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 × 10−5). 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 = −0.008, SE = 0.004, P = 0.0029), lasting 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 GENT2D, 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
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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 age 69.5 ± 9.1 years, and A1C 7.5 ± 1.6%) (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 (Tomami, Japan), Amenityh Tsukioka Hospital (Itoigawa, Japan), Aoi Hospital (Tonami, Toyama, Japan), Amenityh Tsukioka Hospital (Itoigawa, Japan), Hida City Hospital (Hida, 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 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.

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.

### Table 1—Clinical characteristics of the participants

|                           | Type 2 diabetic | Control | P        |
|---------------------------|-----------------|---------|----------|
| n                         | 724             | 763     | <0.0001* |
| 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 | 11.2 ± 7.4 | 0.71 |
| Age at diagnosis (years)  | 51.4 ± 11.6     | 53.4 ± 11.6 | 0.61 |
| Self-reported family history of diabetes (%) | 55.7 | 57.9 | 0.61 |
| BMI (kg/m²)               | 24.5 ± 3.9      | 22.7 ± 3.3 | <0.0001 |
| Maximum BMI (kg/m²)       | 27.4 ± 4.3      | 24.7 ± 3.1 | <0.0001 |
| Waist circumference (cm)  | Males           | 87.1 ± 9.5 | <0.05 |
|                           | Females         | 87.4 ± 11.7 | <0.0001 |
| FPG (mmol/L)              | 7.60 ± 1.88     | 5.33 ± 0.58 | <0.0001 |
| eGFR (ml/min)             | 74.8 ± 21.6     | 72.3 ± 16.3 | <0.05 |
| HOMA-β (%)                | 37.5 ± 40.2     | 60.8 ± 41.2 | <0.0001 |
| HOMA-IR (m²·μU/L²)        | 2.17 ± 1.60     | 1.24 ± 0.78 | <0.0001 |
| F-CPR (mg/mL)             | 1.65 ± 0.85     | 1.49 ± 0.61 | <0.0001 |
| F-CPI                      | 1.25 ± 0.71     | 1.56 ± 0.61 | <0.0001 |
| Complications (%)         | Diabetic nephropathy | 39.3 | 0.01 |
|                           | Diabetic retinopathy | 42.3 | 0.01 |
| Treatment of diabetes (%) | Diet alone      | 13.4 | 0.01 |
|                           | Using oral hypoglycemic agents | 73.9 | 0.01 |
|                           | Sulfonylureas   | 46.7 | 0.01 |
|                           | Thiazolidinediones | 20.3 | 0.01 |
|                           | Biguanides      | 28.5 | 0.01 |
|                           | α-Glucosidase inhibitor | 33.7 | 0.01 |
|                           | Glinide         | 4.9  | 0.01 |
|                           | Using insulin   | 31.4 | 0.01 |
| Presence of hypertension (%) | 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 (%) | 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 χ² test. #HOMA-β and -IR were calculated in all participants except for those treated with insulin therapy. §F-CPR and CPI were calculated in all participants except for those with serum creatinine level >1.5 mg/dL. Determination of hypertension was defined as systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg or having been treated for hypertension. 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.
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 (QiAmp 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: rs2237892 in CDKN1A, rs11111875 in CDKN2B, rs13266634 in SLC30A8, rs7756992 in HHEX, rs10811661 in CDKN2B, rs13266634 in SLC30A8, rs4402960 in IGFBP2, rs7903146 in TCF7L2, rs780094 in GCKR, rs7612463 in UBE2E2, rs7172432 in C2CD4A/B, rs2237892 in KCNQ1, and rs5219 in KCNJ11 (2)). The proportion of the number of SNPs studied was considered statistically significant (P < 0.05).

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 CDKN2B, rs10811661 near CDKN2B, rs13266634 in SLC30A8, and rs2237892 in KCNQ1) were found to be significantly associated with type 2 diabetes (Supplemental Table 3) (P = 1.7 × 10⁻⁵, 7.5 × 10⁻⁶, 2.8 × 10⁻⁷, and 4.1 × 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).

The power of the sample size for the current study to identify the association of previously reported SNP loci 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 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.

The proportion 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 and without adjustments for age, sex, and BMI. Variables with skewed distributions were logtransformed (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 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/).
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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 T-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 (\( \beta \) of ln-HOMA-β = –0.3077, \( SE = 0.0103, P = 0.0003 \)) was statistically significant, whereas the association of the R-GRS with HOMA-IR did not reach a statistically significant level (\( \beta \) 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 (\( \beta \) of maximum BMI 0.225 [95% CI 0.367 to –0.083], \( P = 0.002 \)) and the age at diagnosis (\( \beta \) 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 (\( \beta \) 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 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 HbA1c 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 (\( \beta \) of insulin therapy 0.0131 [95% CI 0.0004–0.0259], \( P = 0.0431 \)) and a younger age at diagnosis (\( \beta \) 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) (\( \beta \) of serum C-peptide –0.036 [–0.065 to –0.007], \( P = 0.0140 \)) and the C-peptide index (CPI) (\( \beta \) 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.
| SNP                  | HOMA-IR Control | HOMA-IR Control and type 2 diabetic subjects | HOMA-IR | HOMA-IR | HOMA-β | HOMA-β |
|---------------------|-----------------|---------------------------------------------|---------|---------|--------|--------|
| rs5219, KCNJ11      | 0.039 (0.037)   | -1.800 (2.039)                              | 0.042   | 0.039   | -2.531| 1.868  |
| Effect (SE)         | 0.297           | 0.378                                       | 0.281   | 0.178   |
| rs7903146, TCF7L2   | -0.050 (0.095)  | 6.088 (5.229)                               | 0.068   | 0.100   | 9.183 | 4.794  |
| Effect (SE)         | 0.599           | 0.245                                       | 0.501   | 0.056   |
| rs1111875, HHEX     | -0.037 (0.040)  | -1.915 (2.192)                              | -0.022  | 0.042   | -1.743| 2.010  |
| Effect (SE)         | 0.354           | 0.383                                       | 0.597   | 0.386   |
| rs13266634, SLC30A8 | 0.015 (0.035)   | -1.283 (1.928)                              | -0.003  | 0.038   | -1.464| 1.789  |
| Effect (SE)         | 0.673           | 0.506                                       | 0.937   | 0.413   |
| rs7750992, CDKAL1   | -0.053 (0.036)  | -1.629 (1.963)                              | -0.046  | 0.038   | -0.937| 1.804  |
| Effect (SE)         | 0.137           | 0.407                                       | 0.219   | 0.604   |
| rs10811661, CDKN2B  | 0.036 (0.036)   | -0.878 (2.001)                              | 0.046   | 0.039   | -1.443| 1.863  |
| Effect (SE)         | 0.328           | 0.661                                       | 0.24    | 0.439   |
| rs4402960, IGF2BP2  | 0.009 (0.040)   | 0.308 (2.170)                               | -0.013  | 0.041   | -0.230| 1.991  |
| Effect (SE)         | 0.813           | 0.887                                       | 0.75    | 0.908   |
| rs2237892, KCNQ1    | 0.027 (0.035)   | 0.552 (1.947)                               | -0.008  | 0.038   | -0.447| 1.833  |
| Effect (SE)         | 0.441           | 0.777                                       | 0.833   | 0.807   |
| rs780094, GCKR      | 0.030 (0.038)   | -0.025 (2.091)                              | 0.022   | 0.040   | -0.275| 1.927  |
| Effect (SE)         | 0.43            | 0.99                                        | 0.585   | 0.887   |
| rs7612463, UBE2E2   | -0.057 (0.052)  | -4.372 (2.831)                              | -0.032  | 0.055   | -4.768| 2.648  |
| Effect (SE)         | 0.271           | 0.123                                       | 0.568   | 0.072   |
| rs7172432, C2CD4A/B | -0.007 (0.037)  | -3.208 (2.023)                              | 0.003   | 0.039   | -2.740| 1.880  |
| Effect (SE)         | 0.851           | 0.113                                       | 0.947   | 0.145   |
| rs2943641, IRS-1    | 0.153 (0.061)   | 3.989 (3.340)                               | 0.128   | 0.065   | 2.993 | 3.131  |
| Effect (SE)         | 0.012           | 0.233                                       | 0.051   | 0.339   |
| rs1801282, PPARG    | 0.097 (0.114)   | 5.243 (6.262)                               | 0.110   | 0.123   | 5.903 | 5.903  |
| Effect (SE)         | 0.394           | 0.403                                       | 0.372   | 0.318   |
| rs8050136, FTO      | 0.117 (0.048)   | 3.885 (2.632)                               | 0.138   | 0.050   | 3.500 | 2.402  |
| Effect (SE)         | 0.015           | 0.14                                        | 0.006   | 0.146   |
| T-GRS               | 0.017 (0.011)   | -0.656 (0.630)                              | 0.016   | 0.012   | -0.868| 0.584  |
| Effect (SE)         | 0.15            | 0.298                                       | 0.179   | 0.138   |
| β-GRS               | 0.003 (0.012)   | -1.213 (0.669)                              | 0.002   | 0.013   | -1.388| 0.618  |
| Effect (SE)         | 0.775           | 0.07                                        | 0.856   | 0.025   |
| R-GRS               | 0.125 (0.035)   | 3.873 (1.917)                               | 0.131   | 0.037   | 3.368 | 1.763  |
| Effect (SE)         | 3.0 × 10^-4 *   | 0.044                                       | 0.4 × 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.
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Table 3—Clinical characteristics of the three groups according to the T-GRS of 14 SNPs in patients with type 2 diabetes

| GRS and index | Low | Intermediate | High | P (ANOVA) | P (multivariate)* |
|---------------|-----|--------------|------|-----------|------------------|
| 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 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 GRS 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 GRS 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. contributed to discussion. A.Takahara, A.Takahara, and H.F. researched data. K.H. researched data. K.T. 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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References
1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047–1053
2. O’Rahilly S, Barroso I, Wareham NJ. Genetic factors in type 2 diabetes: the end of the beginning? Science 2005;307:370–373
3. Chauhan G, Spurgeon CJ, Tabassum R, et al. Impact of common variants of PPARG, KCNJ11, TCF7L2, SLC30A8, HHEX, CDKN2A, IGF2BP2, and CDKAL1 on the risk of type 2 diabetes in 5,164 Indians. Diabetes 2010;59:2068–2074
4. Stancáková A, Kulasmaa T, Paananen J, et al. Association of 18 confirmed susceptibility loci for type 2 diabetes with indices of insulin release, proinsulin conversion, and insulin sensitivity in 5,327 nondiabetic Finnish men. Diabetes 2009;58:2129–2136
5. McCarthy MI. Genomics, type 2 diabetes, and obesity. N Engl J Med 2010;363:2339–2350
6. Sladek R, Rocheleau G, Rung J, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 2007;445:881–885
7. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, et al. A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. Nat Genet 2007;39:770–775
8. Saxena R, Voight BF, Lyssenko V, et al.; Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 2007;316:1331–1336
9. Zeggini E, Weedon MN, Lindgren CM, et al.; Wellcome Trust Case Control Consortium (WTCCC). Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 2007;316:1336–1341
10. Scott LJ, Mohlke KL, Bonnycastle LL, et al. A genome-wide association study of type 2 diabetes. Nat Genet 2007;39:776–781
11. Weedon MN, Palmer CN, Farrall M, et al.; Wellcome Trust Case Control Consortium (WTCCC). Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 2007;316:1336–1341
12. Hansen JB, Jensen MD, Vedel P, et al. Comprehensive meta-analysis of genome-wide association studies identifies two new loci for type 2 diabetes. Nat Genet 2007;39:776–781
13. Buetow KH, Stracke S, Boedtkjer J, et al. Genome-wide association analysis identifies novel risk loci for type 2 diabetes. Science 2007;316:1336–1341
14. Scholl C, Schormann T, Schmidt-Ott S, et al.; German Diabetes Genetics Initiative (DDG). A genome-wide association study for type 2 diabetes identifies another new risk locus. Diabetes 2008;57:1030–1037
15. Hattersley AS, Hattersley AT, Borel T, et al. A genome-wide association study identifies two new loci for type 2 diabetes. Science 2007;316:1336–1341
16. Groop L, Hattersley AS, Borel T, et al. A genome-wide association study identifies two new loci for type 2 diabetes. Science 2007;316:1336–1341
17. Scott LJ, Mohlke KL, Bonnycastle LL, et al. A genome-wide association study of type 2 diabetes. Nat Genet 2007;39:776–781
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diabetes in Finns detects multiple susceptibility variants. Science 2007;316:1341–1345
11. Frayling TM, Timpson NJ, Weedon MN, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007;316:889–894
12. Zeggini E, Scott LJ, Saxena R, et al.; Wellcome Trust Case Control Consortium. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 2008;40:638–645
13. Voight BF, Scott LJ, Steinthorsdottir V, et al.; MAGIC investigators; GIANT Consortium. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet 2010;42:579–589
14. Dupuis J, Langenberg C, Prokopenko I, et al.; DIABOLO Consortium; GIANT Consortium; Global BPgen Consortium; Anders Hamsten on behalf of Procardis Consortium; MAGIC investigators. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 2010;42:105–116
15. Unoki H, Takahashi A, Kawaguchi T, et al. SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. Nat Genet 2008;40:1098–1102
16. Yasuda K, Miyake K, Horikawa Y, et al. Variants in KCNQ1 are associated with susceptibility to type 2 diabetes mellitus. Nat Genet 2008;40:1092–1097
17. Altshuler D, Hirschhorn JN, Klammemark M, et al. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet 2000;26:76–80
18. Gloyd AL, Weedon MN, Owen KR, 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;52:568–572
19. Sandhu MS, Weedon MN, Fawcett KA, et al. Common variants in WFS1 confer risk of type 2 diabetes. Nat Genet 2007;39:951–953
20. Grant SF, Thorleifsson G, Reynisdottir I, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet 2006;38:320–323
21. Weedon MN, McCarthy MI, Hitman G, et al. Combining information from common type 2 diabetes risk polymorphisms improves disease prediction. PLoS Med 2006;3:e374
22. Cauchi S, Meyre D, Durand E, et al. Post genome-wide association studies of novel genes associated with type 2 diabetes show gene-gene interaction and high predictive value. PLoS ONE 2008;3:e2031
23. Lango H, Palmer CN, Morris AD, et al.; UK Type 2 Diabetes Genetics Consortium. Assessing the combined impact of 18 common genetic variants of modest effect sizes on type 2 diabetes risk. Diabetes 2008;57:3129–3135
24. Lyssenko V, Jonsson A, Almgren P, et al. Clinical risk factors, DNA variants, and the development of type 2 diabetes. N Engl J Med 2008;359:2220–2232
25. Miyake K, Yang W, Hara K, et al. Construction of a prediction model for type 2 diabetes mellitus in the Japanese population based on 11 genes with strong evidence of the association. J Hum Genet 2009;54:236–241
26. Hart LM, Simonis-Bik AM, Nijpels G, et al. Combined risk allele score of eight type 2 diabetes genes is associated with reduced first-phase glucose-stimulated insulin secretion during hyperglycemic clamps. Diabetes 2010;59:287–292
27. Takeuchi F, Serizawa M, Yamamoto K, et al. Confirmation of multiple risk Loci and genetic impacts by a genome-wide association study of type 2 diabetes in the Japanese population. Diabetes 2009;58:1690–1699
28. Yamauchi T, Hara K, Maeda S, et al. A genome-wide association study in the Japanese population identifies susceptibility loci for type 2 diabetes at UBE2E2 and C2CD4A-C2CD4B. Nat Genet 2010;42:864–868
29. Horikawa Y, Miyake K, Yasuda K, et al. Replication of genome-wide association studies of type 2 diabetes susceptibility in Japan. J Clin Endocrinol Metab 2008;93:3136–3141
30. Horikoshi M, Hara K, Ito C, et al. Variations in the HHEX gene are associated with increased risk of type 2 diabetes in the Japanese population. Diabetologia 2007;50:2461–2466
31. Omori S, Tanaka Y, Takahashi A, et al. Association of CDKAL1, IGF2BP2, CDKN2A/B, HHEX, SLC30A8, and KCNJ11 with susceptibility to type 2 diabetes in a Japanese population. Diabetes 2008;57:791–795
32. Tabara Y, Osawa H, Kawamoto R, et al. Replication study of candidate genes associated with type 2 diabetes based on genome-wide screening. Diabetes 2009;58:493–498
33. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2003;26(Suppl. 1):S5–S20
34. Maeda S, Tsukada S, Kanazawa A, et al. Genetic variations in the gene encoding TFAP2B are associated with type 2 diabetes mellitus. J Hum Genet 2005;50:283–292
35. Báez S, Tsuchiya Y, Calvo A, et al. Genetic variants involved in gallstone formation and capsaicin metabolism, and the risk of gallbladder cancer in Chilean women. World J Gastroenterol 2010;16:372–378
36. Nielsen DM, Ehm MG, Weir BS. Detecting marker-disease association by testing for Hardy-Weinberg disequilibrium at a marker locus. Am J Hum Genet 1998;63:1531–1540
37. Pascoe L, Frayling TM, Weedon MN, et al.; RISC Consortium. Beta cell glucose sensitivity is decreased by 39% in non-diabetic individuals carrying multiple diabetes-risk alleles compared with those with no risk alleles. Diabetologia 2008;51:1989–1992
38. Haupt A, Staiger H, Schafer SA, et al. The risk allele load accelerates the age-dependent decline in beta cell function. Diabetologia 2009;52:457–462