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Novel *ERBB receptor feedback inhibitor 1 (ERRFI1)* + 808 T/G polymorphism confers protective effect on diabetic nephropathy in a Korean population

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Abstract.
BACKGROUND: The identification and characterization of the gene, *ERRFI1*, in diabetes has not been reported. In this study, we evaluated the relationship between *ERRFI1* polymorphism and characteristics of type 2 diabetes mellitus (T2DM) in Korea.
SUBJECTS AND METHODS: We conduct a case-control study involving T2DM patients ($n = 342$) and controls ($n = 473$).
RESULTS: A novel single nucleotide *ERRFI1* gene polymorphism at +807(T/G) was found. G genotype frequency was 40.1% in the diabetic group and 42.7% in the control group; the difference was not significant ($p = 0.45$). In the diabetic group, the urine albumin to creatinine ratio (ACR) was lower in the G genotype than in the T genotype ($P = 0.004$). In males with T2DM, those with the G genotype displayed lower systolic blood pressure ($P = 0.01$) and higher glomerular filtration rate ($P = 0.048$) compare to those with the T genotype. In females with T2DM, urine ACR was low in those with the G genotype than in those with the T genotype ($P = 0.02$). In the diabetic group, patients who harboring T allele had a 1.81 times higher risk of diabetic nephropathy than the G allele (95% CI 1.11–2.96, $P = 0.02$). In females with T2DM, patients who harboring T allele had a 2.12 times higher risk of diabetic nephropathy (95% CI 1.07–4.1, $P = 0.03$).
CONCLUSIONS: We identify new loci associated with glycemic traits in diabetes and this finding indicates the potential of *ERRFI1* as a novel therapeutic target of diabetic nephropathy.

Keywords: Diabetic nephropathy, ERRFI1, polymorphism, type 2 diabetes

1. Introduction

Type 2 diabetes mellitus (T2DM) is becoming more common every year in developing and western countries due to excessive caloric intake and decreasing physical activity. T2DM is a complex disease characterized by insulin resistance and β-cell failure due to a combination of genetic and environmental factors that adversely influence glucose metabolism. Our studies conducted over a number of years have revealed the important roles played by genetic factors in the development and progression of diabetes. Genome-wide association studies in Europe have identified diabetes associated genes that include *transcription factor 7-like 2 (TCF7L2), pancreatic beta-cell KATP channel sub-*

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units Kir6.2 (KCNJ11), potassium voltage-gated channels, protein tyrosine phosphatase, receptor type D (PTPRD), and *serine racemase (SRR)* [1–3]. Many genes such as *angiotensin-converting enzyme*, *erythropoietin*, and *osteoprotegerin* are associated with diabetic microvascular complication [4–6]. A number of genome-wide association studies on T2DM have been conducted on individuals of European ancestry. The contributions of these genetic variants in other ethnic groups are less clear.

ERBB receptor feedback inhibitor 1 (ERRFI1) is an adaptor protein containing the epidermal growth factor (EGF) receptor, Cdc42 (cell division control protein 42)/Rac interaction and binging (CRIB), Src-homology-3 (SH3), and 14-3-3 binding domain [7]. ERRFI1 is a negative regulator of EGF signaling and an immediate early response gene that is rapidly induced by heterologous arrays of mitogenic and stressful stimuli [7,8]. ERRFI1 is induced by insulin and plays an important role in insulin signaling [9,10]. High level expression of ERRFI1 is thought to trigger cells to initiate hypertrophy in chronic pathological conditions such as diabetes and hypertension [11–13]. Recently, low level of ERRFI1 expression associated with intrahepatic lipid accumulation and hypercholesterolemia [14]. But, the identification and characterization of the *ERRFI1* gene in diabetes has not been reported. In this study, we investigated relationship between *ERRFI1* gene polymorphism and T2DM in a Korean population.

2. Subjects and methods

2.1. Study subjects

The hospital-based, case-control study including 815 Korean subjects comprised 342 T2DM patients and 473 controls. The T2DM group was comprised of patients who visited Chungnam National University Hospital from February 2010 to November 2010. The controls were acquired from a health examination population in the hospital's out-patient clinic. Subjects were considered diabetic and were included in the T2DM group if any of the following criteria were met: (1) history of T2DM, (2) plasma levels of fasting glucose $\geq$ 126 mg/dL, or (3) plasma levels of 2-h postprandial glucose $\geq$ 200 mg/dL. Non-diabetic control subjects had no past history of diabetes and displayed a fasting plasma glucose concentration $<$ 100 mg/dL. Subjects with renal failure requiring regular dialysis, known malignant disease, acute infection, alcohol abuse, and/or thyroid disease were excluded. T2DM patients were receiving life style modification therapy (19.6%), oral hypoglycemic agents (31.9%), insulin alone (24%) and insulin plus oral drug combination therapy (24.5%). In 342 patients with T2DM, 106 patients (31%) were receiving statins (29.2%) and fibrates (1.8%). Written informed consent was given by all of participants after a complete and clear explanation of the nature and purpose of the study and the experimental protocol was provided. The study was approved by the Institutional Review Board of Chungnam National University Hospital.

2.2. Biochemical measurements

The subjects underwent a standardized physical examination and laboratory tests. Weight (without shoes and wearing light outdoor clothing) and height were measured by trained personnel and body mass index (BMI, kg/m^2) was calculated. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in the sitting position after a 5-min rest. All subjects were assessed in the morning following an overnight fast. Venous blood was drawn for measurements of fasting plasma glucose, glycated hemoglobin (HbA1c), aspartate transaminase (AST), alanine transaminase (ALT), total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, blood urea nitrogen (BUN), and serum creatinine (Cr). Triglyceride, TC, and HDL cholesterol were measured enzymatically using a chemistry analyzer (Hitachi 747, Tokyo, Japan). Fasting plasma glucose was measured by the glucose oxidase method. Insulin was measured by immunoradiometric assay (Diabetes Primary Care, Los Angeles, CA, USA). Insulin sensitivity was measured as fasting serum insulin (mU/L), the HOMA-IR and the QUICKI. HOMA-IR was calculated as [fasting insulin (mU/L) $\times$ fasting glucose (mmol/L)] $\div$ 22.5. QUICKI was calculated as $1 \div$ (log fasting insulin $+$ log fasting glucose), with log fasting insulin expressed as mU/L and fasting glucose expressed as mg/dL. Insulin secretion function was measured by HOMA-β, calculated as (20 $\times$ fasting plasma insulin) $\div$ (fasting plasma glucose -3.5) [15], with the fasting parameters expressed as above.

2.3. Genotyping of ERRFI1 sequence variations

Genomic DNA was extracted from blood treated with ethylenediaminetetraacetic acid (EDTA) antico-

Table 1
Primers for amplification and sequencing of ERRFI1 gene

Primer	Sequence (5' to 3')	Tm (°C)
PCR forward primer	CTACCTCCCAGGGAATGAAAGCTA-	72
PCR reverse primer	TAAGGGATTTTTCTCTGCACTTCA	66
Genotyping primer	CTACCTCCCAGGGAATGAAAGCTA	72
Genotyping primer	GGTGCTCTCTCCCTCACACA	60
Genotyping primer	GATCTCAGC TATGTGTCTG	56

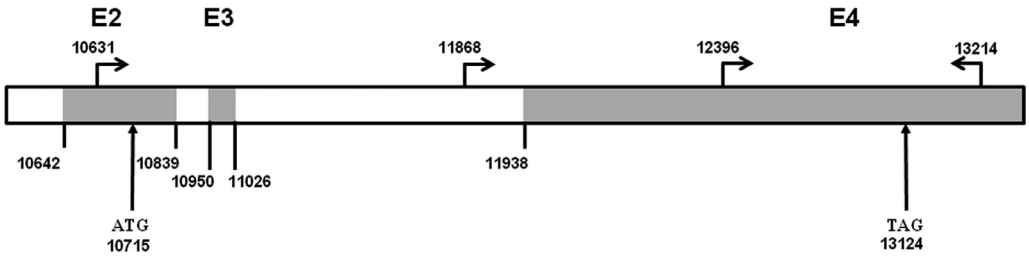

Fig. 1. Schematic representation of the localization of gene-genome specific primer pairs for *ERRFI1*. Gray boxes correspond to exon regions. The arrows indicate the annealing sites and the direction of the primers.

agulant using a standard phenol/chloroform procedure. A Genomic DNA preparation kit (SolGent, Daejeon, Korea) was used according to the manufacturer's instructions and the obtained genomic DNA stored at −70°C until use. Genotypes were analyzed using polymerase chain reaction (PCR) and direct sequencing, as described below, performed without knowledge of case-control status of the patients. ERRFI1 gene primers were designed using the National Center for Biotechnology Information (NCBI) reference sequence NT_021937.19 and are summarized in Table 1 and Fig. 1. The reaction conditions were as follows: 95°C denaturation temperature for 2 min followed by 30 cycles consisting of 95°C for 20 sec, 60°C for 40 sec and 72°C for 2.5 min, and followed by a final extension step of 5 min at 72°C on a PTC-100 apparatus (Bio-Rad Laboratories, Hercules, CA, USA). For DNA sequence analyses, the PCR products were purified by a PCR purification kit (SolGent, Daejeon, Korea) and sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit following the manufacturer's instructions and an ABI PRISM 3730XL DNA Analyzer (ABI, Foster City, CA, USA). The sequences were assembled using the Phred/Phrap/Consed software package from the University of Washington, Seattle [16–18].

2.4. Statistical analyses

All statistical analyses were performed using the SPSS version 18.0 statistical package (SPSS, Chicago, IL, USA). The distribution of genotypes between the T2DM and control groups was compared using the chi-square test. Continuous data are reported as mean ± SD. Categorical data are reported as number of subjects and percentage of individuals affected. The independent-samples t test was used to compare genotype groups in terms of clinical and laboratory characteristics. P value < 0.05 was considered to be significant.

3. Results

3.1. Clinical characteristics

Results of the analyses of clinical characteristics of the diabetic and control groups are presented in Table 2. Higher levels of fasting plasma glucose were noted in the T2DM group, compared with the control group. The height, TC, HDL-cholesterol, and LDL-cholesterol in the T2DM group were lower than in control group. There were no differences in the other clinical characteristics assessed, including mean age, gender, and weight between the T2DM and control groups.

3.2. Genotype distribution of ERRFI1 gene single nucleotide polymorphism (SNP)

A novel *ERRFI1* SNP was found in the third intron located in the promoter downstream of the transcription start site at +808(T/G) (Fig. 2). No other *ERRFI1* SNP was identified. The novel SNP was identified in both the T2DM and control groups. The frequency of

Table 2
Baseline characteristics of the subjects

	Control ($N = 473$)	Diabetes ($N = 342$)	P-value
Age	57.6 ± 14.7	58.9 ± 14.1	0.20
Sex			
Male	242	160	0.22
Female	231	182	
Height (cm)	163.9 ± 9.1	160.2 ± 9.4	0.000
Weight (cm)	64.1 ± 11.8	63.1 ± 12.5	0.27
BMI (kg/m^2)	23.7 ± 3.2	24.5 ± 4.0	0.003
SBP (mmHg)	115.9 ± 13.9	123.1 ± 14.1	0.000
DBP (mmHg)	75.0 ± 9.9	76.7 ± 8.9	0.02
FPB (mg/dL)	90.3 ± 9.6	177.1 ± 80.0	0.000
AST (mg/dL)	20.5 ± 9.8	29.0 ± 24.6	0.000
ALT (mg/dL)	23.7 ± 18.3	28.8 ± 24.4	0.001
Protein (mg/dL)	7.3 ± 0.6	7.3 ± 0.4	0.78
Albumin (mg/dL)	4.4 ± 0.4	4.4 ± 0.2	0.82
BUN (mg/dL)	12.2 ± 7.3	17.0 ± 8.3	0.000
Cr (mg/dL)	0.9 ± 0.2	1.1 ± 0.7	0.000
TC (mg/dL)	182.6 ± 32.4	169.1 ± 46.1	0.000
TG (mg/dL)	116.7 ± 81.9	158.5 ± 121.1	0.000
HDL-C (mg/dL)	56.9 ± 9.1	44.0 ± 13.8	0.000
LDL-C (mg/dL)	102.8 ± 27.8	94.5 ± 35.5	0.000

Data are expressed as mean ± S.D. BMI, Body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; FPG, Fasting plasma glucose; Cr, Creatinine; TC, Total cholesterol; TG, Triglyceride; HDL-C, High density lipoprotein-cholesterol; LDL-C, Low density lipoprotein-cholesterol.

Table 3
Distribution of *ERRFI1* genotypes and alleles

MIG6 genotype		Control ($N = 473$)	Diabetes ($N = 342$)	P-value
Total	T allele	271 (57.3%)	205 (59.9%)	0.45
	G allele	202 (42.7%)	137 (40.1%)	
Male	T allele	125 (51.7%)	102 (63.8%)	0.02
	G allele	117 (48.3%)	58 (36.3%)	
Female	T allele	146 (63.2%)	103 (56.6%)	0.17
	G allele	85 (36.8%)	79 (43.4%)	

the SNP in the total subjects, males, and females was 41.6%, 43.5%, and 39.7% respectively. The distribution of the SNP was not significantly different between the T2DM and control groups ($P = 0.45$). The frequency of the G allele in males with T2DM was significantly lower than in control males (36.2% vs. 48.3%, respectively; $P = 0.02$; Table 3). But, the frequency of the G allele in females with T2DM was not significantly different from females in the control group.

3.3. *Diabetic complication and cardiovascular risk factors associated with ERRFI1 polymorphism in T2DM*

In the entire T2DM cohort, differences in clinical parameters associated with expression of *ERRFI1* polymorphism were compared. The results are summarized in Table 4. Higher levels of fasting plasma glucose and lower levels of urine ACR were evident in the G allele group, compared with the values in the T allele group (fasting plasma glucose: 188.0 ± 77.4 vs. 179.9 ± 81.1, respectively, $P = 0.043$; ACR: 63.7 ± 223.9 vs. 199.9 ± 537.8, respectively, $P = 0.004$). There were no differences in the other clinical characteristics assessed, including mean age, HbA1c, BUN, Cr, and lipid profile between the T2DM and control groups. Moreover, there were no differences in HOMA-IR and QUICKI as surrogate makers of insulin resistance between the G allele and T allele. There was no statistically significant difference in the levels of HOMA-β as a surrogate maker of insulin secretion function between the G allele and T allele (87.8 ± 131.8 vs. 209.2 ± 1315.8, respectively, $P = 0.22$).

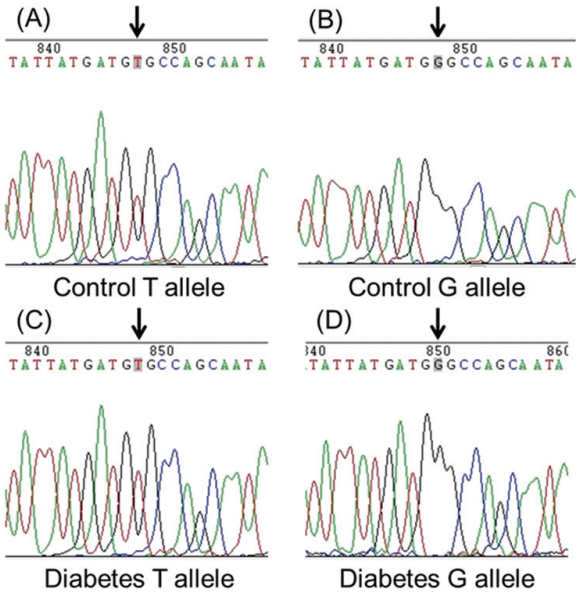

Fig. 2. Sequencing results showing the novel SNP in the region of the third mRNA of *ERRFI1*. Arrows indicate the position of SNP defined according to NT_021937.19 for *ERRFI1*. Control was (A) T allele and (B) G allele. Diabetes was (C) T allele and (D) G allele. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/DMA-120949)

We further examined the relationship between characteristics of T2DM and *ERRFI1* polymorphism according to gender and results are shown in Table 5. Higher levels of GFR and lower levels of SBP were evident in the male diabetic group harboring the G allele, as compared with male diabetics harboring the T allele (GFR: 72.9 ± 31.9 vs. 84.4 ± 34.5, respectively, $P = 0.048$; SBP: 125.8 ± 15.5 vs. 119.6 ± 12.3, respectively, $P = 0.01$). There was no statistically significant difference in the levels of urine ACR and fasting

Table 4
Clinical characteristics of different *ERRFI1* genotypes in diabetic patients

	T allele ($N = 205$)	G allele ($N = 137$)	P-value
Age	58.6 ± 13.7	59.4 ± 14.7	0.59
Sex			
Male	102	58	0.18
Female	103	79	
Height (cm)	160.3 ± 9.0	160.0 ± 10.0	0.85
Weight (cm)	63.0 ± 12.1	63.3 ± 13.1	0.78
BMI (kg/m^2)	24.5 ± 4.1	24.6 ± 3.7	0.84
SBP (mmHg)	124.2 ± 14.5	121.5 ± 13.4	0.11
DBP (mmHg)	76.8 ± 8.8	76.5 ± 9.1	0.79
FPG (mg/dL)	179.9 ± 81.1	188.0 ± 77.4	0.043
HbA1c (%)	9.1 ± 2.4	9.4 ± 2.4	0.40
AST (mg/dL)	30.0 ± 28.0	27.6 ± 18.2	0.37
ALT (mg/dL)	29.1 ± 21.9	28.4 ± 27.9	0.80
Protein (mg/dL)	7.2 ± 0.4	7.4 ± 0.4	0.24
Albumin (mg/dL)	4.4 ± 0.3	4.4 ± 0.2	0.47
BUN (mg/dL)	17.3 ± 9.1	16.6 ± 6.9	0.46
Cr (mg/dL)	1.1 ± 0.8	1.1 ± 0.5	0.38
A/C ratio(mg/g)	199.9 ± 537.8	63.7 ± 223.9	0.004
GFR	70.3 ± 33.5	72.1 ± 33.8	0.65
TC (mg/dL)	170.8 ± 47.8	166.5 ± 43.5	0.41
TG (mg/dL)	155.5 ± 120.1	163.0 ± 122.8	0.58
HDL-C (mg/dL)	44.8 ± 14.4	42.7 ± 12.8	0.18
LDL-C (mg/dL)	96.3 ± 37.3	91.6 ± 32.4	0.23
HOMA-IR	7.45 ± 12.77	7.48 ± 8.84	0.99
QUICKI	0.32 ± 0.05	0.31 ± 0.04	0.20
HOMA-β	209.2 ± 1315.8	87.8 ± 131.8	0.22
Fasting insulin (mU/L)	17.7 ± 23.3	16.4 ± 17.3	0.62
Fasting C-peptide (ng/mL)	1.4 ± 1.3	1.4 ± 1.1	0.66
PP2hr C-peptide (ng/mL)	2.6 ± 2.4	2.3 ± 1.8	0.34

Data are expressed as mean ± S.D. BMI, Body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; FPG, Fasting plasma glucose; Cr, Creatinine; TC, Total cholesterol; TG, Triglyceride; HDL-C, High density lipoprotein-cholesterol; LDL-C, Low density lipoprotein-cholesterol; A/C ratio, Albumin/Creatinine ratio; GFR, glomerular filtration rate; HOMA-IR, homeostasis model assessment: insulin resistance; the quantitative insulin sensitivity check index; QUICKI, the quantitative insulin sensitivity check index; HOMA-β, homeostasis model assessment of insulin resistance β; PP2hr C-peptide, 2-hour postprandial C-peptide.

plasma glucose between male diabetics harboring the G allele and T allele (ACR: 103.3 ± 314.4 vs. 229.5 ± 541.7, respectively, $P = 0.10$; fasting glucose: 196.0 ± 70.3 vs. 173.1 ± 81.7, respectively, $P = 0.08$). Lower levels of urine ACR were evident in female diabetics harboring the G allele, as compared with female diabetics harboring the T allele (32.7 ± 103.7 vs. 172.1 ± 535.9, respectively, $P = 0.02$). There was no statistically significant difference in the levels of TC between female diabetics harboring the G allele and T allele (173.8 ± 35.9 vs. 181.2 ± 42.9, respectively, $P = 0.06$).

Table 6 summarizes the results of association between *ERRFI1* polymorphism and diabetic nephropathy. Novel *ERRFI1* polymorphism was in Hardy-Weinberg equilibrium among diabetic group (Table 6). No significant difference in the minor allele frequency of *ERRFI1* was seen between diabetes with diabetic nephropathy and without diabetic nephropathy. The power was computed for SNP using SNP tools for Microsoft excel [19]. At $\alpha = 0.05$, this sample in total, male, female diabetes provided 67, 20 and 58% power in detecting diabetic nephropathy respectively. The diabetes harboring T allele had a 1.81 times higher risk of diabetic nephropathy (95% CI 1.11–2.96) than the G allele ($P = 0.02$). There was no statistically significant difference in odds ratio between male diabetics harboring the G allele and T allele. The female diabetes harboring T allele had a 2.12 times higher risk of diabetic nephropathy (95% CI 1.07–4.1) than the G allele ($P = 0.03$).

4. Discussion

The present study analyzed the *ERRFI1* SNP in a T2DM case-control cohort comprising 815 Korean individuals. The results reveal that the *ERRFI1* poly-

Table 5
Clinical characteristics of different *ERRFI1* genotypes in male and female diabetic group

	Male			Female		
	T allele ($N = 102$)	G allele ($N = 58$)	P-value	T allele ($N = 103$)	G allele ($N = 79$)	P-value
Age	57.3 ± 13.2	54.8 ± 15.5	0.29	59.3 ± 13.6	60.3 ± 13.8	0.42
Height (cm)	166.0 ± 6.8	168.3 ± 8.6	0.16	155.6 ± 5.9	155.9 ± 6.6	0.39
Weight (cm)	65.4 ± 13.4	70.0 ± 13.9	0.06	58.2 ± 9.1	57.7 ± 9.7	0.62
BMI (kg/m2)	23.7 ± 3.5	24.70 ± 3.72	0.14	24.0 ± 3.6	23.7 ± 3.6	0.44
SBP (mmHg)	125.8 ± 15.5	119.6 ± 12.3	0.01	117.2 ± 14.1	117.4 ± 15.9	0.89
DBP (mmHg)	77.9 ± 8.6	75.5 ± 8.1	0.11	74.1 ± 9.0	74.1 ± 10.4	0.99
FPG (mg/dL)	173.1 ± 81.7	196.0 ± 70.3	0.08	121.6 ± 64.5	133.4 ± 73.4	0.10
HbA1c (%)	9.4 ± 2.5	10.0 ± 2.5	0.15	8.9 ± 2.2	8.8 ± 2.1	0.97
AST (mg/dL)	32.3 ± 35.9	29.2 ± 17.4	0.53	22.4 ± 13.2	22.3 ± 14.3	0.93
ALT (mg/dL)	31.0 ± 24.5	33.8 ± 31.2	0.52	22.5 ± 19.8	21.7 ± 19.1	0.71
Protein (mg/dL)	7.2 ± 0.4	7.6 ± 0.5	0.30	7.3 ± 0.7	7.2 ± 0.8	0.51
Albumin (mg/dL)	4.4 ± 0.3	4.6 ± 0.3	0.36	4.4 ± 0.4	4.4 ± 0.7	0.90
BUN (mg/dL)	17.2 ± 8.4	16.7 ± 6.6	0.69	14.5 ± 11.6	13.8 ± 6.1	0.47
Cr (mg/dL)	1.2 ± 0.6	1.2 ± 0.6	0.84	0.9 ± 0.6	0.9 ± 0.4	0.77
A/C ratio(mg/g)	229.5 ± 541.7	103.3 ± 314.4	0.10	172.1 ± 535.9	32.7 ± 103.7	0.02
GFR	72.9 ± 31.9	84.4 ± 34.5	0.048	71.0 ± 26.2	68.6 ± 26.1	0.38
TC (mg/dL)	162.6 ± 40.4	168.3 ± 50.1	0.44	181.2 ± 42.9	173.8 ± 35.9	0.06
TGs (mg/dL)	146.4 ± 99.0	174.4 ± 131.9	0.16	128.5 ± 42.9	173.8 ± 35.9	0.49
HDL-C (mg/dL)	44.2 ± 14.9	42.0 ± 12.7	0.35	53.5 ± 13.2	51.2 ± 13.1	0.09
LDL-C (mg/dL)	90.4 ± 31.9	88.4 ± 31.0	0.71	103.7 ± 34.1	99.9 ± 31.2	0.25
HOMA-IR	8.15 ± 15.56	7.81 ± 9.05	0.89	6.82 ± 9.58	7.14 ± 8.74	0.83
QUICKI	0.32 ± 0.05	0.31 ± 0.05	0.35	0.32 ± 0.04	0.31 ± 0.04	0.41
HOMA-β	330.5 ± 1877.1	66.7 ± 83.0	0.32	99.6 ± 325.1	104.1 ± 158.4	0.92
Fasting insulin (mU/L)	19.9 ± 29.8	16.4 ± 17.5	0.65	15.6 ± 14.8	16.4 ± 17.1	0.74
Fasting C-peptide (ng/mL)	1.2 ± 0.8	1.4 ± 1.1	0.20	1.7 ± 1.5	1.4 ± 1.0	0.15
PP2hr C-peptide (ng/mL)	2.1 ± 1.7	2.2 ± 1.9	0.68	3.02 ± 2.83	2.39 ± 1.78	0.09

Data are expressed as mean ± S.D. BMI, Body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; FPG, Fasting plasma glucose; Cr, Creatinine; TC, Total cholesterol; TG, Triglyceride; HDL-C, High density lipoprotein-cholesterol; LDL-C, Low density lipoprotein-cholesterol; A/C ratio, Albumin/Creatinine ratio; GFR, glomerular filtration rate; HOMA-IR, homeostasis model assessment: insulin resistance; the quantitative insulin sensitivity check index; QUICKI, the quantitative insulin sensitivity check index; HOMA-β, homeostasis model assessment of insulin resistance β; PP2hr C-peptide, 2-hour postprandial C-peptide.

Table 6
Association of *ERRFI1* SNP with diabetic nephropathy in diabetic group

	Alleles	Controls: Cases	MAF	HWE P	Power	χ^2	OR (95% CI)	P
Total	T allele	132:73	0.39	0.93	0.67	5.79	1.81 (1.11–2.96)	0.02
	G allele	105:32						
Male	T allele	65:37	0.41	0.26	0.20	1.26	1.49 (0.74–3.02)	0.26
	G allele	42:16						
Female	T allele	67:36	0.37	0.24	0.58	4.73	2.12 (1.07–4.1)	0.03
	G allele	63:16						

MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval.

morphism is associated with fasting plasma glucose in T2DM and bestows a protective effect on diabetic nephropathy.

Many genes, such as ADCY5, MADD, ADRA2A, CRY2, FADS1, GLIS3, SLC2A2, PROX1, and C2CD4B, influence fasting plasma glucose in diabetes and are associated with HOMA-β [20,21]. *ERRFI1*, which is located on chromosome 1p36, is an immediate early response gene that can be induced by stressful stimuli and growth factors [7,8]. The ERRFI1 protein inhibits EGF receptor autophosphorylation and EGF-mediated activation of Ras, extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinases (JNK), Akt/PTB, and Rb protein [22]. *ERRFI1* knockout mice display overactivation of the EGF receptor and mitogen-activated protein kinase (MAPK) signaling leading to enhanced cell proliferation [23]. Therefore, ERRFI1 is a protective regulator of EGF receptor overactivation. Also, ERRFI1 is a downstream target of the progesterone receptor and decreased ex-

pression in carcinomas of breast, lung, and kidney [24].

SNP is the simplest form of DNA variation among individuals. SNP may influence promoter activity, messenger RNA stability, and subcellular localization of mRNAs and/or proteins [25]. Thus, SNPs induce loss/gain of function. To date, a total of 263 *ERRFI1* SNP have been reported, yet their functions are unknown. The present study identifies for the first time a novel *ERRFI1* SNP in the third intron located in the promoter downstream of the transcription start site at +808(T/G). This novel *ERRFI1* polymorphism may represent a loss of function, rather than a gain of function.

Diabetes is the most common cause of end-stage renal failure, accounting for 35%–40% of all new cases requiring dialysis therapy throughout the world [26]. Hyperglycemia and systemic hypertension play an important role in the development and progression of diabetic nephropathy [27]. Despite normalization of BP and strict control of blood glucose levels, many patients still progress to end-stage renal disease [28]. Diabetes-induced hemodynamic alterations that include glomerular hypertension, tyrosine kinase activation, and metabolic derangements due to inappropriate activation of the protein kinase C–MAPK pathway and activation of the polyol pathway, increases the accumulation of advanced glycation end products and oxidative stress. The cellular alteration resulted in extracellular matrix accumulation, leading to the development and progression of diabetic nephropathy. ERRFI1 in the kidney increases dramatically soon after the onset of diabetes and continues to increase throughout the progression to diabetic nephropathy [29]. When ERRFI1 is present in sufficient quantity to allow interaction with Cdc42Hs, ERRFI1 then triggers further activation of the stress activated protein kinases (SAPKs), which, in a positive feedback cycle, stimulate more ERRFI1 transcription as well as increases in ERRFI1 mRNA stability [11,30]. Sustained ERRFI1-dependent SAPK pathway activation, arising as a consequence of mechanical stretch or hyperglycemia, may trigger the cell to initiate hypertrophy [28]. Our study showed that the ACR and SBP were low and GFR was high in type 2 diabetes with G allele. This protective effect of *ERRFI1* polymorphism on renal failure in diabetes was observed even with high levels of fasting plasma glucose in diabetes harboring the G allele. Hypofunction of ERRFI1 may disrupt positive feedback of MAPK activity followed by inhibition of extracellular matrix accumulation and prevention of diabetic nephropathy.

The limitation of this study is that renal failure requiring regular dialysis was excluded from examination. Therefore, further research is required to determine whether a novel *ERRFI1* polymorphism prevents diabetic nephropathy in patients including end-stage renal failure.

In conclusion, our study has identified new loci of polymorphism associated with glycemic traits and the novel protective effect of ERRFI1 has great potential to unravel new mechanisms of diabetic renal failure and identify new targets for therapeutic intervention.

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References

[1] Tsai, F.J., et al., A genome-wide association study identifies susceptibility variants for type 2 diabetes in Han Chinese. *PLoS Genet*, 2010. **6**(2): p. e1000847.

[2] Grant, S.F., et al., Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet*, 2006. **38**(3): pp. 320-3.

[3] Gloyn, A.L., et al., Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. *Diabetes*, 2003. **52**(2): pp. 568-72.

[4] Ding, W., et al., Association between two genetic polymorphisms of the renin-angiotensin-aldosterone system and diabetic nephropathy: a meta-analysis. *Mol Biol Rep*, 2012. **39**(2): pp. 1293-303.

[5] Abhary, S., et al., Association between erythropoietin gene polymorphisms and diabetic retinopathy. *Arch Ophthalmol*, 2010. **128**(1): pp. 102-6.

[6] Pitocco, D., et al., Association between osteoprotegerin G1181C and T245G polymorphisms and diabetic charcot neuroarthropathy: a case-control study. *Diabetes Care*, 2009. **32**(9): pp. 1694-7.

[7] Zhang, Y.W., et al., Evidence that MIG-6 is a tumor-suppressor gene. *Oncogene*, 2007. **26**(2): pp. 269-76.

[8] Zhang, Y.W. and G.F. Vande Woude, Mig-6, signal transduction, stress response and cancer. *Cell Cycle*, 2007. **6**(5): pp. 507-13.

[9] Messina, J.L., Insulin and dexamethasone regulation of a rat hepatoma messenger ribonucleic acid: insulin has a transcriptional and a posttranscriptional effect. *Endocrinology*, 1989. **124**(2): pp. 754-61.

[10] Melendez, P.A., et al., Insulin-induced gene 33 mRNA expression in Chinese hamster ovary cells is insulin receptor dependent. *J Cell Biochem*, 2000. **77**(3): pp. 432-44.

[11] Makkinje, A., et al., Gene 33/Mig-6, a transcriptionally inducible adapter protein that binds GTP-Cdc42 and activates SAPK/JNK. A potential marker transcript for chronic pathologic conditions, such as diabetic nephropathy. Possible role

in the response to persistent stress. *J Biol Chem*, 2000. **275**(23): pp. 17838-47.
[12] Wolf, G. and F.N. Ziyadeh, Molecular mechanisms of diabetic renal hypertrophy. *Kidney Int*, 1999. **56**(2): pp. 393-405.
[13] Mahgoub, M.A. and A.S. Abd-Elfattah, Diabetes mellitus and cardiac function. *Mol Cell Biochem*, 1998. **180**(1–2): pp. 59-64.
[14] Ku, B.J., et al., Mig-6 plays a critical role in the regulation of cholesterol homeostasis and bile acid synthesis. *PLoS One*, 2012. **7**(8): p. e42915.
[15] Matthews, D.R., et al., Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, 1985. **28**(7): pp. 412-9.
[16] Ewing, B., et al., Base-calling of automated sequencer traces using phred. I. Accuracy assessment. *Genome Res*, 1998. **8**(3): pp. 175-85.
[17] Ewing, B. and P. Green, Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Res*, 1998. **8**(3): pp. 186-94.
[18] Gordon, D., C. Abajian and P. Green, Consed: a graphical tool for sequence finishing. *Genome Res*, 1998. **8**(3): pp. 195-202.
[19] Chen, B., et al., SNP_tools: A compact tool package for analysis and conversion of genotype data for MS-Excel. *BMC Res Notes*, 2009. **2**: p. 214.
[20] Dupuis, J., et al., New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nat Genet*, 2010. **42**(2): pp. 105-16.
[21] Park, K.S., The search for genetic risk factors of type 2 diabetes mellitus. *Diabetes Metab J*, 2011. **35**(1): pp. 12-22.
[22] Xu, D., A. Makkinje and J.M. Kyriakis, Gene 33 is an endogenous inhibitor of epidermal growth factor (EGF) receptor signaling and mediates dexamethasone-induced suppression of EGF function. *J Biol Chem*, 2005. **280**(4): pp. 2924-33.
[23] Ferby, I., et al., Mig6 is a negative regulator of EGF receptor-mediated skin morphogenesis and tumor formation. *Nat Med*, 2006. **12**(5): pp. 568-73.
[24] Amatschek, S., et al., Tissue-wide expression profiling using cDNA subtraction and microarrays to identify tumor-specific genes. *Cancer Res*, 2004. **64**(3): pp. 844-56.
[25] Shastry, B.S., SNPs: impact on gene function and phenotype. *Methods Mol Biol*, 2009. **578**: pp. 3-22.
[26] Collins, A.J., et al., Excerpts from the US Renal Data System 2009 Annual Data Report. *Am J Kidney Dis*, 2010. **55**(1 Suppl 1): pp. S1-420, A6-7.
[27] Perneger, T.V., et al., End-stage renal disease attributable to diabetes mellitus. *Ann Intern Med*, 1994. **121**(12): pp. 912-8.
[28] Rosolowsky, E.T., et al., Risk for ESRD in type 1 diabetes remains high despite renoprotection. *J Am Soc Nephrol*, 2011. **22**(3): pp. 545-53.
[29] Kikkawa, R., D. Koya and M. Haneda, Progression of diabetic nephropathy. *Am J Kidney Dis*, 2003. **41**(3 Suppl 1): pp. S19-21.
[30] Chen, C.Y., et al., Stabilization of interleukin-2 mRNA by the c-Jun NH2-terminal kinase pathway. *Science*, 1998. **280**(5371): pp. 1945-9.