for up to 6 years. Patients in whom the condition progressed to overt proteinuria (AER ≥200 μg/min) were classified as progressors (case subjects: n = 32, age 60.9 ± 1.7 years, diabetes duration 14.5 ± 1.6 years, BMI 24.9 ± 0.5 kg/m^2) and the remaining patients were defined as nonprogressors (control subjects: n = 168, age 60.4 ± 0.7 years, diabetes duration 12.8 ± 0.7 years, BMI 23.9 ± 0.3 kg/m^2).

Study 4. Patients with type 2 diabetes who regularly visited Tokai University Hospital or its affiliated hospitals were enrolled in this study. All the nephropathy patients (n = 300, age 64.4 ± 0.6 years, diabetes duration 21.9 ± 0.9 years, BMI 22.1 ± 0.2 kg/m^2) were receiving chronic hemodialysis therapy, and the control patients (n = 224, age 65.0 ± 0.7 years, diabetes duration 16.3 ± 0.4 years, BMI 23.4 ± 0.3 kg/m^2) included those with normoalbuminuria, determined by at least two measurements of the urinary ACR, and diabetes for more than 10 years.

All the patients participating in this study provided written informed consent, and the study protocol was approved by the ethics committees of RIKEN Yokohama Institute and of each participating institution.

The clinical characteristics of the subjects are shown in supplementary Table 1 in the online appendix available at http://diabetes.diabetesjournals.org/cgi/content/full/db10-0067/DC1.

Among 13 SNPs previously reported, 11 SNPs were genotyped using multiplex PCR-invader assays, as described previously (9–12). Because rs17412858 and rs2391777 were in absolute linkage disequilibrium (LD) (r^2 = 1) to rs1411766 and rs6492208, respectively, these two SNPs were excluded from the present analyses. Overall success rates of the present assay are >95%, and the concordance rates in selected duplicate samples are 100% for all examined SNPs.

Statistical analyses. We tested the genotype distributions for Hardy-Weinberg equilibrium (HWE) proportions by using the χ^2 test (17). We analyzed the differences between the case and control groups in terms of the distribution of genotypes with an allelic model by using a logistic regression analysis. Combined meta-analysis was performed using the Mantel-Haenszel test with a fixed-effects model or the DerSimonian-Laird method with a random-effects model after testing for heterogeneity.

RESULTS

Among the 11 SNPs examined in the present study, 4 SNPs showed modest deviation from HWE in either the case or control groups (rs451041 and rs739401 in the study 2 control group, rs1411766 in the study 4 case group, and rs1041466 in the case groups of studies 1 and 2; see supplementary Table 2). Regarding two SNPs with modest deviation from HWE in the control group, genotype distribution of combined population (case and control groups in study 2) were in HWE (P = 0.20, P = 0.36, respectively); therefore, we included these data in the present analyses.

As shown in Table 1, six SNPs were observed to be nominally associated with diabetic nephropathy in one of the four studies ($P < 0.05$, rs39059 and rs1888747 in study 3, rs451041 in study 1, rs1411766 in studies 1 and 4, rs7989848 and rs9521445 in study 4). However, none of the examined SNPs exhibited a significant association with diabetic nephropathy in the individual studies after the correction for multiple testing errors. By combining the results of all four studies by a meta-analysis, however, we determined that rs1411766 on chromosome 13q was significantly associated with diabetic nephropathy (nominal P = 0.004, corrected P = 0.04, odds ratio [OR] = 1.26 [95% CI 1.07–1.47]; Table 2) with the same direction of the association as previously described by Pezzolesi et al. We further performed the meta-analysis to combine the present Japanese results with the data previously reported in European American type 1 diabetes. The results indicated that significant heterogeneities were present for ORs of most SNPs examined in this study, except for rs1411766 and rs1041466, between the Japanese type 2 diabetes and European American type 1 diabetes, and that the association of rs1411766 with diabetic nephropathy attained genome-wide significance levels (Table 3).

DISCUSSION

In the present study, we examined four independent Japanese populations to determine the association of four candidate loci with diabetic nephropathy; these loci had been identified through a GWAS on European American subjects with type 1 diabetes. A SNP (rs1411766) in chromosome 13 was observed to be associated with susceptibility to diabetic nephropathy in the Japanese populations.

GWAS conducted in European and East Asian populations have revealed multiple risk-associated loci for many common diseases, including type 2 diabetes (18–21). The success of these studies has confirmed that GWAS is a useful and promising approach for identifying susceptibility genes for common complex traits. Many susceptibility loci identified through GWAS have been confirmed, and these loci have been shown to be common across different ethnic groups (22,23); however, for considerable number of loci, the associations have not been shown to consistently hold true for different ethnic populations (24).

In the present study, we could replicate the results for only one of the four loci from GWAS in European American type 1 diabetes. Because the trend observed in the association of rs1411766 with diabetic nephropathy in our study is consistent with that in a previous report (15), this locus is likely to be a common susceptibility locus for diabetic nephropathy in the case of European American patients with type 1 diabetes and Japanese patients with type 2 diabetes. Interestingly, in this chromosome 13q locus, only one SNP (rs1411766) of five SNPs was signifi-

TABLE 1
Association of candidate SNPs with diabetic nephropathy in 4 independent Japanese populations

SNP (risk allele)* position†	Study 1		Study 2		Study 3		Study 4	
	P	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)
rs39059 (A)	0.11	1.14 (0.97–1.35)	0.83	0.98 (0.84–1.15)	0.03	1.93 (1.08–3.42)	0.39	1.12 (0.87–1.43)
Ch7, 29255470	0.15‡	1.16 (0.95–1.43)	0.88‡	1.01 (0.86–1.20)	0.26‡	1.54 (0.72–3.28)	0.83‡	0.97 (0.72–1.30)
rs39075 (G)	0.23	1.11 (0.94–1.30)	0.94	0.99 (0.85–1.16)	0.07	1.72 (0.96–3.09)	0.44	1.10 (0.86–1.41)
Ch7, 29276692	0.14‡	1.17 (0.95–1.43)	0.77‡	1.03 (0.87–1.21)	0.36‡	1.44 (0.66–3.14)	0.71‡	0.95 (0.70–1.27)
rs1888747 (G)	0.35	1.10 (0.90–1.36)	0.86	0.98 (0.81–1.19)	0.03	0.50 (0.27–0.93)	0.12	0.79 (0.58–1.06)
Ch9, 86155551	0.10‡	1.24 (0.96–1.61)	0.84‡	1.02 (0.83–1.26)	0.004‡	0.28 (0.12–0.67)	0.43‡	0.87 (0.61–1.23)
rs10868025 (A)	0.53	1.06 (0.88–1.28)	0.41	0.93 (0.78–1.11)	0.29	0.73 (0.40–1.31)	0.26	0.86 (0.65–1.12)
Ch9, 86164176	0.31‡	1.12 (0.89–1.42)	0.54‡	0.94 (0.78–1.14)	0.07‡	0.49 (0.22–1.06)	0.33‡	0.85 (0.62–1.18)
rs739401 (C)	0.45	1.07 (0.90–1.28)	0.85	0.98 (0.83–1.16)	0.13	0.63 (0.34–1.15)	0.69	0.95 (0.73–1.23)
Ch11, 3036324	0.30‡	1.12 (0.90–1.39)	0.91‡	1.01 (0.85–1.21)	0.25‡	0.62 (0.27–1.42)	0.82‡	1.04 (0.76–1.42)
rs451041 (A)	0.11	1.17 (0.97–1.41)	0.60	1.05 (0.88–1.25)	0.25	0.69 (0.36–1.31)	0.86	0.98 (0.74–1.29)
Ch11, 3060725	0.049‡	1.26 (1.00–1.59)	0.42‡	1.08 (0.90–1.30)	0.26‡	0.60 (0.25–1.46)	0.91‡	1.02 (0.73–1.43)
rs1041466 (G)	0.11	1.26 (0.95–1.66)	0.51	1.11 (0.82–1.48)	0.16	0.35 (0.08–1.50)	0.92	0.98 (0.67–1.43)
Ch13, 110244322	0.18‡	1.27 (0.90–1.80)	0.18‡	1.27 (0.90–1.80)	0.12‡	0.15 (0.01–1.59)	0.44‡	1.20 (0.76–1.88)
rs1411766 (A)	0.045	1.33 (1.01–1.75)	0.08	1.24 (0.98–1.56)	0.75	0.86 (0.32–2.29)	0.21	1.26 (0.88–1.82)
Ch13, 110252160	0.009‡	1.58 (1.12–2.22)	0.08‡	1.26 (0.98–1.62)	0.33‡	0.50 (0.12–2.01)	0.046‡	1.55 (1.01–2.39)
rs6492208 (T)	0.61	1.05 (0.89–1.23)	0.71	0.97 (0.83–1.14)	0.68	1.13 (0.64–1.98)	0.36	1.12 (0.88–1.43)
Ch13, 110257726	0.74‡	0.97 (0.79–1.19)	0.48‡	0.94 (0.79–1.11)	0.82‡	0.91 (0.40–2.05)	0.13‡	1.25 (0.93–1.67)
rs7989848 (A)	0.75	1.03 (0.87–1.22)	0.999	1.00 (0.85–1.17)	0.79	1.08 (0.60–1.95)	0.11	1.23 (0.95–1.58)
Ch13, 110283468	0.98‡	0.998 (0.81–1.23)	0.60‡	0.96 (0.80–1.14)	0.55‡	0.78 (0.34–1.78)	0.03‡	1.42 (1.05–1.92)
rs9521445 (A)	0.76	1.03 (0.87–1.22)	0.75	1.03 (0.88–1.20)	0.97	0.99 (0.55–1.78)	0.09	1.25 (0.97–1.62)
Ch13, 110285534	0.84‡	0.98 (0.79–1.21)	0.82‡	0.98 (0.82–1.16)	0.45‡	0.72 (0.30–1.69)	0.02‡	1.45 (1.07–1.97)

*Risk allele reported in the GoKinD populations. †Data from the National Center for Biotechnology Information database (Entrez SNP, Genome Reference Consortium Human Build 37.1; http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp). ‡Adjusted for age, sex, BMI, and duration of type 2 diabetes in studies 1, 3, and 4, and for age, sex, and BMI in study 2.

cantly associated with diabetic nephropathy in Japanese subjects with type 2 diabetes, whereas the other four SNPs did not exhibit any association with the disease. When we reviewed the HapMap database (http://www.hapmap.org/index.html), we observed that rs1411766 was in moderate LD to rs1041466 ($r^2 = 0.55$ in JPT) and in lower LD with the other three SNPs ($r^2 = 0.12$–0.20 in JPT, 0.30–0.37 in CEU, see supplementary Fig. 1); this suggested that rs1411766 was more directly linked to causative variations.

In this study, for the remaining three loci, the statistical power to detect true association of the present study is estimated to be >0.95 in the case of SNPs with a minor allele frequency 0.2–0.5 under the following conditions: *1*) the cutoff value is set at a significant level after Bonferroni's correction ($P = 0.0044$), *2*) there is a genotypic relative risk (γ) of 1.3–1.4, and *3*) the prevalence of diabetic nephropathy is assumed to be 10% (CaTS power calculator, http://www.sph.umich.edu/csg/abecasis/CaTS/). In addition, combined analyses of the present data with data from the original study by Pezzolesi et al. (15) using the Mantel-Haenszel test did not further strengthen the original associations of these three loci with diabetic nephropathy (Table 3), whereas the association of rs1411766 with the disease became more significant ($P = 4.9 \times 10^{-8}$, OR 1.34 [95% Cl 1.21–1.49]). Therefore, the effects of these three loci might be specific for populations with a European ancestry or for subjects with type 1 diabetes. Because the association of these loci did not attain the genome-wide significance levels ($<5 \times 10^{-8}$) in the original report (15), further replication studies in type 1 diabetes are necessary to know whether those loci are true

TABLE 2
Results of a meta-analysis of four independent Japanese studies on the association of 11 SNPs with diabetic nephropathy

SNP (risk allele)*	Proteinuria (Studies 1, 2, and 3)			Proteinuria + ESRD (all studies)		
	P	OR (95% CI)	*P* for heterogeneity	*P*	OR (95% CI)	*P* for heterogeneity
rs39059 (A)	0.19	1.08 (0.96–1.20)	0.06	0.12	1.08 (0.98–1.20)	0.12
rs39075 (G)	0.28	1.06 (0.95–1.19)	0.18	0.19	1.07 (0.97–1.18)	0.31
rs1888747 (G)	0.99	1.00 (0.87–1.15)	0.06	0.51	0.96 (0.85–1.09)	0.05
rs10868025 (A)	0.68	0.97 (0.86–1.10)	0.37	0.40	0.95 (0.85–1.07)	0.44
rs739401 (C)	0.96	1.00 (0.89–1.13)	0.24	0.91	0.99 (0.89–1.11)	0.38
rs451041 (A)	0.22	1.08 (0.95–1.23)	0.26	0.30	1.06 (0.95–1.19)	0.37
rs1041466 (G)	0.16	1.15 (0.95–1.41)	0.21	0.23	1.11 (0.93–1.33)	0.29
rs1411766 (A)	0.01	1.26 (1.06–1.50)	0.69	0.004	1.26 (1.07–1.47)	0.86
rs6492208 (T)	0.90	1.01 (0.90–1.13)	0.78	0.62	1.03 (0.93–1.14)	0.78
rs7989848 (A)	0.82	1.01 (0.90–1.14)	0.96	0.39	1.05 (0.94–1.16)	0.59
rs9521445 (A)	0.69	1.02 (0.91–1.15)	0.99	0.29	1.06 (0.95–1.17)	0.58

Meta-analysis was performed using the Mantel-Haenszel test. *Risk allele reported in the GoKinD populations. ESRD, end-stage renal disease.

TABLE 3
Results of a meta-analysis of 4 independent Japanese studies and U.S.-GoKinD study on the association of 11 SNPs with diabetic nephropathy

SNP (risk allele)*	Risk allele frequencies (case/control)					Meta-analysis		
	Study 1	Study 2	Study 3	Study 4	GoKind*	*P* for heterogeneity	Original *P*†	Combined *P*
rs39059 (A)	0.55/0.52	0.51/0.52	0.68/0.52	0.56/0.53	0.68/0.60	0.0099	6.7×10^{-6}	0.038‡
rs39075 (G)	0.55/0.52	0.53/0.53	0.68/0.56	0.57/0.55	0.65/0.57	0.010	1.5×10^{-6}	0.051‡
rs1888747 (G)	0.81/0.80	0.80/0.80	0.70/0.82	0.77/0.81	0.74/0.66	5.8×10^{-5}	1.3×10^{-6}	0.912‡
rs10868025 (A)	0.74/0.73	0.72/0.73	0.67/0.73	0.70/0.73	0.66/0.58	0.00027	1.1×10^{-6}	0.851‡
rs739401 (C)	0.33/0.32	0.33/0.33	0.28/0.39	0.32/0.33	0.55/0.48	0.0053	1.7×10^{-5}	0.606‡
rs451041 (A)	0.27/0.24	0.27/0.26	0.23/0.31	0.26/0.27	0.55/0.47	0.033	8.1×10^{-6}	0.166‡
rs1041466 (G)	0.11/0.09	0.11/0.10	0.03/0.09	0.12/0.12	0.49/0.41	0.13	2.6×10^{-6}	8.3×10^{-6}
rs1411766 (A)	0.12/0.09	0.13/0.11	0.09/0.10	0.15/0.12	0.39/0.32	0.77	1.9×10^{-6}	4.9×10^{-8}
rs6492208 (T)	0.43/0.42	0.43/0.44	0.41/0.39	0.45/0.42	0.63/0.56	0.015	5.4×10^{-6}	0.152‡
rs7989848 (A)	0.37/0.37	0.38/0.38	0.33/0.32	0.40/0.35	0.57/0.49	0.032	1.2×10^{-5}	0.084‡
rs9521445 (A)	0.36/0.36	0.38/0.37	0.31/0.31	0.39/0.34	0.55/0.47	0.026	3.7×10^{-6}	0.072‡

Meta-analysis was performed using the Mantel-Haenszel test with a fixed-effects model, or the ‡DerSimonian-Laird method with a random effect model. *Risk allele reported in the GoKinD populations. †*P* values for allelic association model are calculated from the data in original report by Pezzolesi et al. (14).

susceptibility loci. Moreover, compared with a study in subjects with type 1 diabetes, phenotype determination is likely more complicated for nephropathy study in type 2 diabetes. Therefore, phenotype differences might exist between the US-GoKinD study and our present study. Further investigation is required to elucidate the contribution of these loci in conferring susceptibility to diabetic nephropathy.

In summary, we confirmed the association of the A allele of rs1411766 near the *IRS2* locus on chromosome 13q with susceptibility to diabetic nephropathy in Japanese subjects with type 2 diabetes. These data suggest that this locus (rs1411766) may be a common locus involved in susceptibility to diabetic nephropathy among patients with type 1 or type 2 diabetes across different ethnic groups.

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