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Clinical and genetic determinants of urinary glucose excretion in patients with diabetes mellitus

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ABSTRACT

Aims/Introduction: Glucosuria is a representative symptom in diabetes patients with poor glycemic control and in those treated with sodium–glucose cotransporter 2 inhibitors. Renal threshold levels of glucose excretion are known to vary among individuals, but factors contributing to glucosuria are not well characterized. The present study aimed to clarify clinical and genetic determinants of glucosuria in individuals with diabetes mellitus.

Materials and Methods: The 24-h urinary glucose excretion was measured in 135 hospitalized patients on admission, with continuous measurement for five consecutive days in 75 patients. Genetic and clinical factors contributing to glucosuria were studied. As a genetic factor, *SLC5A2* polymorphism was genotyped. A total of 476 participants (266 participants with type 2 diabetes and 210 healthy controls) were additionally genotyped for the association study of *SLC5A2* with type 2 diabetes. A meta-analysis was carried out with the present study and previous association studies.

Results: Multiple regression analysis showed that the independent variables of average blood glucose ($\beta = 0.41$, $P = 1.4 \times 10^{-7}$), estimated glomerular filtration rate ($\beta = 0.28$, $P = 6.0 \times 10^{-5}$), sex ($\beta = 0.28$, $P = 5.7 \times 10^{-5}$) and *SLC5A2* rs9934336 polymorphism ($\beta = 0.17$, $P = 0.02$) were significantly correlated with urinary glucose excretion. The frequency of the A allele of rs9934336 tended to be lower in participants with type 2 diabetes than in controls (odds ratio 0.78, 95% confidence interval 0.53–1.13, not significant), and meta-analysis showed a significant association between the A allele and type 2 diabetes (summary odds ratio for minor allele [A] 0.86, 95% confidence interval 0.78–0.94, $P < 0.002$).

Conclusions: Blood glucose, estimated glomerular filtration rate, sex and *SLC5A2* polymorphism were independent determinants of glucosuria in diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a common metabolic disorder that is characterized by chronic hyperglycemia as a result of reduced insulin secretion, decreased glucose utilization and increased glucose production. The kidneys play a critical role in maintaining glucose homeostasis by the production of glucose through gluconeogenesis in the renal cortex[1], utilization of glucose mainly in the renal medulla[2] and reabsorption of glucose from glomerular filtrate[3]. In healthy individuals, approximately 180 g/day of glucose is filtered by the kidneys, and most of the glucose is reabsorbed into the circulation. Urine is, therefore, essentially free from glucose under normal conditions. The renal threshold level of glucose excretion is known to vary among healthy individuals and is reported to increase with age, especially among women[4]. Reabsorption of glucose from glomerular filtrate occurs by means of sodium–glucose cotransporters (SGLTs) in the proximal convoluted tubule in the kidney cortex[5]. SGLT2, encoded by *SLC5A2*, is almost entirely confined to the first segment (S1) of the proximal tubules, where it mediates reabsorption of >90% of the filtered glucose[5]. SGLT1 is expressed in the more distal segments (S2–3) of the proximal tubule, where it mediates the reabsorption of glucose that has not been

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reabsorbed by SGLT2[6]. Glucosuria is a prominent feature of poorly controlled diabetes, as well as diabetes treated with SGLT2 inhibitors. There is evidence, however, that the renal threshold of glucose excretion is higher in the diabetic state, leading to a reduction of urinary glucose excretion[7–9]. Rare genetic mutations were also reported to affect renal threshold of glucose excretion leading to variation in the magnitude of urinary glucose excretion[10–12]. However, besides these rare mutations, the effects of clinical factors and common genetic variants on urinary glucose excretion in the diabetic state are largely unknown. With the increasing use of SGLT2 inhibitors for the treatment of diabetes, as well as in kidney and cardiovascular diseases, clinical and genetic determinants have become important fundamentals for the treatment with and clinical outcomes of SGLT2 inhibitors. Identification of determinants of urinary glucose excretion is expected to increase our understanding of interindividual variation in the efficacy and safety of SGLT2 inhibitors, leading to individualization of treatment with SGLT2 inhibitors. The present study, therefore, aimed to clarify the determinants of urinary glucose excretion based on data from the 24-h collection of urine in hospitalized individuals with diabetes mellitus.

METHODS

Participants

Urinary glucose excretion for 24-h periods was studied in 135 hospitalized participants with diabetes mellitus at day 1 after hospitalization (Table 1). Of these, 75 participants were prospectively studied for changes in urinary glucose excretion with 24-h samples collected for five consecutive days after hospitalization. A total of 476 participants (266 with type 2 diabetes and 210 of healthy controls) were additionally studied for the association of *SLC5A2* polymorphisms with susceptibility to type 2 diabetes (Table 1). The age, body mass index and fasting blood glucose of healthy controls (72 women and 138 men) were 42.5 ± 11.3 years, 22.4 ± 2.8 kg/m^2 and 87.7 ± 7.8 mg/dL, respectively (mean ± SD). This study was approved by the appropriate ethics committees, and written informed consent was obtained from all participants.

Study protocol

On admission, all the patients for urinary glucose excretion study (n = 135) were treated with a standard diet for diabetes mellitus recommended by the Japan Diabetes Society[13]. On day 1 of hospitalization, 24-h urine samples were collected. In a subset of patients (n = 75), 24-h urine samples were prospectively collected for five consecutive days to study longitudinal changes in urinary glucose excretion.

The daily blood glucose profile was self-monitored at four points during the day – before each meal and at bedtime – and the average blood glucose was calculated. Continuous glucose monitoring (CGM; FreeStyle Libre Pro, Abbott Japan LLC, Tokyo, Japan) was also carried out in a subset of the patients (n = 50), and the area under the curve (AUC) of the glucose value was calculated. AUC values >160 mg/dL, corresponding to the average glucose level for renal threshold of glucose excretion, were used for regression analysis.

Any glucose-lowering therapy, except for SGLT2 inhibitors, was allowed for glycemic control after hospitalization in the present study.

Table 1 | Clinical characteristics of participants with diabetes mellitus for 24-h urine collection to study glucose excretion and additional genotyping

	Participants for urine collection (n = 135)	Participants for additional genotyping (n = 266)
Female/male	78/57	116/150
Age (years)	63.6 ± 13.8	63.9 ± 12.6
Duration of diabetes (years)	13.2 ± 11.1	13.8 ± 10.7
BMI	24.7 ± 4.9	24.7 ± 4.5
HbA1c (%)	9.4 ± 1.8	9.0 ± 1.8
Fasting blood glucose (mg/dL)	152.1 ± 41.8	149.7 ± 48.4
eGFR (mL/min/1.73 m^2)	71.4 ± 27.5	72.2 ± 30.2
Type of diabetes (type 1/type 2/other)	13/111/11	0/266/0
Glucose-lowering therapy, n (%)		
Insulin	64 (47.4)	
Metformin	50 (37.0)	
DPP4 inhibitor	41 (30.3)	
Sulfonylurea	30 (22.2)	
α-Glucosidase inhibitor	16 (11.8)	
GLP-1 receptor agonist	12 (8.8)	
Glinide	7 (5.1)	
Thiazolidine	2 (1.4)	
		Mean ± SD

BMI, body mass index; DPP4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide-1; HbA1c, glycated hemoglobin.

Genetic analysis

The genotypes of 321 SNPs on *SLC5A2* (SGLT2 gene) were extracted from 414 Asian individuals in the 1,000 Genomes Browser (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/), and four tag single-nucleotide polymorphisms (SNPs; rs9934336, rs3813007, rs3813008 and rs118162329; Figure 1) were selected by SNP Annotation and Proxy Search (https://omictools.com/snap-3-tool). Genomic deoxyribonucleic acid was extracted from peripheral leukocytes using a standard phenol–chloroform method from the participants, with their informed consent, and stored at 4°C, at the Kindai University Faculty of Medicine. *SLC5A2* polymorphisms (rs9934336, rs3813007, rs3813008 and rs118162329) were genotyped by TaqMan® SNP genotyping assay according to the manufacturer's instructions (Applied Biosystems, Tokyo, Japan).

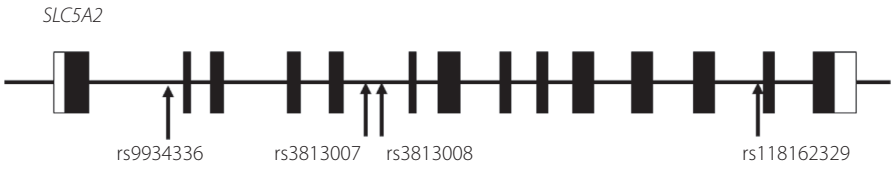


Figure 1 | Location of polymorphisms of *SLC5A2*. Location of tag single-nucleotide polymorphisms (rs9934336, rs3813007, rs3813008 and rs118162329) in *SLC5A2*. Minor allele frequencies were 12.6% (rs9934336), 36.4% (rs3813007), 20.8% (rs3813008) and 0% (rs118162329) in 135 participants with diabetes.

Selection of studies for meta-analysis

To carry out a meta-analysis, published literature was searched using PubMed with the key words "*SLC5A2*", "rs9934336" and "diabetes", followed by a complimentary search of the reference list of the selected articles. The study by Ordelheide *et al.*[14] was excluded from meta-analysis in the present study, because of the association study of *SLC5A2* polymorphisms with the glucagon concentration.

Statistical analysis

Continuous variables were presented as the mean and standard deviation for normally distributed data or as the median for non-normally distributed data. One-way repeated measures ANOVA, Friedman test, Kruskal–Wallis test or Mann–Whitney *U*-test were applied to determine the significance of differences in the comparison of continuous variables. The urinary glucose excretion was log-transformed as a dependent variable before regression analysis, because it was not normally distributed. Multiple linear regression analysis with the forward–backward stepwise selection method was carried out to identify variables that were independently associated with a change in the urinary glucose excretion. Allele frequency was estimated by direct counting. The χ^2-test and Fisher's exact probability test were applied to determine the significance of differences in the distribution of the number of participants and alleles. Observed and expected genotype frequencies were compared by the Hardy–Weinberg equilibrium using χ^2 analysis. No significant deviation from equilibrium was observed in the present study. For calculation of the summary odds ratio according to the genotype groups from case–control studies, we adopted a fixed model using the Mantel–Haenszel method[15]. Publication bias was assessed by Begg and Mazumdar's rank correlation test, and Egger's regression test. Statistical tests were carried out using JMP Pro® version 14.0.0 (SAS Institute Inc., Cary, NC, USA) software and R version 4.0.2 (The R Project for Statistical Computing, Vienna, Austria), and statistical significance was defined as $P < 0.05$.

RESULTS

Temporal changes of the urinary glucose excretion

To study temporal changes of urinary glucose excretion relative to the blood glucose level, urinary glucose excretion was measured for five consecutive days after hospitalization ($n = 75$). The urinary glucose excretion continuously decreased from day 1 to day 5, with significantly lower levels at day 4 and 5 than that at day 1 (Figure 2a: all participants, $n = 75$; and Figure 2b: participants with a urinary glucose excretion value >0.5 g/day at day 1, $n = 48$). The fasting blood glucose did not change until day 4, and significantly decreased at day 5 for all participants (Figure 2a), but it significantly decreased from day 3 to 5 for participants with a urinary glucose excretion value >0.5 g/day measured at day 1 (Figure 2b). In contrast, the average blood glucose at four points a day (before breakfast, before lunch, before dinner and before sleep) decreased continuously, and was significantly lower at days 3, 4 and 5 than that at day 1 (Figure 2a,b). The urine volume was stable through day 1 to day 5 (1328 ± 598, 1323 ± 637, 1309 ± 531, 1279 ± 530 and 1284 ± 453 mL/day, $n = 75$). When the urinary glucose excretion of each patient was followed, marked interindividual variation was noticed from patient-to-patient (Figure 3a). When two rapidly changing variables, urinary glucose excretion and the average blood glucose, were correlated with each other, marked interindividual variation was observed in the correlation (Figure 3b), indicating variation in the renal threshold of glucose excretion.

Correlation of clinical variables with urinary glucose excretion

To study the factors affecting interindividual variation in the glucose excretion, the correlation of continuous variables with urinary glucose excretion was analyzed by simple linear regression analysis by using urine collection data at day 1 of hospitalization. All the four independent variables related to blood glucose were significantly correlated with urinary glucose excretion. Among these, the highest multiple correlation coefficient (r) was observed in AUC >160 mg/dL of glucose value measured by continuous glucose monitoring ($r = 0.57$, $P < 0.0003$, $n = 50$), the second highest in the average blood glucose of four points of self-monitoring blood glucose ($r = 0.48$, $P = 3.1 \times 10^{-9}$, $n = 135$), the third in fasting blood glucose ($r = 0.35$, $P = 3.2 \times 10^{-5}$, $n = 135$) and the fourth in glycated hemoglobin (HbA1c; $r = 0.29$, $P < 0.0007$, $n = 135$; Table 2). The two independent variables related to estimated glomerular filtration rate (eGFR) were significantly correlated with urinary glucose excretion. The multiple correlation coefficient was higher in eGFR ($r = 0.31$, $P = 0.0003$, $n = 135$) than serum creatinine ($r = -0.17$, $P < 0.05$, $n = 135$). The age of

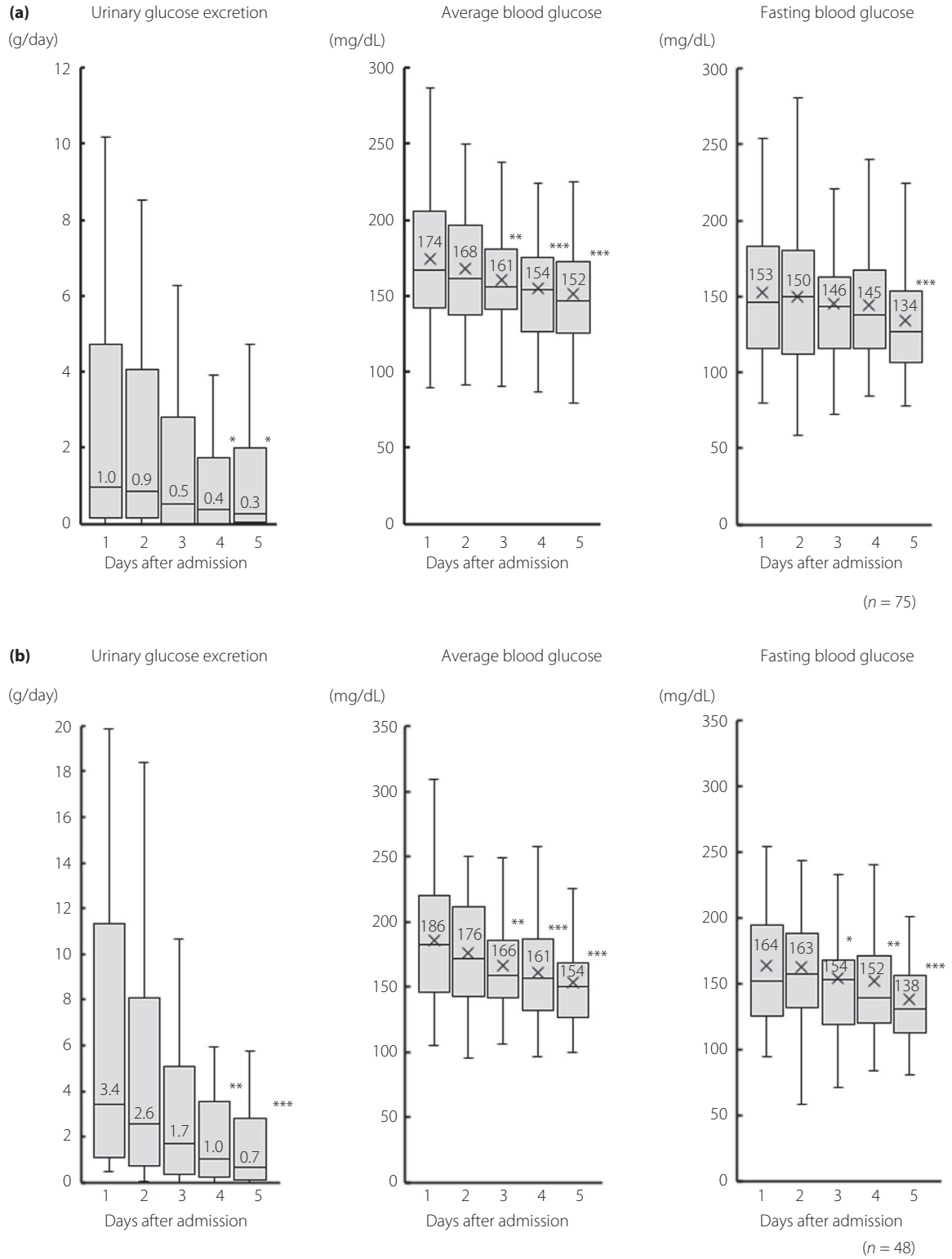


Figure 2 | Changes in urinary glucose excretion, average blood glucose at four points during the day and fasting blood glucose for five consecutive days after hospitalization. (a) All participants (n = 75). (b) Participants with urinary glucose excretion >0.5 g/day at day 1 (n = 48). *P < 0.05, **P < 0.005, ***P < 0.001 versus day 1. The Friedman test (multiple comparison by Scheffé) was carried out for urinary glucose excretion, and one-way repeated measures ANOVA (multiple comparison by Bonferroni) was carried out for average blood glucose and fasting blood glucose. Bars and numbers in each box are the median, and x represents the mean value.

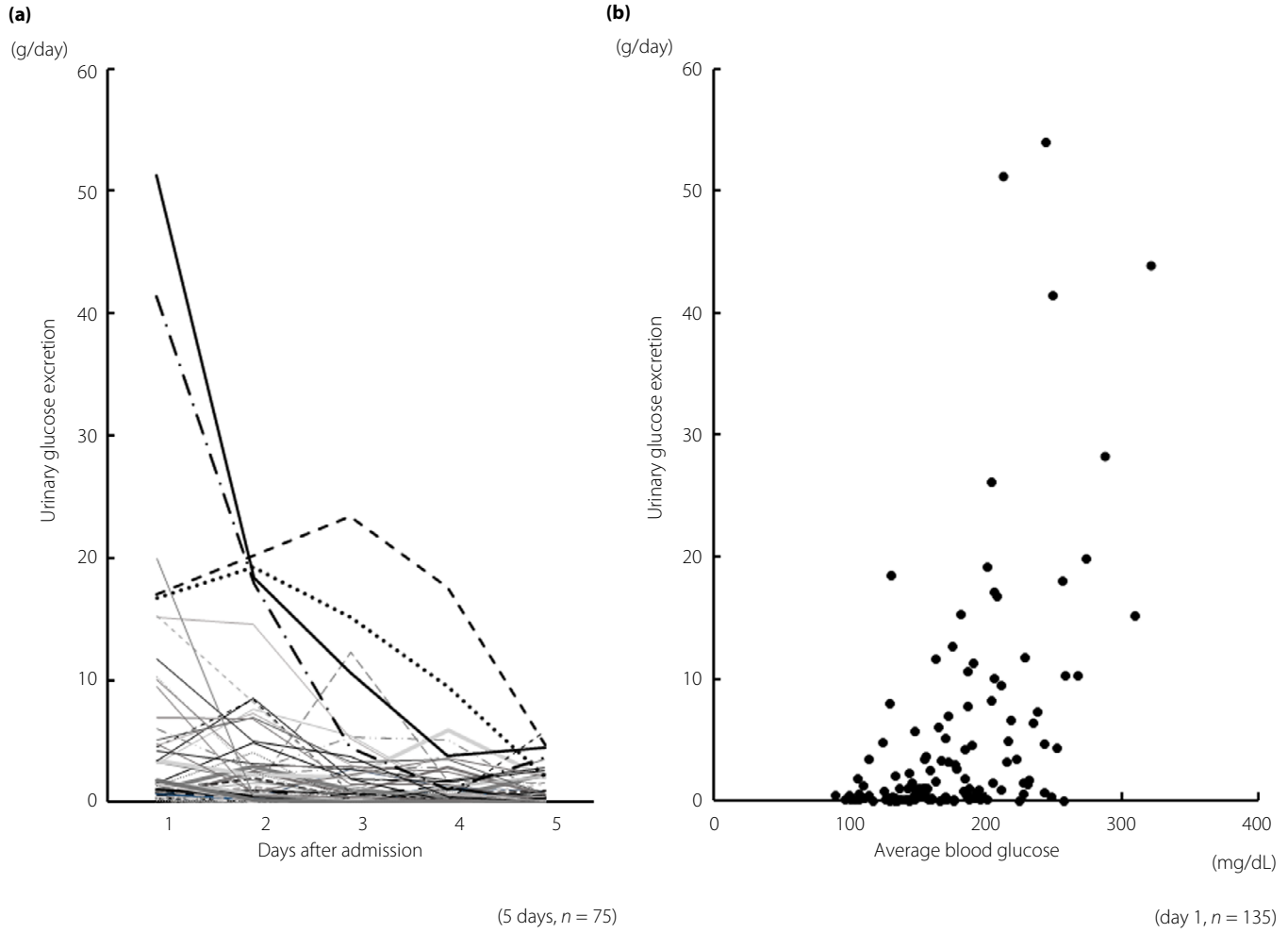


Figure 3 | Interindividual variation in urinary glucose excretion. (a) Changes in urinary glucose excretion of each participant for five consecutive days (n = 75). (b) Correlation between urinary glucose excretion and mean blood glucose on day 1 (n = 135).

participants was also negatively correlated with urinary glucose excretion (r = –0.24, P < 0.006, n = 135). No significant correlation was observed between urinary glucose excretion and the duration of diabetes, urine volume and body mass index (Table 2).

Multiple linear regression analysis of urinary glucose excretion

To study the significance of clinical and genetic determinants on urinary glucose excretion in patients with diabetes, multiple independent variables including not only clinical variables, but also *SLC5A2* polymorphisms as a genetic factor were analyzed by multiple linear regression analysis (forward–backward stepwise selection method). As for the independent variable of blood glucose control, the average blood glucose was selected because of the second highest multiple correlation coefficient (β = 0.48) and the largest number of participants tested (n = 135) by simple regression analysis (Table 2). In addition to the continuous variables tested in simple regression analysis, sex, type of diabetes and genotypes of *SLC5A2* polymorphisms (rs9934336, rs3813007 and rs3713008) were tested in multiple regression analysis by using dummy variables. The SNP, rs11816232, was not adopted, because all participants showed the T/T genotype. At the most, 12 independent variables were applied for the analysis in 135 participants as model 1, and four independent variables showed a significant correlation with urinary glucose excretion (average blood glucose, eGFR, sex and rs9934336 genotype; Table 3). eGFR is an estimated value by using age, sex and serum creatinine, and average blood glucose and HbA1c could be correlated with each other. Three other models with different sets of independent variables were adopted, and no linear combination was confirmed among independent variables in all the four models. The independent variables, showing significant correlation with urinary glucose excretion (average blood glucose, eGFR, sex and rs9934336

genotype), were identical in all models tested (Table 3). The highest standard partial regression coefficient (β) was observed in average blood glucose (β = 0.41, $P = 1.4 \times 10^{-7}$), the second highest in eGFR (β = 0.28, $P = 6.0 \times 10^{-5}$), the third in sex (β = 0.28, $P = 5.7 \times 10^{-5}$) and the fourth in rs9934336 (β = 0.17, $P = 0.02$) in models 1, 2 and 3. Age, body mass index, duration of diabetes, types of diabetes, serum creatinine, HbA1c and other polymorphisms (rs3813007 and rs3813008) were dismissed as independent variables of urinary glucose excretion by forward–backward stepwise selection method.

Table 2 | Simple regression analysis for urinary glucose excretion in participants with diabetes mellitus

Variables	Multiple correlation coefficient	*P*-value
Average blood glucose† (mg/dL)	0.48	3.1×10^{-9}
fasting blood glucose (mg/dL)	0.35	3.2×10^{-5}
AUC of CGM‡	0.57	<0.0003
eGFR (mL/min/1.73 m^2)	0.31	0.0003
HbA1c (%)	0.29	<0.0007
Age (years)	–0.24	<0.006
Serum creatinine (mg/dL)	–0.17	<0.05
Duration of diabetes (years)	0.12	NS
Urine volume (mL)	0.12	NS
BMI	0.003	NS
		$n = 135$

BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; NS, not significant †Average blood glucose at four points during the day (before breakfast, before lunch, before dinner and before sleep). ‡Area under the curve (AUC) >160 mg/dL of glucose value measured by continuous glucose monitoring (CGM; $n = 50$).

SLC5A2 genotypes and urinary glucose excretion

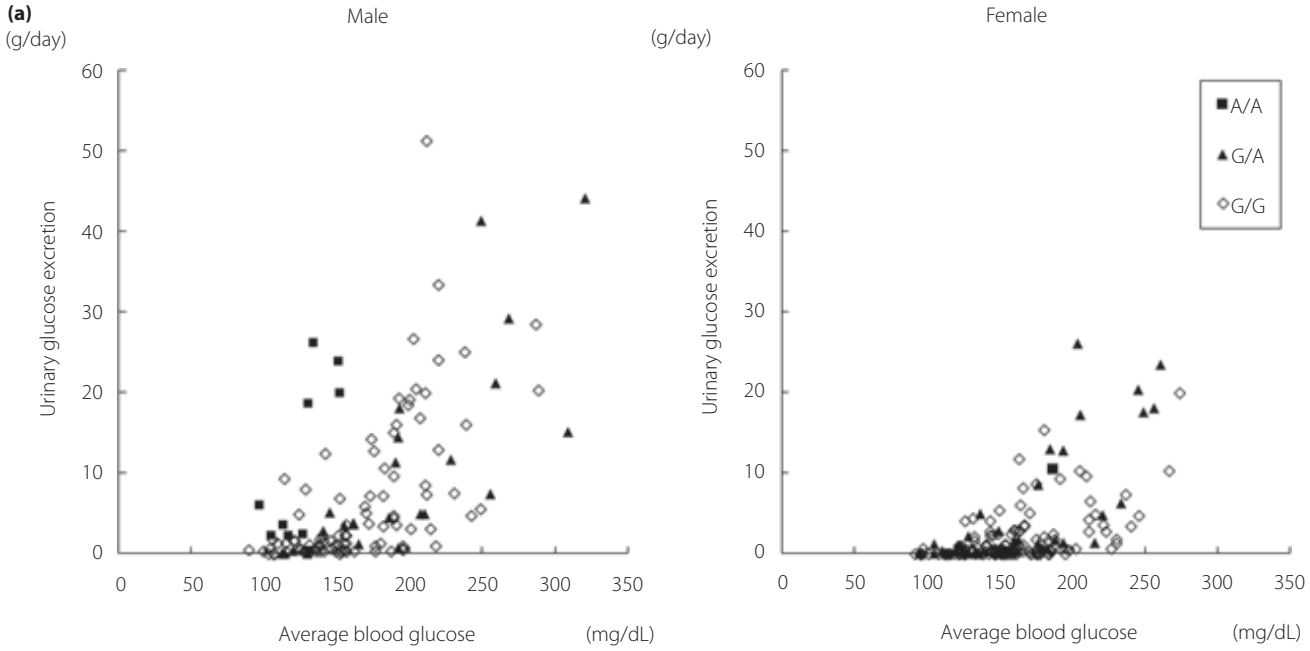
Based on the results observed by multiple regression analysis, at least four independent determinants (blood glucose, eGFR, sex and *SLC5A2* polymorphism) are significantly correlated with the amount of urinary glucose excretion. To further study the contribution of genotypes of *SLC5A2* polymorphism on urinary glucose excretion, the correlation of urinary glucose excretion with average blood glucose was shown for the participants with preserved eGFR (eGFR >60 mL/min/1.73 m^2) in stratification by rs9934336 genotypes (day 1–5). Participants with A allele (A/A or G/A) tended to show a higher level of urinary glucose excretion at low average blood glucose levels, especially in men (Figure 4a). The urinary glucose excretion of participants with preserved eGFR (day 1) was significantly higher in diabetes patients with the A/A or G/A genotype than those with the G/G genotype ($P = 0.02$, Mann–Whitney *U*-test; Figure 4b, middle panel). The significant difference was still observed when the urinary glucose excretion was divided by average blood glucose to correct the effect of blood glucose level on glucosuria ($P = 0.02$, Mann–Whitney *U*-test; Figure 4b, right panel). The

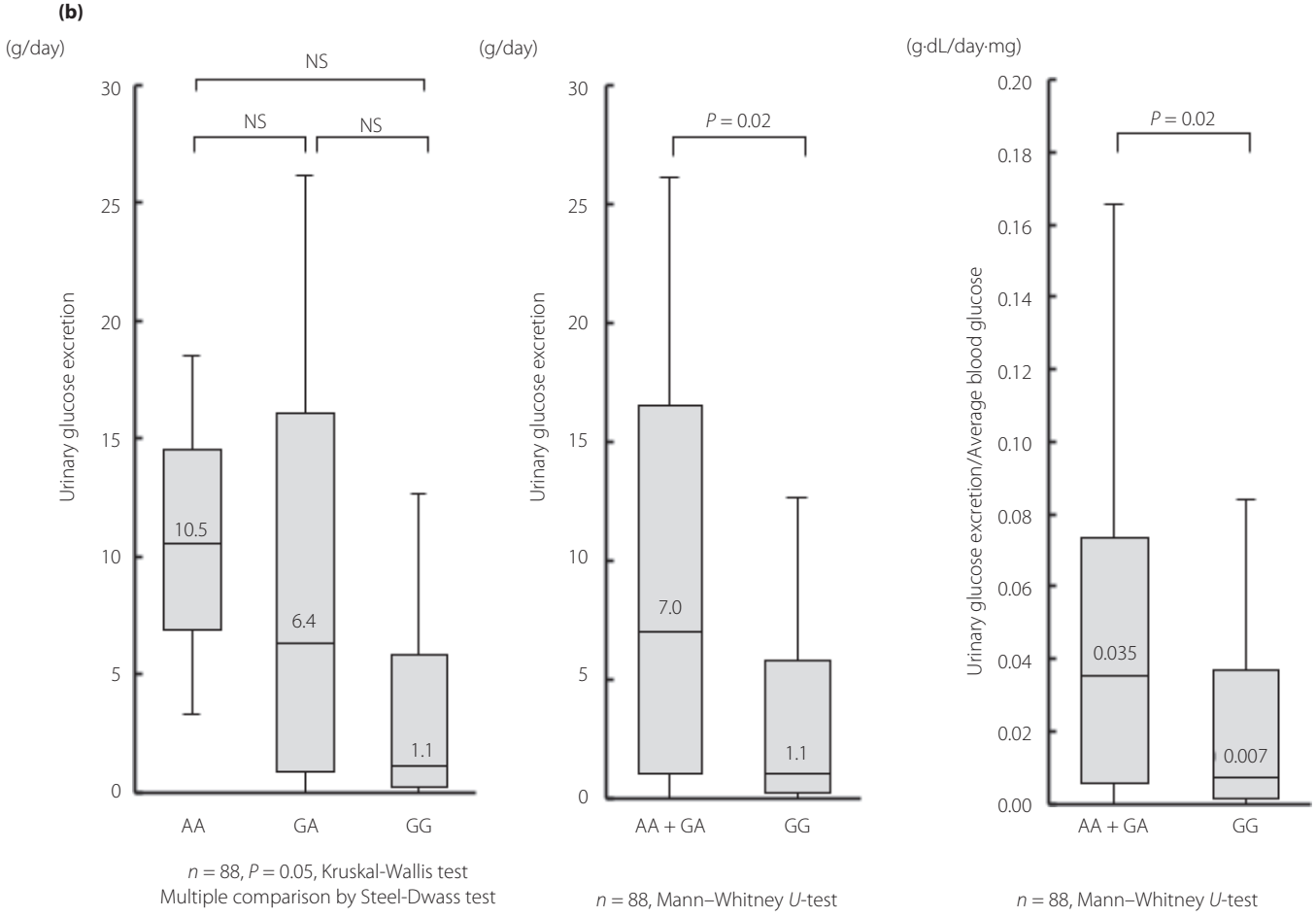
Table 3 | Multiple linear regression analysis for urine glucose excretion in individuals with diabetes mellitus

Variables		Average blood glucose†	eGFR	Sex	rs9934336	rs3813007	HbA1c
Dummy variables				0: Female 1: Male	0: G/G 1: G/A or A/A	0: A/A 1: A/T or T/T	
Model 1	β	0.41	0.28	0.28	0.17	0.13	0.14
	P	1.4×10^{-7}	6.0×10^{-5}	5.7×10^{-5}	0.02	0.084	0.058
Model 2	β	0.41	0.28	0.28	0.17	0.13	0.14
	P	1.4×10^{-7}	6.0×10^{-5}	5.7×10^{-5}	0.02	0.084	0.058
Model 3	β	0.41	0.28	0.28	0.17	0.13	0.14
	P	1.4×10^{-7}	6.0×10^{-5}	5.7×10^{-5}	0.02	0.084	0.058
Model 4	β	0.46	0.30	0.27	0.15	0.12	–
	P	1.9×10^{-10}	1.9×10^{-5}	0.0001	0.038	NS	

Independent variables for each model were listed below. No linear combination was observed between independent variables in all models. Model 1: age, sex, body mass index, duration of diabetes, type of diabetes, average blood glucose (av.BG), serum creatinine, estimated glomerular filtration rate (eGFR), glycated hemoglobin, rs9934336, rs3813007 and rs3813008. Model 2: sex, body mass index, duration of diabetes, av.BG, eGFR, glycated hemoglobin, rs9934336, rs3813007 and rs3813008. Model 3: age, sex, av.BG, eGFR, glycated hemoglobin, rs9934336, rs3813007 and rs3813008. Model 4: sex, type of diabetes, av.BG, eGFR, rs9934336 and rs3813007. β, Standard partial regression coefficient; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; NS: not significant. †Average blood glucose at point points during the day (average blood glucose).

Figure 4 | *SLC5A2* genotypes and urinary glucose excretion. (a) The relationship between urinary glucose excretion and average blood glucose stratified by genotypes of *SLC5A2* rs9934336. Urinary glucose excretion (vertical axis) and average blood glucose (horizontal axis) were shown in men (left panel) and women (right panel). (b) The urinary glucose excretion in participants with preserved estimated glomerular filtration rate stratified by genotypes of *SLC5A2* rs9934336. Bar and number in each box represent medians of urinary glucose excretion. Preserved estimated glomerular filtration rate was defined the estimated glomerular filtration rate >60 mL/min/1.73 m^2. NS, not significant.





n = 88, *P* = 0.05, Kruskal-Wallis test
Multiple comparison by Steel-Dwass test

n = 88, Mann–Whitney *U*-test

n = 88, Mann–Whitney *U*-test

significant difference was not apparent between the A/A or G/A and G/G genotypes in participants with a modest or severe decline in eGFR (<60 mL/min/1.73 m^2), probably due to low urinary glucose excretion regardless of the genotypes (n = 47, median ± interquartile range 0.1 ± 0.4 vs 0.3 ± 1.5 g/day, P = 0.74, Mann–Whitney U-test).

Association of *SLC5A2* polymorphism with susceptibility to type 2 diabetes

In addition to urinary glucose excretion, previous studies suggested the contribution of *SLC5A2* polymorphism to glucose tolerance and diabetes[16,17]. Four SNPs of *SLC5A2* were therefore genotyped in an additional 476 participants (266 participants with type 2 diabetes and 210 healthy controls) for the association study with susceptibility to type 2 diabetes. Among four SNPs, the minor allele (A) of rs9934336 tended to be more frequent in controls (12.6%) than in participants with type 2 diabetes (10.1%, odds ratio 0.78, 95% confidence interval 0.53–1.13, P = 0.18; Table 4). Meta-analysis including present data and previous reports[16–18]; however, showed a statistically significant association of *SLC5A2* rs9934336 polymorphism with type 2 diabetes (summary odds ratio for minor allele (A) 0.86, 95% confidence interval 0.78–0.94, P < 0.002; Figure 5). A fixed effects model (Mantel–Haenszel method) was adopted for meta-analysis, because no heterogeneity was observed in association with *SLC5A2* rs9934336 among the studies tested (I^2 = 0.0%, P = 0.634). No significant publication bias was shown in the meta-analysis (P = 0.75 by the Begg and Mazumdar rank correlation test, and P = 0.08 by Egger's regression test).

DISCUSSION

Based on the data of urine collection for five consecutive days after hospitalization, it was observed that the urinary glucose excretion was significantly reduced when average blood glucose level declined <160 mg/dL at day 4, suggesting the threshold of glucosuria in the diabetic state as a whole in the present study. In addition, marked interindividual variation was noticed from patient-to-patient. The present study showed the significance of clinical and genetic determinants of the urinary glucose excretion by simple and multiple regression analysis. The highest multiple correlation coefficient was observed by the area under the curve >160 mg/dL of blood glucose measured by continuous glucose monitoring (AUC of CGM, r = 0.57, P = 0.0003; Table 2), and the average blood glucose at four points during the day was comparable to the AUC of CGM (r = 0.48, $P = 3.1 \times 10^{-9}$) and convenient for data collection. Fasting blood glucose (r = 0.35, $P = 3.2 \times 10^{-5}$), as well as HbA1c (r = 0.29, P < 0.0007), showed a lower multiple correlation coefficient than the AUC of CGM, or average blood glucose at four points during the day. From a practical point of view, HbA1c could be an useful surrogate for blood glucose in outpatients because of the significant correlation with urinary glucose excretion (r = 0.29, P < 0.0007), although this correlation is weaker than that with blood glucose levels.

In addition to the variables related to blood glucose, the negative correlation between age and urinary glucose excretion was observed by simple regression analysis (r = –0.24, P < 0.006), consistent with a previous study showing that the renal threshold of urinary glucose excretion rises with age[4]. The urinary glucose excretion was, however, also significantly correlated with eGFR (r = 0.31, P = 0.0003; Table 2), and renal function is known to decline with aging. Age has been dismissed as an independent variable of urinary glucose excretion by multiple regression analysis in the present study, suggesting that eGFR is a major determinant of age-related decrease in urinary glucose excretion.

Multiple regression analysis showed that sex is another independent variable, indicating higher urinary glucose excretion in men than in women, as reported earlier[4,11]. The hypothesis regarding the insufficiency of urine collection in women has been excluded, because the urinary creatinine excretion in the present study (men 1.01 ± 0.31 g/day, women 0.70 ± 0.27 g/day) was compatible with that in previous reports[19,20]. A previous study showed that female-dominant expression of SGLT2 protein is post-transcriptionally upregulated by sex hormones after puberty in renal proximal tubules of a rat model[21], indicating the possible mechanism of difference in the threshold of urinary glucose excretion between women and men.

In addition to clinical factors, the present study showed the contribution of a genetic factor to the amount of urinary glucose excretion, with the individuals with the G/G genotype of *SLC5A2* rs9934336 showing lower urinary glucose excretion (Table 4; Figure 4b). Although previous studies have shown the contribution of rare mutations of *SLC5A2* to renal glucosuria[22,23], the current study suggests the common variant of

Table 4 | Association study of *SLC5A2* with susceptibility to type 2 diabetes in the Japanese population

	Minor allele	Minor allele frequency		Odds ratio	95% CI	*P*-value
		Controls (n = 210)	Type 2 diabetes (n = 377)			
rs9934336	A	12.6	10.1	0.78	0.53–1.13	0.18
rs3813007	T	42.6	41.8	0.97	0.76–1.23	0.78
rs3813008	A	19.3	19.6	1.02	0.76–1.38	0.89
rs118162329	A	0.9	0.4	0.42	0.10–1.79	0.24
					χ^2-test or Fisher's exact probability test	

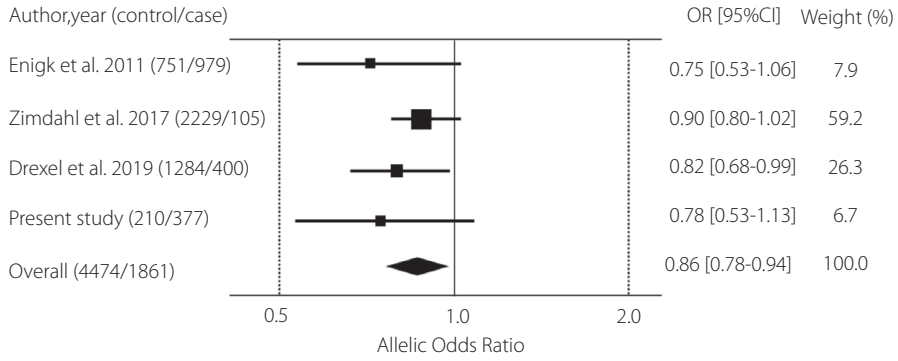


Figure 5 | Meta-analysis of the association of *SLC5A2* rs9934336 polymorphism with susceptibility to type 2 diabetes. $P < 0.002$, $I^2 = 0\%$. CI, confidence interval; OR, odds ratio.

SLC5A2 as a novel determinant of urinary glucose excretion. Minor allele frequency of rs9934336 is as high as 10–13%, indicating that this polymorphism is common among diabetes patients in the usual clinical setting. Among clinical and genetic determinants identified in the present study, the average blood glucose levels showed the most significant effect on urinary glucose excretion (Figure 4a). However, the significant effect of rs9934336 on urinary glucose excretion was still observed when the urinary glucose excretion was corrected for average blood glucose (A/A + G/A vs G/G, $P = 0.02$, Mann–Whitney *U*-test; Figure 4b, right panel), suggesting that rs9934336 genotypes affect the threshold of glucosuria, with the G/G genotype reducing the urinary glucose excretion under the same blood glucose levels.

In addition to its effect on urinary glucose excretion, the contribution of rs9934336 to glucose tolerance has previously been reported. Individuals with[17] and without[16] diabetes and harboring the G/G genotype of rs9934336 polymorphism had been reported to show higher blood glucose level after oral glucose tolerance test. The present meta-analysis showed a significant association of *SLC5A2* rs9934336 polymorphism with type 2 diabetes (summary odds ratio for A allele 0.86, 95% confidence interval 0.78–0.94, $P < 0.002$; Figure 5), suggesting that allele A is protective for the development of type 2 diabetes, possibly due to improved glucose tolerance caused by higher urinary glucose excretion. The heterogeneity of the association was not observed between the studies, aside from the ethnic differences ($I^2 = 0\%$), suggesting no genetic heterogeneity in the association of *SLC5A2* with susceptibility to type 2 diabetes across ethnicities.

To our best knowledge, the present study provided the first evidence showing that the A allele of *SLC5A2* rs9934336 is protective against the development of type 2 diabetes in the Japanese population. These observations are expected to deepen our understanding of the novel pathophysiological mechanism of glucosuria in the development of diabetes. The disease-associated G allele of *SLC5A2* rs9934336 could contribute to the development of type 2 diabetes by reducing the amount of urinary glucose excretion. As the present study reports that a lower urinary glucose excretion predicts a better response to SGLT2 inhibitor[24], individuals with the G allele of *SLC5A2* rs9934336 might also have the possibility to respond to the SGLT2 inhibitor better than individuals with the A allele, suggesting a new aspect for precision medicine and treatment of type 2 diabetes. As for the molecular function of the SNP, rs9934336, located in intron 1 of SGLT2, was estimated to create a possible intronic splicing enhancer site. However, it probably has no impact on splicing due to its deep intronic position, 121 bp upstream to the 5′ end of exon 2, as ascertained by *in silico* splice site analysis[17]. According to the dataset of the Genotype Tissue Expression project expression quantitative trait locus[25], there was no gene with expression regulated by rs9934336. Therefore, the molecular mechanism of rs9934336 itself on the function of SGLT2 is unclear. An alternative functional variant in the haplotype might affect the activity of SGLT2, because rs9934336 was identified as a tag SNP of *SLC5A2*.

The administration of SGLT2 inhibitors has been reported to increase glucose reabsorption by SGLT1 in the distal segments (S2–3) of the proximal tubule[26], suggesting that SGLT1 might compensate for part of the effect of polymorphisms in the SGLT2 gene (*SLC5A2*) on glucose excretion. The interaction between rs9934336 polymorphisms in the SGLT2 gene with variants in the SGLT1 gene is yet to be investigated.

With the increasing use of SGLT inhibitors in the treatment of diabetes, as well as cardiovascular and renal complications, further studies are necessary to clarify the contribution of the common variant of *SLC5A2* to the efficacy and safety of SGLT inhibitors.

In conclusion, these observations showed that blood glucose, eGFR, sex and *SLC5A2* polymorphism are the independent determinants of urinary glucose excretion in diabetes mellitus.

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DISCLOSURE

The authors declare no conflict of interest.

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