Association of Solute Carrier Family 12 (Sodium/Chloride) Member 3 With Diabetic Nephropathy, Identified by Genome-Wide Analyses of Single Nucleotide Polymorphisms

Nobue Tanaka,[1,2,3] Tetsuya Babazono,[2] Susumu Saito,[4] Akihiro Sekine,[4] Tatsuhiko Tsunoda,[5] Masakazu Haneda,[6] Yasushi Tanaka,[7] Tomoaki Fujioka,[8] Kohei Kaku,[9] Ryuzou Kawamori,[7] Ryuichi Kikkawa,[6] Yasuhiko Iwamoto,[2] Yusuke Nakamura,[3,4] and Shiro Maeda[1]

To identify genetic elements that might confer susceptibility to diabetic nephropathy, we performed a genome-wide analysis of gene-based single nucleotide polymorphisms (SNPs) in a large cohort of Japanese patients with diabetes. In case-control association studies, patients with type 2 diabetes were divided into two groups, one having retinopathy as well as overt nephropathy and the other (the control group) having diabetic retinopathy but with no signs of renal involvement. Genotyping of these patients at >55,000 SNP loci indicated a gene encoding solute carrier family 12 member 3 (SLC12A3) to be a good candidate for the susceptibility to diabetic nephropathy, in view of a significant association of one landmark SNP located in the 24th intron (χ^2 = 15.4, P = 0.000087, odds ratio = 2.53 [95% CI 1.57–4.09]). Subsequent analysis of additional genetic variations in this gene identified several SNPs that were significantly associated with nephropathy, especially one in exon 23 (+78 G to A: Arg913Gln, χ^2 = 18.5, P = 0.00002, odds ratio = 2.53 [95% CI 1.64–3.90]). The results implicated that substitution of Arg913 to Gln in the *SLC12A3* gene might reduce the risk to develop diabetic nephropathy and suggested that the gene product might be a potential target for the prevention or treatment of this disease. *Diabetes* 52:2848–2853, 2003

From the [1]Laboratory for Diabetic Nephropathy, SNP Research Center, the Institute of Physical and Chemical Research, Kanagawa, Japan; the [2]Diabetes Center, Tokyo Women's Medical University, Tokyo, Japan; the [3]Laboratory for Molecular Medicine, Human Genome Center, the Institute of Medical Science, University of Tokyo, Tokyo, Japan; the [4]Laboratory for Genotyping, SNP Research Center, the Institute of Physical and Chemical Research, Kanagawa, Japan; the [5]Laboratory for Medical Informatics, SNP Research Center, the Institute of Physical and Chemical Research, Kanagawa, Japan; the [6]Department of Medicine, Shiga University of Medical Science, Shiga, Japan; the [7]Department of Medicine, Metabolism & Endocrinology, School of Medicine, Juntendo University, Tokyo, Japan; the [8]Department of Urology, Iwate Medical University School of Medicine, Iwate, Japan; and the [9]Division of Endocrinology and Metabolism, Department of Internal Medicine, Kawasaki Medical School, Okayama, Japan.

Address correspondence and reprint requests to Shiro Maeda, Laboratory for Diabetic Nephropathy, SNP Research center, the Institute of Physical and Chemical Research, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan. E-mail: smaeda@src.riken.go.jp.

Received for publication 14 May 2003 and accepted in revised form 22 July 2003.

AER, albumin excretion rate; ESRD, end-stage renal disease; LD, linkage disequilibrium; SNP, single nucleotide polymorphism.

© 2003 by the American Diabetes Association.

Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) in Western countries as well as in Japan (1–3). The pathogenesis of diabetic nephropathy seems to be multifactorial; several genetic and environmental factors probably contribute to its development and the progression, although the precise mechanisms are unknown. Since cumulative epidemiological findings have provided evidence that genetic susceptibility plays an important role in the pathogenesis of diabetic nephropathy (4,5), efforts to identify the gene(s) involved in the development and progression of diabetic nephropathy in both type 1 and type 2 diabetes have been extensive (6,7), but no definitive results have yet emerged.

The sequencing of entire human genome has been completed (8,9), and a large body of information has accumulated regarding genes of known or unknown functions. Single nucleotide polymorphisms (SNPs) are the type of genetic variation that occurs most frequently through our genome; these sites are considered to be potentially useful as markers to identify genes that in some forms may confer susceptibility to common, etiologically complex diseases such as diabetes (10,11).

After developing at our center a system of high-throughput SNP genotyping that combines the invader assay with multiplex PCRs (12–14), we began to undertake genome-wide association studies using SNPs to identify loci involved in susceptibility to common diseases. In the study reported here, we showed the results of a genome-wide SNP genotyping using a large panel of Japanese patients with type 2 diabetes to identify the gene(s) that confers susceptibility to diabetic nephropathy. Our data suggest that the gene encoding SLC12A3, a thiazide-sensitive sodium-chloride co-transporter whose mutant form causes Gitelman syndrome (MIM600968) (15), is likely to contribute to genetic susceptibility to diabetic nephropathy.

RESEARCH DESIGN AND METHODS

DNA preparation and SNP genotyping. DNA samples were obtained from peripheral blood of the patients with type 2 diabetes who come regularly to the outpatient clinic of Shiga University of Medical Science, Tokyo Women's Medical University, Juntendo University, Kawasaki Medical School, Iwate Medical University, or Chibanishi General Hospital. All patients provided

TABLE 1
Clinical characteristics of the patients

	Case (1^{st})	Case (2^{nd})	Control (1^{st})	Control (2^{nd})
n	94	466	94	266
Sex (M:F)	63:31	305:161	37:57*	125:141*
Age	57.9 ± 12.5	59.6 ± 13.5	62.7 ± 9.9	62.9 ± 12.0
Duration (years)	18.6 ± 9.7	17.3 ± 10.4	16.2 ± 8.4	14.6 ± 9.3
HbA_{1c} (%)	7.7 ± 1.3	7.8 ± 1.1	7.4 ± 1.1	7.1 ± 1.2
S-Cr (mg/dl)	1.37 ± 0.83	1.48 ± 1.23	0.66 ± 0.20†	0.67 ± 0.15†
SBP (mmHg)	145 ± 21	137 ± 27	136 ± 19.0	134 ± 20
DBP (mmHg)	77 ± 13	74 ± 13	75 ± 10	75 ± 10

Data are means ± SD unless otherwise indicated. Statistical significance between any two groups was analyzed by one-way ANOVA followed by Sheffe's test. *χ^2 test; $P < 0.001$ vs. case (1^{st}), vs. case (2^{nd}).

informed consent before enrolling in this study, and DNA extraction was performed according to standard phenol-chloroform procedures. Diabetic patients were divided into two groups according to the following diagnostic criteria: *1*) nephropathy cases, i.e., patients with diabetic retinopathy as well as overt nephropathy indicated by urinary albumin excretion rates (AERs) ≥200 μg/min or urinary albumin-to-creatinine ratio (Alb/Cr) ≥300 mg/g Cr, or under chronic renal replacement therapy; and *2*) control subjects, patients with diabetic retinopathy but showing no evidence of renal dysfunction, i.e., AER <20 μg/min or Alb/Cr <30 mg/g Cr. The SNPs for genotyping were randomly selected from our gene-based SNP database (snp.ims.u-tokyo.ac.jp) (16,17). The genotype of each SNP locus was analyzed with invader assay as previously described (14).

Our first screening involved genotyping 94 nephropathy patients and 94 control subjects for 56648 SNP loci. By evaluating the statistical data using a 2×2 contingency table, we selected SNPs that showed significant differences in allelic frequencies between the nephropathy and control groups. Then we analyzed in a larger number of subjects to clarify statistical significance. The study protocol was approved by the ethics committees of the Institute of Physical and Chemical Research and each participating institution.

Identification of polymorphisms in the *SLC12A3* gene and genotyping. On the basis of the GenBank information pertaining to the genomic sequence containing the *SLC12A3* gene (accession no. AC_012181.6), we designed PCR primers to amplify fragments of this DNA. Repetitive elements were excluded from the search by invoking the REPEAT MASKER computer program in the manner described by Bedell et al. (18). PCR experiments and DNA sequencing were carried out as previously described (19). Genotyping of each SNP was performed with invader assay or in some cases with direct sequencing.

Analysis of haplotype structure. Analysis of haplotype structure was carried out by estimating haplotype phasing using Expectation Maximization (EM) algorithm (20) and by constructing haplotype blocks as previously described (21) with the following modifications. First, we clustered neighboring SNPs that were linked absolutely into a single representative SNP to simplify further analyses. Second, we added the constraint that the set of common haplotypes of max(N+1,2 [0.5 N]), where N was the number of SNPs in the block, would cover >90% of the population. We counted the frequency of each haplotype for patients and control subjects independently.

Statistical analysis. Statistical analysis for association study, for haplotype frequencies and Hardy-Weinberg equilibrium, and for calculation of linkage disequilibrium (LD) coefficients D′ were described previously (22,23). For analyzing clinical data, statistical significance between any two groups was analyzed by one-way ANOVA followed by Scheffe's test.

RESULTS

Clinical characteristics of each group are summarized in Table 1. First, we genotyped 56,648 SNP loci for 94 nephropathy patients and 94 control subjects. A total of 402 SNP loci that had shown $P < 0.01$ between the two groups were further analyzed using a larger number of patients. This process excluded most of the 402 SNP loci because of the higher *P* value (>0.01), but five SNP loci still showed a significant association ($P < 0.01$). Among them, one SNP locus in the 24th intron of the *SLC12A3* gene on chromosome 16q13 is most strongly associated with diabetic nephropathy (G vs. A, $\chi^2 = 15.4$, $P = 0.000087$, odds ratio = 2.53, 95% CI 1.57–4.09; Table 2).

Subsequent LD analysis of 40 SNPs around this landmark SNP in the *SLC12A3* gene revealed that the LD in this region extended to ~100 kb upstream and to 50 kb downstream of the landmark SNP site (Fig. 1). Therefore, the critical region for susceptibility to diabetic nephropathy seemed to lie within this 150-kb LD block, which contained three genes (*SLC12A3*, *KIAA0095*, and the gene encoding homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1 [*HERPUD1*]). We then analyzed 16 SNPs in *KIAA0095* and 15 in *HERPUD1*, as well as five in the gene encoding cholesteryl ester transfer protein for the association with diabetic nephropathy; the third one had been proposed as a candidate for diabetic nephropathy (24). We found no significant association of these additional SNPs with diabetic nephropathy ($P > 0.01$; data not shown) and concluded that *SLC12A3* itself was the most likely candidate for conferring susceptibility to diabetic nephropathy.

To validate further the candidacy of *SLC12A3* for diabetic nephropathy, we searched for additional polymorphisms in the gene and identified 65 novel SNPs, making a total of 78 in this gene, and four insertion/deletion polymorphisms (Fig. 2). From among those SNPs, we selected 26 for the Invader assays (one SNP from the 5′ flanking region, one in the first intron, 21 in other introns, and three in exons) and successfully genotyped for a total of 553 nephropathy patients and 317 control subjects. In addition, we analyzed the three exonic SNPs as well as two in the first intron by direct sequencing. Four of these 31 SNPs showed significant associations with diabetic nephropathy; particularly, one SNP in the 23rd exon, which substituted an amino acid (+78, Arg913Gln), was strongly associated ($\chi^2 = 18.5$, $P = 0.00002$, odds ratio = 2.53, 95% CI 1.64–3.90; Table 3). The four SNPs were in quasi-complete LD. We then selected 18 SNPs with the minor allelic frequency of >5% and analyzed the haplotype structure. Eleven of the 18 SNP loci, including the one in exon 23, constituted one haplotype block, and 10 common

TABLE 2
P values for the landmark SNP in *SLC12A3*

	GG	GA	AA	G	A
First screening					
Nephropathy	88 (95.7%)	4 (4.3%)	0 (0%)	0.98	0.02
Control	77 (83.7%)	15 (16.3%)	0 (0%)	0.92	0.08

	χ^2	*P*	Odds ratio	95% CI
G vs. A	6.7	0.0095	3.99	1.30–12.28

	GG	GA	AA	G	A
Second screening					
Nephropathy	436 (93.6%)	28 (6.0%)	2 (0.4%)	0.97	0.03
Control	205 (84.7%)	34 (14.0%)	3 (1.2%)	0.92	0.08

	χ^2	*P*	Odds ratio	95% CI
G vs. A	15.4	0.000087	2.53	1.57–4.09

Cases are expressed as number of subjects. Percentages of the totals are shown in parentheses.

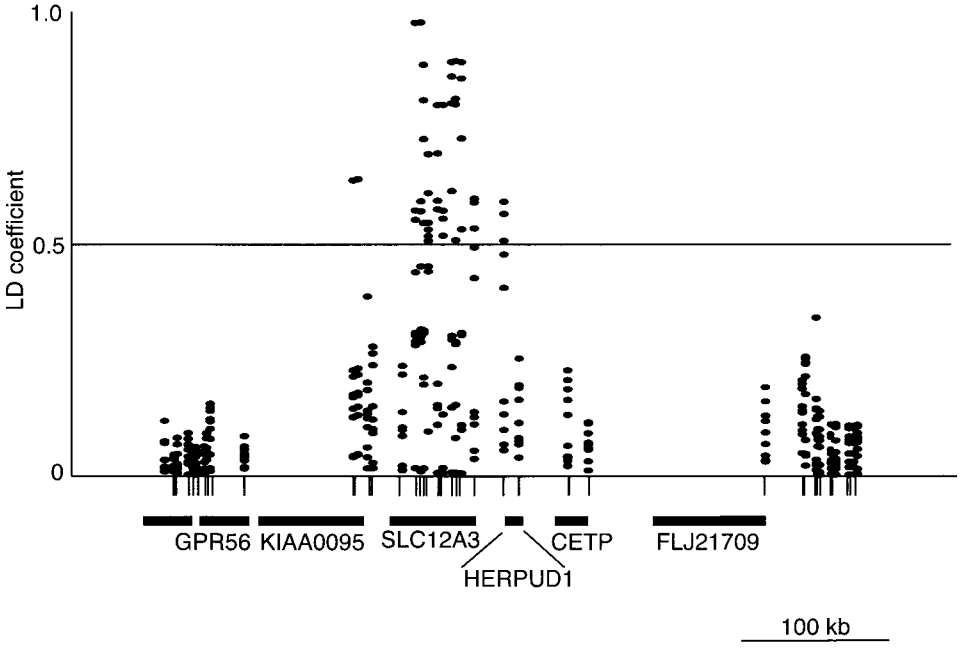

FIG. 1. LD mapping around the *SLC12A3* gene. LD coefficients (D′) between nine SNPs in the *SLC12A3* gene and other SNPs were calculated and plotted. SNPs with minor-allele frequencies <0.15 were not included in the calculations. Other genes in the region are indicated with size approximations.

haplotypes of this block accounted for >90% of the study population (Fig. 3). A subsequent investigation of these haplotypes and diabetic nephropathy identified a significant association of haplotype 7. However, this association was less significant than that observed by the Arg913Gln SNP alone. The genotypic distributions of SNPs shown in the tables all were in Hardy-Weinberg equilibrium.

DISCUSSION

In a genome-wide case-control association study using SNPs as genetic markers, we identified the *SLC12A3* gene as a candidate for conferring susceptibility to diabetic nephropathy. Our data also suggested that an amino-acid substitution of Gln for Arg at codon 913 might contribute directly to the reduction of risk of diabetic nephropathy by affecting the function of the gene product, a thiazide-sensitive Na^+-Cl^- co-transporter.

The results presented here provide the evidence that a genome-wide case-control association study using gene-based SNPs as genetic markers is a powerful strategy for identifying genes associated with susceptibility to common diseases. Epidemiological finding had strongly suggested a contribution of genetic factors to the development and progression of diabetic nephropathy (4,5), but worldwide efforts have so far failed to identify any solid evidence to indicate a gene susceptible to the disease. Results even conflicted in many cases, probably because sample sizes were often inappropriate. However, the ma-

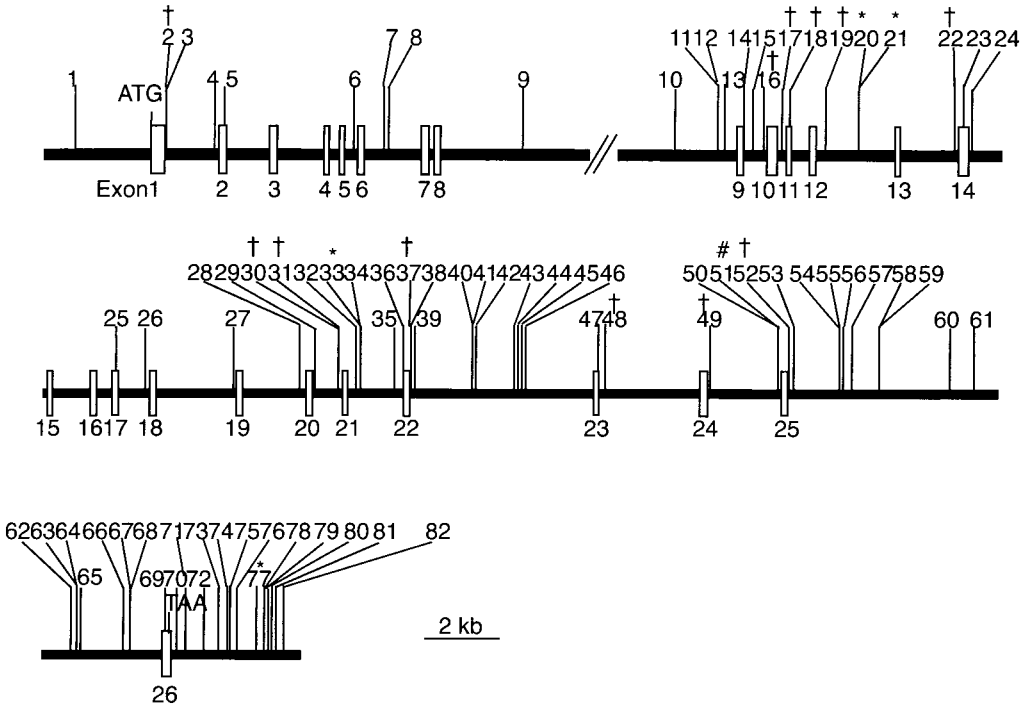

FIG. 2. Genetic variations in the *SLC12A3* gene. #Landmark SNP; *insertion/deletion polymorphisms; †SNPs analyzed in the first screening; no symbol, SNPs identified in the extensive search of the gene's genomic sequence after primary results were positive.

TABLE 3
Association of SNPs in the *SLC12A3* gene with diabetic nephropathy

		GG(Arg/Arg)	GA(Arg/Gln)	AA(Gln/Gln)	G	A
SNP47: Exon 23 + 78 G/A (Arg913Gln)						
Nephropathy		517 (93.5%)	35 (6.3%)	1 (0.2%)	0.97	0.03
Control		267 (84.2%)	49 (15.5%)	1 (0.3%)	0.92	0.08
	χ^2	*P*		Odds ratio		95% CI
2 × 3	19.5	0.00006				
G vs. A	18.5	0.00002		2.53		1.64–3.90
		GG	GA	AA	G	A
SNP51: intron 24 + 1870 G/A						
Nephropathy		475 (92.8%)	34 (6.6%)	3 (0.6%)	0.96	0.04
Control		266 (84.2%)	43 (13.6%)	7 (2.2%)	0.91	0.09
	χ^2	*P*		Odds ratio		95% CI
2 × 3	16.1	0.0003				
G vs. A	18.5	0.00002		2.44		1.60–2.3.70
		CC	CT	TT	C	T
SNP52: intron 25 + 13 C/T						
Nephropathy		442 (86.3%)	67 (13.1%)	3 (0.6%)	0.93	0.07
Control		245 (77.8%)	66 (20.9%)	4 (1.3%)	0.88	0.12
	χ^2	*P*		Odds ratio		95% CI
2 × 3	10.3	0.006				
G vs. A	10.3	0.001		1.73		1.23–2.44
		CC	CT	TT	C	T
SNP58: intron 25 + 2500 C/T						
Nephropathy		472 (93.1%)	33 (6.7%)	1 (0.2%)	0.97	0.03
Control		272 (85.7%)	47 (14.0%)	1 (0.3%)	0.92	0.08
	χ^2	*P*		Odds ratio		95% CI
2 × 3	15.1	0.0005				
G vs. A	14.3	0.0001		2.31		1.48–3.61

Cases are expressed as numbers of subjects. Percentages of the totals are shown in parentheses.

jor cause of failures to obtain solid conclusions is possibly that multiple genetic factors are involved in diabetic nephropathy in a complex manner and the power of each factor is too weak to be identified. Therefore, approaches other than standard candidate-gene analysis or family-based linkage analysis seem to be required to identify genes that are involved in susceptibility to common diseases such as this one. The strategy that we used for this study had two major advantages over previous investigations. We started by analyzing a huge number of loci (56,648 SNPs) on a genome-wide basis, using a high-throughput genotyping system developed in our institute (14). Second, all of the SNPs contributing to this report are the gene-based SNPs in the Japanese population (16,17); therefore, we were able to screen for the candidate genes more efficiently than other SNP databases.

In a large-scale genome-wide association study for common diseases such as in this study, the elimination for type 1 error should be concerned. Hence, we performed correction of multiple-testing error for our whole screening process according to the following calculations. Overall *P* values (*P* 1st × *P* 2nd) × number of test (test 1st + test 2nd) = 0.0095 × 0.000087 × (56,648 + 402) = 0.047. Therefore, we thought that the association of this locus with diabetic nephropathy was statistically significant.

Another possible cause to generate spurious association in case-control studies is related to the power of the study protocol to detect the association. In evaluation of polymorphisms whose contribution is modest to the disease susceptibility, >1,000 subjects might be necessary for each patient and control group if the minor allelic frequencies are relatively small (<10%) like the one in this study. However, we could observe the positive association of this locus with diabetic nephropathy consistently in two independent sets of case-control groups; therefore, we believe that the possibility of false positive is not likely in this case.

The *SLC12A3* gene, at chromosome 16q13, encodes a thiazide-sensitive Na^+-Cl^- co-transporter that mediates reabsorption of Na^+ and of Cl^- at the renal distal convoluted tubule; this molecule is the target of thiazide diuretics. Mutations in the *SLC12A3* gene are responsible for Gitelman syndrome (15), which is inherited as an autosomal recessive trait characterized by hypokalemia, met-

	*	Control	Case	χ^2	*p* value
haplotype 1	A G C G G T C G G C T	0.32	0.34	0.3	0.56
haplotype 2	G A T G G T C G G C T	0.15	0.16	0.1	0.75
haplotype 3	G G C G A C C G A C T	0.13	0.19	3.3	0.07
haplotype 4	G A T G G T C G G C C	0.1	0.08	0.4	0.54
haplotype 5	A A C G G T C G G C T	0.09	0.1	0.1	0.73
haplotype 6	G G T G A C C G A C T	0.06	0.06	0.01	0.92
haplotype 7	G G C A A T T A G T T	0.04	0.006	9.0	0.0027
haplotype 8	G A C G G T C A A C T	0.02	0.02	0.001	0.97
haplotype 9	G A C A A T T A G T T	0.02	0.003	4.4	0.03
haplotype 10	G A C G A T C A A C T	0.02	0.02	0.001	0.97

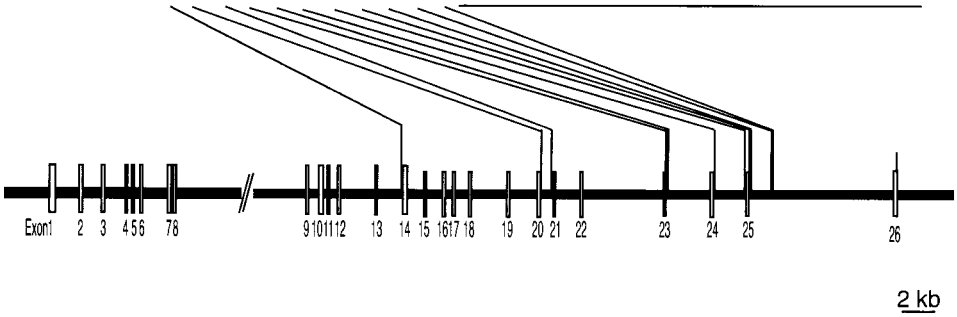

FIG. 3. Analysis of haplotype structure and estimated haplotype frequencies within the *SLC12A3* gene. Eleven variations constituted one haplotype block. The asterisk highlights the SNP at exon 23 + 78 G/A (Arg913Gln).

abolic alkalosis, hypomagnesemia, hypocalciuria, and volume depletion. Simmons et al. (15) reported 16 mutations of the *SLC12A3* gene among 12 patients with this syndrome, and others have identified additional mutations (25–27). Loss of function of this thiazide-sensitive Na^+-Cl^- co-transporter consistently results in fluid and electrolyte abnormalities, characteristics of Gitelman syndrome. Although the substitution of Gln for Arg at codon 913 is among the mutations identified in patients with Gitelman syndrome (25), no individuals in our test population showed clinical Gitelman syndrome even though two of them were homozygous for the substituted allele. Therefore, this substitution is not the cause of Gitelman syndrome but a polymorphism. In fact, higher frequency of the substituted allele in our control subjects (8% in control vs. 3% in nephropathy; $\chi^2 = 18.5$, $P = 0.00002$) suggested that the carrier of the substituted allele might be protected from the progression of nephropathy through a chronic and mild decrease in the activity of this ion transporter.

The precise mechanism accounting for the reduced risk of diabetic nephropathy in patients with the minor allele (913Gln) is unclear. It was reported that the loss of function of this thiazide-sensitive Na^+-Cl^- co-transporter significantly reduced arterial blood pressures in sodium-restricted mice (28); therefore, one can hypothesize that substitution of 913Arg to Gln could affect the blood pressure in humans under pathological conditions such as diabetes. Furthermore, thiazide diuretics are widely used for the treatment of patients with hypertension. Because elevated blood pressure is an independent risk factor for development and progression of diabetic nephropathy (29), this substitution might contribute to the reduced susceptibility for renal disease by decreasing arterial blood pressure in type 2 diabetic subjects. Alternatively, this transporter could regulate reabsorption of unknown molecules, and a sustained decrease in its activity might lead to chronic accumulation of nephrotoxic substances in patients with type 2 diabetes. Our cross-sectional data suggest that the former is not the case (data not shown), but prospective randomized trials will be necessary to clarify the precise mechanism by which this genetic polymorphism contributes to susceptibility for diabetic nephropathy.

In summary, we identified the *SLC12A3* gene as a candidate for conferring susceptibility to diabetic nephropathy, on the basis of a genome-wide case-control association study using gene-based SNPs. The evidence presented here, when combined with previous reports, suggests that *SLC12A3* should be a target for new drugs to aid in the prevention and treatment of diabetic nephropathy.

ACKNOWLEDGMENTS

This work was supported by a grant from the Japanese Millennium Project.

We acknowledge Akio Kanazawa, Shuichi Tsukada, Kana Ohashi, Yoshihiro Kawamura, Atsuyuki Shimazaki, Shuichi Iwashita, and the technical stuff at the SNP Research Center, The Institute of Physical and Chemical Research.

REFERENCES

1. Akmal M: Hemodialysis in diabetic patients. *Am J Kidney Dis* 38 (Suppl. 1):S195–S199, 2001
2. Excerpts from the United States Renal Data System 2001 Annual Data Report: Atlas of end-stage renal disease in the United States. *Am J Kidney Dis* 38 (Suppl. 3):S1–S176, 2001
3. Patient registration committee: Japanese society for dialysis therapy: the current state of chronic dialysis treatment in Japan. *J Jpn Soc Dial Ther* 35:1155–1184, 2002
4. Krolewski AS, Waram JH, Rand LI, Kahn CR: Epidemiologic approach to the etiology of type 1 diabetes mellitus and its complications. *N Engl J Med* 317:1390–1398, 1987
5. Quinn M, Angelico MC, Warram JH, Krolewski AS: Familial factors determine the development of diabetic nephropathy in patients with IDDM. *Diabetologia* 39:940–945, 1996
6. Moczulski DK, Rogus JJ, Antonellis A, Warram JH, Krolewski AS: Major

susceptibility locus for nephropathy in type 1 diabetes on chromosome 3q. *Diabetes* 47:1164–1169, 1998
7. Imperatore G, Hanson RL, Pettitt DJ, Kobes S, Bennett PH, Knowler WC, the Pima Diabetes Genes Group: Sib-pair linkage analysis for susceptibility genes for microvascular complications among Pima Indians with type 2 diabetes. *Diabetes* 47:821–830, 1998
8. International human genome sequencing consortium: Initial sequencing and analysis of the human genome. *Nature* 409:860–921, 2001
9. Venter JC, et al: The sequence of the human genome. *Science* 291:1304–1351, 2001
10. Risch N, Merikangas K: The future of genetic studies of complex human disease. *Science* 273:1516–1517, 1996
11. Collins FS, Guyer MS, Chakravarti A: Variations on a theme: cataloging human DNA sequence variation. *Science* 278:1580–1581, 1997
12. Lyamichev V, Mast AL, Hall JG, Prudent JR, Kaiser MW, Takova T, Kwiatkowski RW, Sander TJ, de Arruda M, Arco DA, Neri BP, Brow MA: Polymorphism identification and quantitative detection of genomic DNA by invasive cleavage of oligonucleotide probes. *Nat Biotechnol* 17:292–296, 1999
13. Mein CA, Barratt BJ, Dunn MG, Siegmund T, Smith AN, Esposito L, Nutland S, Stevens HE, Wilson AJ, Phillips MS, Jarvis N, Law S, de Arruda M, Todd JA: Evaluation of single nucleotide polymorphism typing with invader on PCR amplicons and its automation. *Genome Res* 10:330–343, 2000
14. Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y: A high-throughput SNP typing system for genome-wide association studies. *J Hum Genet* 46:471–477, 2001
15. Simon DB, Nelson-Williams C, Johnson-Bia M, Ellison D, Karet FE, Morey-Molina A, Vaara I, Iwata F, Cushner HM, Koolen M, Gainza FJ, Giterman HJ, Liften RP: Gitelman's variant of Bartters syndrome, inherited hypokalaemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotransporter. *Nat Genet* 12:24–30, 1996
16. Hirakawa M, Tanaka T, Hashimoto Y, Kuroda M, Takagi T, Nakamura Y: JSNP: a database of common gene variations in the Japanese population. *Nucleic Acids Res* 30:158–162, 2002
17. Haga H, Yamada R, Ohnishi Y, Nakamura Y, Tanaka T: Gene-based SNP discovery as part of the Japanese Millennium Genome Project: identification of 190,562 genetic variations in the human genome. *J Hum Genet* 47:605–610, 2002
18. Bedell JA, Korf I, Gish W: MaskerAid: a performance enhancement to RepeatMasker. *Bioinformatics* 16:1040–1041, 2000
19. Ohnishi Y, Tanaka T, Yamada R, Suematsu K, Minami M, Fujii K, Hoki N, Kodama K, Nagata S, Hayashi T, Kinoshita N, Sato Hiroshi, Sato Hideyuki, Kuzuya T, Takeda H, Hori M, Nakamura Y: Identification of 187 single nucleotide polymorphisms (SNPs) among 41 candidate genes for ischemic heart disease in the Japanese population. *J Hum Genet* 106:288–292, 2000
20. Excoffier L, Slatkin M: Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. *Mol Biol Evol* 12:921–927, 1995
21. Daly MJ, Rioux JD, Schaffner SF, Hudson TJ, Lander ES: High-resolution haplotype structure in the human genome. *Nat Genet* 29:229–232, 2001
22. Devlin B, Risch N: A comparison of linkage disequilibrium measures for fine-scale mapping. *Genomics* 29:311–322, 1995
23. Nielsen DM, Ehm MG, Weir BS: Detecting marker-disease association by testing for Hardy-Weinberg disequilibrium at a marker locus. *Am J Hum Genet* 63:1531–1540, 1998
24. Kahri JK, Groop PH, Elliott T, Viberti GC, Taskinen MR: Plasma cholesteryl ester transfer protein and its relationship to plasma lipoprotein A-I-containing lipoproteins in IDDM patients with microalbuminuria and clinical nephropathy. *Diabetes Care* 17:412–419, 1994
25. Lemmink HH, Knoers NV, Karolyi L, van Dijk H, Niaudet P, Antignac C, Guay-Woodford LM, Goodyer PR, Carel JC, Hermes A, Seyberth HW, Monnens LA, van den Heuvel LP: Novel mutations in the thiazide-sensitive NaCl cotransporter gene in patients with Giterman syndrome with predominant localization to the C-terminal domain. *Kidney Int* 54:720–730, 1998
26. Takaushi K, Kure G, Kato T, Taniyama Y, Takahashi N, Ikeda Y, Abe T, Narisawa K, Mutamatsu Y, Abe K: Association of a mutation in thiazide-sensitive cotransporter with familial Gitelman's syndrome. *J Clin Endocrinol Metab* 81:4496–4499, 1996
27. Mastroianni N, Bettinelli A, Bianchetti M, Colussi G, de Fusco M, Sereni F, Ballabio A, Casari G: Novel molecular variants of the Na-Cl cotransporter gene are responsible for Gitelman syndrome. *Am J Hum Genet* 59:1019–1026, 1996
28. Schultheis PJ, Lorenz JN, Meneton P, Nieman ML, Riddle TM, Flagella M, Duffy JJ, Doetschman T, Miller ML, Shull GE: Phenotype resembling Gitelman's syndrome in mice lacking the apical Na+-Cl-cotransporter of the distal convoluted tubule. *J Biol Chem* 273:29150–29155, 1998
29. UK Prospective Diabetes Study Group: Tight control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *BMJ* 317:703–713, 1998