Genetic Risk Score Constructed Using 14 Susceptibility Alleles for Type 2 Diabetes Is Associated With the Early Onset of Diabetes and May Predict the Future Requirement of Insulin Injections Among Japanese Individuals

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OBJECTIVE—We evaluated the clinical usefulness of a genetic risk score (GRS) based on 14 well-established variants for type 2 diabetes.

RESEARCH DESIGN AND METHODS—We analyzed 14 SNPs at *HHEX*, *CDKAL1*, *CDKN2B*, *SLC30A8*, *KCNJ11*, *IGF2BP2*, *PPARG*, *TCF7L2*, *FTO*, *KCNQ1*, *IRS-1*, *GCKR*, *UBE2E2*, and *C2CD4A/B* in 1,487 Japanese individuals (724 patients with type 2 diabetes and 763 control subjects). A GRS was calculated according to the number of risk alleles by counting all 14 SNPs (T-GRS) as well as 11 SNPs related to β-cell function (β-GRS) and then assessing the association between each GRS and the clinical features.

RESULTS—Among the 14 SNPs, 4 SNPs were significantly associated with type 2 diabetes in the present Japanese sample ($P < 0.0036$). The T-GRS was significantly associated with type 2 diabetes ($P = 5.9 \times 10^{-21}$). Among the subjects with type 2 diabetes, the β-GRS was associated with individuals receiving insulin therapy (β = 0.0131, SE = 0.006, $P = 0.0431$), age at diagnosis (β = −0.608, SE = 0.204, $P = 0.0029$), fasting serum C-peptide level (β = −0.032, SE = 0.0140, $P = 0.022$), and C-peptide index (β = −0.031, SE = 0.012, $P = 0.0125$).

CONCLUSIONS—Our data suggest that the β-GRS is associated with reduced β-cell functions and may be useful for selecting patients who should receive more aggressive β-cell–preserving therapy.

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Type 2 diabetes affects nearly 300 million individuals worldwide, and its prevalence continues to increase in many countries, including Japan (1). Although the precise mechanisms underlying the development and progression of type 2 diabetes have not been elucidated, a combination of multiple genetic and/or environmental factors contribute to the pathogenesis of the disease (2,3). Impaired insulin secretion and insulin resistance, the two main pathophysiological mechanisms leading to type 2 diabetes, have a significant genetic component (4).

Recent studies have confirmed ~40 genetic loci associated with type 2 diabetes (5); most of these loci were discovered in genome-wide association studies (6–16), with the exception of *PPARG* (17), *KCNJ11* (18), and *WFS1* (19), which were identified using candidate gene approaches, and *TCF7L2*, which was discovered using a linkage-positional cloning strategy (20). Among them, many loci (at least 10), such as *MTNR1B*, *SLC30A8*, *THADA*, *TCF7L2*, *KCNQ1*, *CAMK1D*, *CDKAL1*, *IGF2BP2*, *HNF1B*, and *CENTD2*, have been shown to be associated with impaired β-cell functions, whereas only a few loci such as *PPARG*, *IRS1*, and *FTO* have been associated with insulin resistance (13).

Although the molecular mechanisms responsible for the susceptibility effect can be well assigned for some loci, such as those at *KCNJ11* and *SLC30A8*, the mechanisms by which most genetic loci contribute to the development of type 2 diabetes are not understood.

Recently, the construction of a genetic risk score (GRS) using information on these diabetes susceptibility loci has been shown to be useful for evaluating the risk of the development of type 2 diabetes in individuals (21–26). However, the currently available genetic information is

obviously insufficient for predicting the development of type 2 diabetes, and little is known about the detailed relationship between the GRS and the clinical features of type 2 diabetes. In the current study, we selected 14 well-replicated and well-established genetic variants associated with type 2 diabetes in the Japanese population (25,27–32) and constructed a GRS, which may predict mechanism (β-cell function and insulin resistance) of diabetes development, to evaluate the possibility that currently available genetic information can be translated into clinical practice.

RESEARCH DESIGN AND METHODS

—All patients with type 2 diabetes who regularly attended the outpatient clinics in five hospitals—University of Toyama Hospital (Toyama, Japan), Shakaihoken Takaoka Hospital, Saiseikai Takaoka Hospital, Nanto City Hospital (Nanto, Japan), and Asahi General Hospital (Asahi-machi, Japan)—were asked to participate in this study. Among them, informed consent was obtained from 724 patients between January 2008 and December 2009, and these 724 patients were enrolled in the current study as case subjects (62.3% male, mean ± SD age 64.9 ± 11.1 years, and A1C 7.5 ± 1.3%) (Table 1). We also enrolled control individuals (n = 763) selected from subjects who had undergone an annual health check-up at the Itoigawa General Hospital (Itoigawa, Japan), Aoi Hospital (Tonami, Japan), Amenithy Tsukioka Hospital (Toyama, Japan), Hida City Hospital (Hida, Japan), Sakurai Hospital (Kurobe, Japan), Hokuriku chuo Hospital (Oyabe, Japan), and the above five hospitals. The inclusion criteria for the nondiabetic control subjects were as follows: *1*) >50 years of age, *2*) A1C values <6.0%, *3*) no family history of type 2 diabetes in first- and second-degree relatives, and *4*) no past history of a diagnosis of diabetes. Diabetes was diagnosed based on the 1998 American Diabetes Association criteria (33). The exclusion criteria for the case subjects with diabetes were diabetes caused by *1*) liver dysfunction, *2*) steroids and other drugs that might increase glucose levels, *3*) malignancy, *4*) monogenic disorders known to cause diabetes, and *5*) individuals who tested positive for anti-GAD antibody. Characteristics of the participants are presented in Table 1.

We also performed an examination of another cohort for the association of GRS with type 2 diabetes (homeostasis model assessment [HOMA] of β-cell function or HOMA of insulin resistance [HOMA-IR]), which was conducted in Tokyo University, Tokyo, Japan (30) (type 2 diabetes cases, n = 1,182, 59.6% male, age 65.3 ± 9.5 years, and A1C 7.7 ± 1.6%; nondiabetic subjects, n = 859, 44.4% male, age 69.5 ± 6.8 years, and A1C 5.6 ± 0.2%) (Supplementary Table 1). The inclusion criteria for the nondiabetic control subjects and the exclusion criteria for the case subjects with diabetes were identical between the two studies, except for the age of control individuals >60 years in the Tokyo University study.

Table 1—*Clinical characteristics of the participants*

	Type 2 diabetic	Control	*P*
n	724	763	
Sex (male/female)	451/273	359/404	<0.0001*
Age (years)	64.9 ± 11.1	72.5 ± 9.0	<0.001
Duration of diabetes (years)	13.6 ± 9.1		
Age at diagnosis (years)	51.4 ± 11.6		
Self-reported family history of diabetes (%)	55.7		
BMI (kg/m^2)	24.5 ± 3.9	22.7 ± 3.3	<0.0001
Maximum BMI (kg/m^2)	27.4 ± 4.3	24.7 ± 3.1	<0.0001
Waist circumference (cm)			
Males	87.1 ± 9.5	85.3 ± 8.1	<0.05
Females	87.4 ± 11.7	81.2 ± 9.1	<0.0001
FPG (mmol/L)	7.60 ± 1.88	5.33 ± 0.58	<0.0001
A1C (%)	7.53 ± 1.25	5.54 ± 0.25	<0.0001
eGFR (mL/min)	74.8 ± 21.6	72.3 ± 16.3	<0.05
HOMA-β (%)[a]	37.5 ± 40.2	60.8 ± 41.2	<0.0001
HOMA-IR (mol · μU/L^2)[a]	2.17 ± 1.60	1.24 ± 0.78	<0.0001
F-CPR (ng/mL)[b]	1.65 ± 0.85	1.49 ± 0.61	<0.0001
F-CPI[b]	1.25 ± 0.71	1.56 ± 0.61	<0.0001
Complications (%)			
Diabetic nephropathy	39.3		
Diabetic retinopathy	42.3		
Treatment of diabetes (%)			
Diet alone	13.4		
Using oral hypoglycemic agents	73.9		
Sulfonylureas	46.7		
Thiazolidinediones	20.3		
Biguanides	28.5		
α-Glucosidase inhibitor	33.7		
Glinide	4.9		
Using insulin	31.4		
Presence of hypertension (%)[c]	76.1	63.2	<0.0001*
Systolic blood pressure (mmHg)	130 ± 16	130 ± 18	0.5755
Diastolic blood pressure (mmHg)	75 ± 11	76 ± 11	<0.01
Presence of dyslipidemia (%)[d]	78.9	64.9	<0.0001*
LDL cholesterol (mg/dL)	112 ± 26	119 ± 29	<0.0001
HDL cholesterol (mg/dL)	53.9 ± 16.3	60.6 ± 16.5	<0.0001
Triglycerides (mg/dL)	122 ± 74	109 ± 67	<0.0001

Data are means ± SD. The value for A1C (%) was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated using the following formula: A1C (%) = A1C (Japan Diabetes Society) (%) + 0.4%. CPI was calculated using the following equation: CPI = (F-CPR/FPG) × 100. eGFR, estimated glomerular filtration rate. *Pearson χ^2 test. [a]HOMA-β and -IR were calculated in all participants except for those treated with insulin therapy. [b]F-CPR and CPI were calculated in all participants except for those with serum creatinine level >1.5 mg/dL. [c]Determination of hypertension was defined as systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg or having been treated for hypertension. [d]Determination of dyslipidemia was defined as serum LDL cholesterol ≥120 mg/dL, serum triglycerides ≥150 mg/dL, or HDL cholesterol <40 mg/dL or having been treated for dyslipidemia.

Collection of clinical information

We obtained clinical information including the current BMI, maximum BMI, family history of diabetes, age at diagnosis, blood chemistry (including plasma glucose, insulin level, serum C-peptide, and serum creatinine) at fasting state, diabetes complications, and use of antidiabetes drugs.

Patients who were required to inject >10 units of insulin a day continuously were regarded as undergoing insulin therapy.

Diabetic nephropathy was defined as having a urinary albumin-to-creatinine ratio ≥30 mg/gCr, determined in at least two consecutive overnight samples collected over a 3- to 6-month period. Patients diagnosed as having a urinary tract infection, other glomerular diseases, or gross hematuria were excluded.

All patients underwent ophthalmologic examinations, including funduscopic examination. We defined nonproliferative diabetic retinopathy, proliferative diabetic retinopathy, and a history of photocoagulation or vitrectomy as indicating the presence of diabetic retinopathy. All the study procedures were approved by the ethics committee of the University of Toyama, and informed consent was obtained from all of the participants.

Genotyping assay

Genomic DNA was extracted from peripheral blood (QIAamp DNA blood kit; QIAGEN, Hilden, Germany). We selected 14 single nucleotide polymorphisms (SNPs) at genetic loci that had been previously shown to be robustly associated with type 2 diabetes in seven recent studies performed in Japanese populations (25,27–32). The following SNPs were examined: in *KCNJ11* (rs5219), in *HHEX* (rs1111875), in *CDKAL1* (rs7756992), near *CDKN2B* (rs10811661), in *SLC30A8* (rs13266634), in *IGF2BP2* (rs4402960), in *PPARG* (rs1801282), in *TCF7L2* (rs7903146), in *FTO* (rs8050136), near *IRS-1* (rs2943641), in *GCKR* (rs780094), in *UBE2E2* (rs7612463), in *C2CD4A-C2CD4B* (rs7172432), and in *KCNQ1* (rs2237892). The genotyping of these SNPs was performed using TaqMan SNP Genotyping assays (Applied Biosystems, Foster City, CA) or a multiplex-PCR-invader assay as described previously (34,35).

The success rates for these assays were >95%, and the concordance rate, based on duplicate comparisons in 763 control participants and 724 type 2 diabetic patients, was 99.4%. A tagging approach to detect all variations completely covering each genomic region has not been used. Although no apparent deviations in the genotype distributions from Hardy-Weinberg equilibrium (HWE) were observed for all of the SNPs ($P \geq 0.001$) (6), some of them had borderline results for the HWE test (rs13266634 in control; rs2237892 in control) (Supplementary Table 2).

Construction of GRS

We combined the information on the 14 SNPs using an allele count model (21). To construct the GRS, we summed the number of risk alleles of all 14 SNPs included in this study in each individual, assuming an equal and additive effect of each allele (T-GRS). The T-GRS was distributed normally in both the control and the diabetic subjects.

We further classified these 14 genetic variants into two categories: *1*) 11 β-cell function–related SNPs (rs1111875 in *HHEX*, rs7756992 in *CDKAL1*, rs10811661 in *CDKN2B*, rs13266634 in *SLC30A8*, rs4402960 in *IGF2BP2*, rs7903146 in *TCF7L2*, rs780094 in *GCKR*, rs7612463 in *UBE2E2*, rs7172432 in *C2CD4A/B*, rs2237892 in *KCNQ1*, and rs5219 in *KCNJ11*) and *2*) three insulin resistance/obesity-related variants (rs1801282 in *PPARG*, rs8050136 in *FTO*, and rs2943641 in *IRS-1*), based on previously reported information (13). We then calculated the GRS of the β-cell function–related SNPs (β-GRS) and the insulin resistance and obesity-related SNPs (R-GRS). The β-GRS and R-GRS were also distributed normally in both the control and diabetic groups.

Statistical analysis

Differences in clinical features, such as the insulin secretory capacity and age at the time of the diagnosis of diabetes, between the risk allele groups were determined using ANOVA and multiple regression analysis after adjustments for related covariables. Results with *P* values <0.05 were considered statistically significant.

We performed HWE tests according to the method described by Nielsen et al. (36). The proportions of genotypes for each SNP were compared between the type 2 diabetic case and the nondiabetic control subjects using a multiple logistic regression analysis with or without adjustments for age, sex, and BMI. The allele-specific odds ratios (ORs) were calculated using logistic regression with or without adjustments for age, sex, and BMI. Variables with skewed distributions were logarithmically (natural) transformed for further analyses. Quantitative trait analyses were performed using a multiple linear regression analysis with or without adjustments for related covariables. Bonferroni correction was applied to correct for multiple testing errors, and $P < 0.0036$ (0.05 divided by 14: the total number of SNPs studied) was considered significant.

The effects of the GRS on the clinical features and quantitative metabolic traits were examined by calculating the β values for the risk allele score using linear generalized estimating equations. *P* values <0.05 were considered statistically significant for this analysis.

The statistical analyses were performed using JMP for Windows version 8.00 software (SAS Institute, Cary, NC). The power of the sample size for the current study to identify the association of previously reported SNP loci with type 2 diabetes was calculated using "CaTS power calculator for genetic studies" software (http://www.sph.umich.edu/csg/abecasis/CaTS/).

RESULTS

Associations of each of the 14 SNPs with type 2 diabetes and quantitative metabolic traits

Among the 14 SNPs from 14 loci, 4 SNPs (rs7756992 in *CDKAL1*, rs10811661 near *CDKN2B*, rs13266634 in *SLC30A8*, and rs2237892 in *KCNQ1*) were found to be significantly associated with type 2 diabetes (Supplementary Table 3) ($P = 1.7 \times 10^{-5}$, 7.5×10^{-6}, 2.8×10^{-3}, and 1.4×10^{-7}, respectively) after adjustments for age, sex, and BMI; the association of rs2237892 in *KCNQ1* was the strongest in the present Japanese sample, as reported previously (16,28). rs4402960 in *IGF2BP2*, rs2943641 near *IRS-1*, rs780094 in *GCKR*, and rs5219 in *KCNJ11* showed a nominal association with type 2 diabetes ($P = 0.010$, $P = 0.028$, $P = 0.013$, and $P = 0.033$, respectively), and rs7172432 in *C2CD4A/B* tended to be associated with type 2 diabetes ($P = 0.073$). As for rs7903146 in *TCF7L2*, rs1111875 in *HHEX*, rs1801282 in *PPARG*, rs8050136 in *FTO*, and rs7612463 in *UBE2E2*, we were unable to detect any SNPs that were significantly associated with type 2 diabetes in the present Japanese sample ($P = 0.659$, 0.773, 0.997, 0.187, and 0.207, respectively). The effect directions of the abovementioned SNPs, with the exception of rs1111875 in *HHEX*, were consistent with those in previous reports (OR >1, $P = 9.2 \times 10^{-4}$, binomial test) (16,28).

We next studied the associations of the T-GRS (equivalent to the sum of the risk alleles of the 14 SNPs studied here), the β-GRS (equivalent to the sum of the 11 β-cell function–related genes), and the R-GRS (equivalent to the sum of the three obesity and insulin resistance-related genes) with the development of type 2

diabetes. The T-GRS and β-GRS were significantly associated with the development of type 2 diabetes (T-GRS OR 1.26 [95% CI 1.20–1.33], $P = 5.9 \times 10^{-21}$ [Supplementary Fig. 1]; β-GRS 1.26 [1.20–1.33], $P = 1.1 \times 10^{-19}$ [Supplementary Table 3]; and R-GRS, nominally associated with the development of type 2 diabetes, 1.18 [1.02–1.37], $P = 0.024$ [Supplementary Table 3]). We further determined that when all of the participants were stratified according to the β-GRS (high-risk genetic group [H]-β-GRS ≥12; intermediate risk [I], 12 > β-GRS ≥ 10; and low risk [L]-β-GRS <10) or the R-GRS (H-R-GRS ≥5; I, 5 > R-GRS ≥ 4; and L-R-GRS <4 (Supplementary Table 4), the risk of developing diabetes in the H-β-GRS and the H-R-GRS groups ($n = 108$) was 6.2-fold higher than in the L-β-GRS and the L-R-GRS groups ($n = 78$) (Supplementary Fig. 2). Interestingly, an effect of the R-GRS was only seen in the L-β-GRS group (OR 1.43 [95% CI 1.06–1.95], $P = 0.02$) and not in the H-β-GRS groups (1.17 [0.85–1.61], $P = 0.34$) (Supplementary Fig. 2), suggesting that the β-GRS has a predominant effect on conferring susceptibility to type 2 diabetes over the R-GRS. To statistically evaluate the interaction between β-GRS and R-GRS, we performed a stepwise logistic regression analysis using strategies of both forward selection (addition of each parameter) and backward selection (starting from all parameters). The results indicated that significant interaction was observed when we added β-GRS to R-GRS ($P < 0.001$), whereas the effect of addition of R-GRS to β-GRS was modest ($P = 0.03$).

We next examined the associations of each genetic variant with quantitative metabolic traits related to type 2 diabetes. None of the SNPs had a significant effect on the HOMA-β or HOMA-IR by themselves, but the β-GRS and R-GRS showed stronger association with the HOMA-β ($P = 0.025$) and HOMA-IR ($P = 0.0004$), respectively, than single SNP alone, in control individuals and patients with type 2 diabetes who were not treated with medications (Table 2). We further examined the association of the three types of GRS with type 2 diabetes and quantitative traits in a previously published independent cohort, which was conducted in Tokyo University. In this cohort, the association between T-GRS and type 2 diabetes (OR 1.18 [95% CI 1.13–1.24], $P = 2.08 \times 10^{-12}$) and β-GRS and HOMA-β (β of ln-HOMA-β = −0.0377, SE = 0.0103, $P = 0.0003$) was statistically significant, whereas the association of the R-GRS with HOMA-IR did not reach a statistically significant level (β of ln-HOMA-IR = 0.0294, SE = 0.0290, $P = 0.3120$) (Supplementary Table 5).

Investigation of combined effects of GRS on the clinical features of type 2 diabetes

We next examined the association of the T-GRS with clinical features, such as the maximum BMI, the age at the time of diagnosis, and the individuals presently receiving insulin therapy (Supplementary Table 6). Significant inverse correlations were observed between the T-GRS and the maximum BMI (β of maximum BMI −0.225 [95% CI −0.367 to −0.083], $P = 0.002$) and the age at diagnosis (β of age at diagnosis −0.663 [−1.048 to −0.278], $P = 0.0008$). We also found that the individuals receiving insulin therapy were positively associated with the T-GRS (β of insulin therapy 0.249 [0.025–0.473], $P = 0.029$).

We then divided all the participants into three approximately equally sized strata according to the T-GRS: L-T-GRS, I-T-GRS, and H-T-GRS genetic groups, as described in Supplementary Table 4. The characteristics of the three groups are shown in Table 3. In the H-T-GRS group, the duration of diabetes was significantly longer ($P < 0.01$) and the current BMI was lower ($P < 0.05$) than those in the L-T-GRS group (Table 3). We next studied the association of the T-GRS with clinical features such as the maximum BMI, the age at the time of diagnosis, and the percentage of individuals receiving insulin therapy (Table 3). We found that the maximum BMI in the H-T-GRS group (27.1 ± 4.2) was significantly lower than that in the L-T-GRS group (28.5 ± 4.6) ($P < 0.01$). In addition, the age at the time of the diagnosis of diabetes in the H-T-GRS group (49.8 ± 12.4 years) was significantly younger than that in the L-T-GRS group (52.5 ± 11.4 years) ($P < 0.001$) after adjustments for sex and the maximum BMI (Table 3). The percentage of individuals receiving insulin therapy in the H-T-GRS group (34.9%) was greater than that in the L-T-GRS group (22.7%) ($P < 0.05$) after adjustments for age, sex, current BMI, duration of diabetes, class of antihyperglycemic drugs, and present HbA_{1c} level.

We next examined the associations of the genetic risk score of β-cell function–related SNPs (β-GRS) with the clinical features (Table 4). We found that the β-GRS was associated with individuals receiving insulin therapy (β of insulin therapy 0.0131 [95% CI 0.0004–0.0259], $P = 0.0431$) and a younger age at diagnosis (β of age at diagnosis −0.608 [−1.008 to −0.208], $P = 0.0029$). Furthermore, we found a significant inverse correlation between the β-GRS and β-cell function–related parameters including the fasting serum C-peptide (F-CPR) (β of serum C-peptide −0.036 [−0.065 to −0.007], $P = 0.0140$) and the C-peptide index (CPI) (β of CPI −0.031 [−0.056 to −0.005], $P = 0.0179$) after adjustments for age, sex, BMI, duration of diabetes, class of antihyperglycemic drugs, fasting plasma glucose, the presence of diabetic nephropathy, and the presence of diabetic retinopathy. We also examined the association of T-GRS with these parameters, but as expected the β-GRS had stronger effects on basal insulin secretion than the T-GRS (Supplementary Table 6). The R-GRS was not associated with any parameters (Table 4).

We further tried, as much as possible, to include all information of European study–derived type 2 diabetes variants in the GRS. Overall, the 36 SNP GRS constructed with the 14 SNPs and additional 22 SNPs, however, did not show stronger association with each metabolic trait than the original T-, β-, and R-GRS in this study (Supplementary Tables 7 and 8).

CONCLUSIONS—In the current study, we examined 14 SNP loci, which were robustly shown to be susceptibility loci for type 2 diabetes in the Japanese population, and constructed a GRS to evaluate the usefulness of this genetic information in clinical practice. We found that most SNPs (13 of 14) showed a directionally consistent association with the results of previous reports (6–16), and constructed GRS (T-GRS) showed a much stronger association with type 2 diabetes than any of the single SNPs alone. The T-GRS was also associated with age at the time of the diagnosis of diabetes. Additionally, we found that a β-GRS, consisting of eleven β-cell function–related SNPs, was associated with requirement of insulin therapy and a reduced basal insulin secretion level in Japanese patients with type 2 diabetes.

Currently, >40 loci have been confirmed as susceptibility loci for type 2 diabetes in populations of European origin (5), but the integration of this information can only explain ~10% of type 2 diabetes

Table 2—*Association of the 14 SNPs with quantitative traits related to glucose metabolism in control subjects and diabetic subjects*

	Control[a]		Control and type 2 diabetic subjects without medication[b]	
	HOMA-IR	HOMA-β	HOMA-IR	HOMA-β
rs5219, *KCNJ11*				
Effect (SE)	0.039 (0.037)	−1.800 (2.039)	0.042 (0.039)	−2.531 (1.868)
P	0.297	0.378	0.281	0.178
rs7903146, *TCF7L2*				
Effect (SE)	−0.050 (0.095)	6.088 (5.229)	0.068 (0.100)	9.183 (4.794)
P	0.599	0.245	0.501	0.056
rs1111875, *HHEX*				
Effect (SE)	−0.037 (0.040)	−1.915 (2.192)	−0.022 (0.042)	−1.743 (2.010)
P	0.354	0.383	0.597	0.386
rs13266634, *SLC30A8*				
Effect (SE)	0.015 (0.035)	−1.283 (1.928)	−0.003 (0.038)	−1.464 (1.789)
P	0.673	0.506	0.937	0.413
rs7756992, *CDKAL1*				
Effect (SE)	−0.053 (0.036)	−1.629 (1.963)	−0.046 (0.038)	−0.937 (1.804)
P	0.137	0.407	0.219	0.604
rs10811661, *CDKN2B*				
Effect (SE)	0.036 (0.036)	−0.878 (2.001)	0.046 (0.039)	−1.443 (1.863)
P	0.328	0.661	0.24	0.439
rs4402960, *IGF2BP2*				
Effect (SE)	0.009 (0.040)	0.308 (2.170)	−0.013 (0.041)	−0.230 (1.991)
P	0.813	0.887	0.75	0.908
rs2237892, *KCNQ1*				
Effect (SE)	0.027 (0.035)	0.552 (1.947)	−0.008 (0.038)	−0.447 (1.833)
P	0.441	0.777	0.833	0.807
rs780094, *GCKR*				
Effect (SE)	0.030 (0.038)	−0.025 (2.091)	0.022 (0.040)	−0.275 (1.927)
P	0.43	0.99	0.585	0.887
rs7612463, *UBE2E2*				
Effect (SE)	−0.057 (0.052)	−4.372 (2.831)	−0.032 (0.055)	−4.768 (2.648)
P	0.271	0.123	0.568	0.072
rs7172432, *C2CD4A/B*				
Effect (SE)	−0.007 (0.037)	−3.208 (2.023)	0.003 (0.039)	−2.740 (1.880)
P	0.851	0.113	0.947	0.145
rs2943641, *IRS-1*				
Effect (SE)	0.153 (0.061)	3.989 (3.340)	0.128 (0.065)	2.993 (3.131)
P	0.012	0.233	0.051	0.339
rs1801282, *PPARG*				
Effect (SE)	0.097 (0.114)	5.243 (6.262)	0.110 (0.123)	5.903 (5.903)
P	0.394	0.403	0.372	0.318
rs8050136, *FTO*				
Effect (SE)	0.117 (0.048)	3.885 (2.632)	0.138 (0.050)	3.500 (2.402)
P	0.015	0.14	0.006	0.146
T-GRS				
Effect (SE)	0.017 (0.011)	−0.656 (0.630)	0.016 (0.012)	−0.868 (0.584)
P	0.15	0.298	0.179	0.138
β-GRS				
Effect (SE)	0.003 (0.012)	−1.213 (0.669)	0.002 (0.013)	−1.388 (0.618)
P	0.775	0.07	0.856	0.025[c]
R-GRS				
Effect (SE)	0.125 (0.035)	3.873 (1.917)	0.131 (0.037)	3.368 (1.763)
P	3.0×10^{-4}*	0.044	4.0×10^{-4}†	0.056

Results of linear regression analyses. The effect size corresponds to the β-coefficient (SE) per copy of the type 2 diabetes risk allele and was calculated using a linear regression analysis. [a]*n* = 763 (adjusted for sex, age, and BMI). [b]*n* = 860 (adjusted for age, sex, BMI, and disease status). [c]*P* = 0.03687 after 100,000 permutations, *P* = 0.03108 after Bonferroni correction. **P* = 0.00633 after 100,000 permutations, *P* = 0.00469 after Bonferroni correction. †*P* = 0.00568 after 100,000 permutations, *P* = 0.00425 after Bonferroni correction.

Table 3—*Clinical characteristics of the three groups according to the T-GRS of 14 SNPs in patients with type 2 diabetes*

GRS	Low	Intermediate	High	*P* (ANOVA)	*P* (multivariate)*
No. of risk alleles	≤13	14–15	≥16		
n	176	244	304		
Sex (male/female)	113/63	155/89	183/121	0.607	
Age (years)	64.95 ± 11.53	65.29 ± 10.23	64.46 ± 11.52	0.684	
BMI (kg/m^2)	25.25 ± 4.27	24.43 ± 3.34	24.21 ± 4.02	0.016	
Self-reported family history of diabetes (%)	51.46	60.67	54.03	0.137	
Duration of diabetes (years)	12.49 ± 8.65	12.72 ± 8.52	14.82 ± 14.82	0.006	
FPG (mmol/L)	7.48 ± 1.89	7.62 ± 1.73	7.66 ± 1.99	0.628	
A1C (%)	7.53 ± 1.26	7.48 ± 1.22	7.56 ± 1.26	0.783	
Diabetic nephropathy (%)	42.44	38.66	37.59	0.650	
Diabetic retinopathy (%)	43.14	40.61	43.11	0.824	
Insulin secretagogue (%)	50.57	50.00	52.96	0.764	
Age at diagnosis (years)	52.49 ± 11.42	52.65 ± 10.49	49.81 ± 12.43	0.008	<0.001
Maximum BMI (kg/m^2)	28.52 ± 4.63	27.05 ± 3.93	27.13 ± 4.23	0.001	0.002
Insulin requirement (%)	22.73	32.79	34.87	0.018	0.044

Data are means ± SD or *n*/*n* unless otherwise indicated. The value for A1C (%) was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated using the following formula: A1C (%) = A1C (Japan Diabetes Society [JDS]) (%) + 0.4%. **P* value for comparison of adjusted data. Age at diagnosis was adjusted for sex and maximum BMI. Maximum BMI was adjusted for sex. The ratio of insulin therapy was adjusted for age, sex, BMI, duration of diabetes, class of antihyperglycemic drug, and A1C level.

heritability; therefore, currently available genetic information for type 2 diabetes is likely insufficient for predicting disease progression and has failed to provide a significant impact on human health care in the general population to date.

Previously, several groups investigated the impact of a GRS for loci related to β-cell function and reported that the GRS was significantly associated with glucose-stimulated insulin secretion (GSIS) in nondiabetic subjects or the subjects with impaired glucose tolerance (4,26,37,38). However, the effects of the GRS on clinical features have not been evaluated in patients with type 2 diabetes. In the current study, we demonstrated for the first time that a β-GRS was associated with individuals receiving insulin therapy and possessing reduced basal insulin secretion, as evaluated using the F-CPR or CPI, among type 2 diabetic subjects. Thus, the β-GRS may be useful for predicting future reductions in basal insulin secretion, resulting in the need for insulin injections to control plasma glucose levels. Interestingly, the association of β-GRS with the reduction in basal insulin secretion long after the onset (e.g., >10 years) was independent of confounding factors, such as age, sex, BMI, duration of diabetes, presence of microvascular complications, and the use of insulin secretagogues. Of note, since the presence of microvascular complications reflects chronic hyperglycemia, the declining β-cell function in individuals with higher β-GRS may not be a consequence of the relatively longer terms of hyperglycemia. Thus, we think that the evaluation of β-GRS at an earlier

Table 4—*Association of β-GRS and R-GRS with quantitative metabolic traits and clinical information in diabetic subjects*

GRS and index	β†	SE	*P*	Covariables
β-GRS				
F-CPR	−0.036	0.014	0.0140	Age, sex, BMI, duration of diabetes, class of antihyperglycemic drug, FPG, the presence of diabetic nephropathy, and the presence of diabetic retinopathy
CPI	−0.031	0.0123	0.0179	Age, sex, BMI, duration of diabetes, class of antihyperglycemic drug, the presence of diabetic nephropathy, and the presence of diabetic retinopathy
Age at diagnosis	−0.608	0.204	0.0029	Sex and maximum BMI
Maximum BMI	−0.263	0.075	0.0004	Sex
Insulin requirement	0.013	0.006	0.0431	Age, sex, BMI, duration of diabetes, class of antihyperglycemic drug, and A1C
R-GRS				
F-CPR	0.018	0.042	0.676	Age, sex, BMI, duration of diabetes, class of antihyperglycemic drug, FPG, the presence of diabetic nephropathy, and the presence of diabetic retinopathy
CPI	0.015	0.037	0.696	Age, sex, BMI, duration of diabetes, class of antihyperglycemic drug and the presence of diabetic nephropathy, and the presence of diabetic retinopathy
Age at diagnosis	−0.891	0.599	0.137	Sex and maximum BMI
Maximum BMI	0.196	0.222	0.380	Sex
Insulin requirement	0.013	0.019	0.507	Age, sex, BMI, duration of diabetes, class of antihyperglycemic drug, and A1C

CPI was calculated using the following equation: CPI = [F-CPR/FPG] × 100. F-CPR and CPI were calculated in all diabetic subjects except for those with serum creatinine level >1.5 mg/dL. †Regression coefficient adjusted for covariables.

stage of the disease may be useful, and patients with a higher β-GRS should be strongly encouraged to receive specialized therapies, such as intensive lifestyle modifications and/or the earlier introduction of β-cell–preserving therapy, such as the use of glucagon-like peptide 1 receptor agonists or medications that ameliorate insulin resistance.

In the current study, we were able to replicate the previously reported associations of 8 of the 14 loci in a Japanese population (4 significantly [$P < 0.0036$] and 4 modestly [$P < 0.05$]) (25,27–32). As for rs7903146 in *TCF7L2*, rs1111875 in *HHEX*, rs1801282 in *PPARG*, rs8050136 in *FTO*, and rs7612463 in *UBE2E2*, which were reported to be associated with type 2 diabetes in previous Japanese reports (25,28,30), we were unable to detect any SNPs that were significantly associated with type 2 diabetes in the present Japanese sample ($P = 0.659$, 0.773, 0.997, 0.187, and 0.207, respectively). However, since the effect directions of most of the SNP loci (13 of 14) were consistent with the results of previous reports and the estimated study power was 15−81% for the 6 unreplicated SNPs (Supplementary Table 2), the lack of replication might be explained by the insufficient power of the current study. In the quantitative trait analyses using control individuals and type 2 diabetic patients with no medications, we did not observe any significant association between each of the single SNPs and glycemic traits, but the β-GRS and R-GRS showed stronger association with the HOMA-β ($P = 0.025$) and HOMA-IR ($P = 0.0004$), respectively, indicating that the constructed β-GRS and R-GRS in the current study were appropriate and useful for evaluating the genetic effects on susceptibility to the disease or on related quantitative traits, even among a relatively small study population. The association of the three types of GRS with the quantitative traits could also be consistently observed in an independent cohort, which was conducted in Tokyo University (28,30), further validating the usefulness of the GRS.

Since HOMA indices have some limitations as indicators of β-cell functions or peripheral insulin sensitivity, evaluation of other independent measures of insulin secretion or resistance, such as 2-h glucose and insulin measurements, is required to confirm our findings. We demonstrated that the β-GRS was associated with a reduced basal insulin secretion in diabetic subjects with an average disease duration of 13.6 years. We also observed that the β-GRS was inversely associated with the GSIS determined by disposition index (β of ln–disposition index −0.102 [95% CI −0.006 to −0.194], $P = 0.038$, after adjustments for age, sex, FPG, and BMI) at the onset of diabetes ($n = 134$ [unpublished results]); therefore, the β-GRS may be involved in the GSIS at the onset of diabetes, and a further reduction in basal insulin secretion in patients with a higher β-GRS long after the onset of diabetes (>10 years) may contribute to the need for insulin injections. A cohort study involving a larger number of subjects is needed to clarify this point.

In conclusion, we have shown that the β-GRS, as determined using eleven β-cell function–related loci, is associated with a lower basal insulin secretion and the percentage of individuals requiring insulin therapy among Japanese subjects with type 2 diabetes. These results suggest that the evaluation of β-GRS at an earlier stage of the disease may be useful, and patients with a higher β-GRS should receive specialized therapy, including guidance regarding intensive lifestyle modifications and β-cell–preserving therapy.

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M.I. wrote the manuscript and researched data. S.M. researched data, wrote the manuscript, and edited the manuscript. Y.K. researched data. A.Takan., H.K., S.M., and K.H. contributed to discussion. A.Takah. researched data. H.F. researched data. K.H. researched data and reviewed the manuscript. T.K. reviewed the manuscript. K.T. wrote the manuscript and reviewed and edited the manuscript. K.T. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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