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Replication Study in a Japanese Population to Evaluate the Association between 10 SNP Loci, Identified in European Genome-Wide Association Studies, and Type 2 Diabetes

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Abstract

Aim

We performed a replication study in a Japanese population to evaluate the association between type 2 diabetes and 7 susceptibility loci originally identified by European genome-wide association study (GWAS) in 2012: *ZMIZ1*, *KLHDC5*, *TLE1*, *ANKRD55*, *CILP2*, *MC4R*, and *BCAR1*. We also examined the association of 3 additional loci: *CCND2* and *GIPR*, identified in sex-differentiated analyses, and *LAMA1*, which was shown to be associated with non-obese European type 2 diabetes.

Methods

We genotyped 6,972 Japanese participants (4,280 type 2 diabetes patients and 2,692 controls) for each of the 10 single nucleotide polymorphisms (SNPs): rs12571751 in *ZMIZ1*, rs10842994 near *KLHDC5*, rs2796441 near *TLE1*, rs459193 near *ANKRD55*, rs10401969 in *CILP2*, rs12970134 near *MC4R*, rs7202877 near *BCAR1*, rs11063069 near *CCND2*, rs8108269 near *GIPR*, and rs8090011 in *LAMA1* using a multiplex polymerase chain reaction invader assay. The association of each SNP locus with the disease was evaluated using a logistic regression analysis.

Results

All SNPs examined in this study had the same direction of effect (odds ratio > 1.0, p = 9.77 × 10^{-4}, binomial test), as in the original reports. Among them, rs12571751 in *ZMIZ1* was significantly associated with type 2 diabetes [p = 0.0041, odds ratio = 1.123, 95% confidence interval 1.037–1.215, adjusted for sex, age and body mass index (BMI)], but we did not observe significant association of the remaining 9 SNP loci with type 2 diabetes in the present Japanese population (p ≥ 0.005). A genetic risk score, constructed from the sum of risk alleles for the 7 SNP loci identified by un-stratified analyses in the European GWAS meta-analysis were associated with type 2 diabetes in the present Japanese population (p = 2.3 × 10^{-4}, adjusted for sex, age and BMI).

Conclusions

ZMIZ1 locus has a significant effect on conferring susceptibility to type 2 diabetes also in the Japanese population.

Introduction

Genetic susceptibility plays an important role in the development and/or progression of type 2 diabetes. Genome-wide association studies (GWAS) for type 2 diabetes have been extensively conducted worldwide, and over 80 susceptibility loci have been identified [1–19]. The association between susceptibility loci for type 2 diabetes identified in European GWAS and the disease have been evaluated in the Japanese population, and many European GWAS-derived loci were found to be associated with type 2 diabetes in Japanese populations, whereas others were not [8,11,18,20–29]

In 2012, a European GWAS meta-analysis identified 8 novel loci: rs12571751 in the zinc finger miz-domain containing 1 gene (*ZMIZ1*), rs10842994 near the kelch domain containing 5 gene (*KLHDC5*), rs2796441 near the transducin-like enhancer of split 1 gene (*TLE1*), rs459193 near the ankyrin repeat domain-containing protein 55 gene (*ANKRD55*), rs10401969 in the cartilage intermediate layer protein 2 gene (*CILP2*), rs12970134 near the melancortin 4 receptor gene (*MC4R*), rs7202877 near the breast cancer antiestrogen resistance 1 gene (*BCAR1*), rs516946 in the ankyrin 1 (*ANK1*) [16]. Subsequently, in a sex-differentiated analysis followed by a meta-analysis, rs11063069 near the cyclin D2 gene (*CCND2*) and rs8108269 near the gastric inhibitory polypeptide receptor gene (*GIPR*) were shown to be associated with type 2 diabetes with a genome-wide significance level, and the effect of *CCND2* locus was stronger in male, whereas the association of the *GIPR* locus was more significant in female [16]. Moreover, rs8090011 in the laminin alpha-1 gene (*LAMA1*) has been shown to be associated with non-obese European type 2 diabetes [15]. Among the 11 loci, an independent Japanese GWAS identified *ANK1* as a susceptibility locus for type 2 diabetes [17]. However, the remaining 10 SNP loci have not been well evaluated in the Japanese population, although the association of 4 SNP loci (rs12571751, rs2796441, rs4591937 and rs7202877) with type 2 diabetes has been suggested [18].

In this study, we examined the association of these 10 susceptibility loci, originally identified in European GWAS, with type 2 diabetes in a Japanese population.

Materials and Methods

Participants and DNA Preparation

We enrolled 4,280 type 2 diabetes patients who regularly visited the outpatient clinics of the Shiga University of Medical Science, Kawasaki Medical School, St. Marianna University, Juntendo University, and the University of Toyama or who were registered in BioBank Japan [11]. Diabetes mellitus was diagnosed according to the World Health Organization (WHO) criteria [30], and type 2 diabetes was clinically defined as a gradual adult-onset diabetes. Patients who tested positive for antibodies to glutamic acid decarboxylase or who were diagnosed with a monogenic form of the disease, such as mitochondrial disease or maturity-onset diabetes of the young, were excluded from the present study. We also recruited 2,692 controls who underwent annual health check-ups at Keio University, St. Marianna University, or Toyama University Hospital or from the general Japanese population registered in the Japanese single nucleotide polymorphism (SNP) database [11]. Peripheral blood samples for genomic DNA were extracted using the standard phenol-chloroform procedure. Obesity was defined as body mass index (BMI) $\geq$ 25 kg/m^2 according to the criteria of the Japan Society for the Study of Obesity [31].

Ethics Statements

All participants agreed to the protocol of this study and provided written informed consent. The study protocol was approved by the ethics committees of RIKEN Yokohama Institutes and each of the participating institutes: Shiga University of Medical Science, Kawasaki Medical School, St. Marianna University, Juntendo University, the University of Toyama, and Keio University.

SNP Genotyping

We selected 10 autosomal SNPs identified by European GWAS in 2012, including, rs12571751 in *ZMIZ1*, rs10842994 near *KLHDC5*, rs2796441 near *TLE1*, rs459193 near *ANKRD55*, rs10401969 in *CILP2*, rs12970134 near *MC4R*, rs7202877 near *BCAR1*, rs11063069 near *CCND2*, rs8108269 near *GIPR* [16], and rs8090011 in *LAMA1* [15].

Genotyping was performed using the multiplex-polymerase chain reaction (PCR) invader assay as reported previously [32]. The genotyping success rates for all of the SNPs were over 95.8% (S1 Table). The genotype concordance rates in the 64 duplicated samples were 100%. We did not observe any significant correlation among any SNP combinations in terms of calculated regression co-efficient (r square) in this analysis.

Statistical Analysis

We performed Hardy-Weinberg equilibrium (HWE) tests according to the method described by Nielsen et al. [33]. Differences in the genotype distribution of each SNP between cases and controls were evaluated by a logistic regression analysis with or without adjustment for age, sex, and BMI. The association of each SNP with quantitative traits, fasting plasma glucose (FPG), BMI, the homeostasis model assessment of beta-cell function (HOMA-β), and the HOMA of insulin resistance (HOMA-IR) [34,35] was evaluated by multiple linear regression analysis. Because the values of these traits in the present Japanese population showed a skewed distribution, we used log-transformed values for the analyses. Genotypes of each SNP were scored using an additive model (0, 1, and 2 for the homozygous of non-effect allele, heterozygous, and homozygous of effect allele, respectively). Statistical analyses were performed using StatView software (SAS Institute, Cary, NC, USA).

Significance was determined by Bonferroni's method for correcting multiple testing errors, and $p < 0.005$ (0.05 divided by 10) was considered statistically significant.

Results

Clinical characteristics of the participants are shown in Table 1. The genotype distributions of all SNPs were in accordance with the Hardy-Weinberg equilibrium proportions, except for rs12571751 in type 2 diabetes cases (Table 2).

Table 1. Clinical characteristic of participants.

	Sample size (case/control)	Type 2 diabetes	Controls	*p* value
n		4,280	2,692	
Sex (M:F)		2,645:1,635	1,526:1,166	< 0.0001[b]
Age (year)[a]	4,280/2,692	63.0 ± 11.4	50.6 ± 16.2	< 0.0001[b]
BMI (kg/m²)[a]	4,280/2,692	24.3 ± 4.1	22.8 ± 3.2	< 0.0001[b]
HbA1c (%)[a]	3,169/571	7.9 ± 2.7	5.3 ± 0.7	< 0.0001[c]
FPG (mmol/L)[a]	1,930/1,139	8.3 ± 2.9	5.3 ± 0.6	< 0.0001[c]
Duration of diabetes (year)[a]	2,891/-	13.7 ± 9.7	-	

[a] Data are mean ± standard deviation.

[b] Student's unpaired t-test

[c] Mann-Whitney U test

BMI: body mass index, HbA1c: Glycated hemoglobin, FPG: fasting plasma glucose

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Table 2. Genotype distributions of 10 SNPs in case and control groups.

SNP	Nearby gene[a]	Allele1/Allele2(Risk Allele)	Allele 11/12/22 (%)		P for HWE test	
			Type 2 diabetes	Controls	Type 2 diabetes	Controls
rs12571751	ZMIZ1	A/G (A)	1233/2098/783 (30.0/51.0/19.0)	725/1260/580 (28.3/49.1/22.6)	0.038	0.466
rs10842994	KLHDC5	C/T (C)	2878/1193/121 (68.7/28.5/2.88)	1770/795/93 (66.6/29.9/3.5)	0.844	0.749
rs2796441	TLE1	G/A (G)	620/2032/1532 (14.8/48.6/36.6)	355/1264/1021 (13.4/47.9/38.7)	0.201	0.244
rs459193	ANKRD55	G/A (G)	971/2108/1065 (23.4/50.9/25.7)	595/1282/762 (22.5/48.6/28.9)	0.249	0.208
rs10401969	CILP2	C/T (C)	43/753/3411 (1.0/17.9/81.1)	32/463/2168 (1.2/17.4/81.4)	0.840	0.198
rs12970134	MC4R	A/G (A)	117/1181/2876 (2.8/28.3/68.9)	66/723/1854 (2.5/27.4/70.1)	0.748	0.652
rs7202877	BCAR1	T/G (T)	2584/1349/193 (62.6/32.7/4.7)	1645/881/127 (62.0/33.2/4.8)	0.32	0.517
rs11063069	CCND2	A/G (G)	4038/216/3 (94.86/5.07/0.07)	2552/129/4 (95.0/4.8/0.2)	0.949	0.080
rs8108269	GIPR	G/T (G)	1723/1853/514 (42.1/45.3/12.6)	1057/1230/333 (40.4/46.9/12.7)	0.648	0.397
rs8090011	LAMA1	C/G (G)	341/1756/2108 (8.1/41.8/50.1)	213/1149/1308 (8.0/43.0/49.0)	0.354	0.073

[a] Information in the original report is shown.

HWE: Hardy-Weinberg equilibrium

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Table 3. Association of 7 SNP loci with type 2 diabetes in a Japanese population.

SNP	Nearby gene[a]	Risk Allele[b]	RAF (case/control)	Unadjusted		Adjusted[c]	
				p value	OR(95%CI)	p value	OR (95%CI)
rs12571751	ZMIZ1	A	0.55/0.53	2.7×10^{-3}	1.114(1.038–1.195)	4.1×10^{-3}	1.123 (1.037–1.215)
rs10842994	KLHDC5	C	0.83/0.82	0.046	1.096 (1.002–1.199)	0.028	1.120 (1.013–1.238)
rs2796441	TLE1	G	0.39/0.37	0.043	1.077 (1.002–1.157)	0.052	1.083 (0.999–1.173)
rs459193	ANKRD5	G	0.49/0.47	0.021	1.085 (1.012–1.163)	0.084	1.071 (0.991–1.157)
rs10401969	CILP2	C	0.100/0.099	0.884	1.009 (0.900–1.130)	0.719	1.024 (0.900–1.164)
rs12970134	MC4R	A	0.170/0.168	0.235	1.058 (0.964–1.161)	0.844	1.011 (0.911–1.121)
rs7202877	BCAR1	T	0.790/0.786	0.614	1.022 (0.940–1.111)	0.548	1.029 (0.937–1.130)
GRS-7[d,f]				3.0×10^{-5}	1.071 (1.037–1.106)	2.3×10^{-4}	1.070 (1.032–1.109)
GRS-6[e,f]				1.6×10^{-3}	1.059 (1.022–1.098)	7.9×10^{-3}	1.056 (1.014–1.099)

The results of logistic regression analysis are shown.

[a] Information in the original report is shown.

[b] Risk allele reported in the previous reports.

[c] Adjusted for age, sex and BMI.

[d] The genetic risk score (GRS-7) was calculated according to the number of risk alleles by counting the 7 European genome-wide association study derived SNPs.

[e] The genetic risk score (GRS-6) was calculated according to the number of risk alleles by counting the 6 European genome-wide association study derived SNPs, rs10842994, rs2796441, rs459193, rs10401969, rs12970134, rs7202877

[f] Individuals who had complete genotype data for the 7 SNPs were used for the analyses (n = 6,200)

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Of the 7 SNPs previously shown to be associated with type 2 diabetes in European GWAS un-stratified analyses; rs12571751 in *ZMIZ1*, rs10842994 near *KLHDC5*, rs2796441 near *TLE1*, rs459193 near *ANKRD55*, rs10401969 in *CILP2*, rs12970134 near *MC4R* and rs7202877 near *BCAR1* [16], all 7 SNPs had the same direction of effect (odds ratio > 1.0 adjusted for sex, age and BMI) as in the original reports (p = 7.81×10^{-3}, binomial test, Table 3, S2 Table). Among them, rs12571751 in *ZMIZ1* was significantly associated with type 2 diabetes even after Bonferroni's correction in this study (p = 4.1×10^{-3}, odds ratio [OR] = 1.123, 95% confidence interval [CI] 1.037–1.215, adjusted for sex, age and BMI; Table 3). Rs10842994 near *KLHDC5* was nominally associated with type 2 diabetes, but did not show a statistically significant association after Bonferroni's correction ($0.005 \leq p < 0.05$, adjusted for sex, age and BMI; Table 3). The remaining 5 SNPs were not associated with type 2 diabetes ($p \geq 0.05$, adjusted for sex, age and BMI; Table 3). When we constructed a genetic risk score (GRS) by summing the number of risk alleles for the 7 SNPs (GRS-7), the GRS-7 was significantly associated with type 2 diabetes in the present Japanese population (p = 2.3×10^{-4}, adjusted for sex, age and BMI; Table 3). We also constructed GRS-6 by excluding rs12571751 in *ZMIZ1* from the GRS-7, and observed that the GRS-6 was associated with type 2 diabetes in the Japanese population (p = 7.9×10^{-3}, adjusted for sex, age and BMI; Table 3).

The remaining 3 SNPs, rs11063069 near *CCND2* and rs8108269 near *GIPR*, which were identified in sex-differentiated European GWAS [16], and rs8090011 in *LAMA1*, which was shown to be associated with non-obese European type 2 diabetes [15], were not associated with type 2 diabetes in the un-stratified analyses in this study ($p \geq 0.05$, adjusted for sex, age and BMI; Tables 4 and 5).

In subsequent sex-differentiated analyses, rs8108269 near *GIPR* was nominally associated with type 2 diabetes in female, but the association was not significant after Bonferroni's correction (p = 0.038, adjusted for age and BMI; Table 4, S3 Table). Rs11063069 was not associated

Table 4. Sex differentiated analysis for the association of rs11063069 near *CCND2* and rs8108269 near *GIPR* with type 2 diabetes.

SNP	Nearby gene[a]	Risk Allele[b]	All[c]		Male[d] (n = 4,171)		Female[d] (n = 2,801)	
			P value	OR(95%CI)	P value	OR(95%CI)	P value	OR (95%CI)
rs11063069	CCND2	G	0.42	1.104(0.868–1.405)	0.962	1.008 (0.741–1.370)	0.291	1.236 (0.834–1.830)
rs8108269	GIPR	G	0.369	1.038(0.957–1.125)	0.650	0.976 (0.881–1.083)	0.038	1.152 (1.008–1.318)

[a] Information in the original report is shown.

[b] Risk allele reported in the previous reports

[c] Results of logistic regression analysis with adjustment for age, sex, and BMI are shown

[d] Results of logistic regression analysis with adjustment for age and BMI are shown

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Table 5. BMI stratified analysis for the association of rs8090011 in *LAMA1* with type 2 diabetes.

SNP	Nearby gene	Risk Allele[a]	All		BMI < 25 (case = 2,627 control = 2,692)		BMI ≥ 25 (case = 1,653 control = 2,692)	
			P value	OR(95%CI)	P value	OR(95%CI)	P value	OR(95%CI)
rs8090011	LAMA1	G	0.504	1.030 (0.945–1.122)	0.511	1.034 (0.936–1.141)	0.777	1.020 (0.888–1.173)

Results of logistic regression analysis with adjustment for age, sex and BMI are shown

[a] Risk allele reported in the previous reports

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with type 2 diabetes in the sex-differentiated analyses (p ≥ 0.05, adjusted for age and BMI; Table 4, S3 Table). In BMI-stratified analyses, we did not observe any association of rs8090011 in *LAMA1* with type 2 diabetes (p ≥ 0.05, adjusted for sex, age and BMI; Table 5, S3 Table). We also performed BMI-stratified analyses using cutoff value of 30 kg/m^2, but did not observe any association of rs8090011 in *LAMA1* with type 2 diabetes (S4 Table).

We further examined the association of the 10 SNPs with quantitative glycemic traits, HOMA-IR, HOMA-β and FPG using control participants (Tables 6, 7 and 8). Rs11063069-G near *CCND2* was significantly associated with a decrease of HOMA-β in male (p = 0.0048, adjusted for age and BMI; Table 7). Rs8090011-G in *LAMA1* was nominally associated with a decrease of HOMA-β, and with an increase of FPG in un-stratified analyses (0.005 ≤ p < 0.05 adjusted for sex, age and BMI; Table 8). The remaining 8 SNP loci and the GRS-7 were not associated with these glycemic traits in this study (p ≥ 0.05, Tables 6, 7 and 8). We also examined the association of the risk allele of 10 SNP loci with BMI, but none of 10 SNP loci was associated with BMI (p ≥ 0.05 adjusted for sex and age; S5 Table).

Discussion

In this study, we examined the association of 7 SNPs, rs12571751 in *ZMIZ1*, rs10842994 near *KLHDC5*, rs2796441 near *TLE1*, rs459193 near *ANKRD55*, rs10401969 in *CILP2*, rs12970134 near *MC4R*, and rs7202877 near *BCAR1*, with susceptibility to type 2 diabetes in a Japanese population, and found that rs12571751 in *ZMIZ1* was significantly associated with type 2 diabetes in the Japanese population. We also examined the effects of 3 additional SNP loci, rs11063069 near *CCND2* and rs8108269 near *GIPR*, which were identified in sex-differentiated analyses [16], and rs8090011 in *LAMA1*, which was shown to be associated with non-obese

Table 6. Association of 7 SNP loci with quantitative traits related to glucose metabolism in control individuals.

SNP	Nearby gene[a]	Risk Allele[b]	HOMA-IR[c] (n = 802)		HOMA-β[c] (n = 802)		FPG[c] (n = 1,144)	
			Effect (SE)	p value	Effect (SE)	p value	Effect (SE)	p value
rs12571751	ZMIZ1	A	-0.030 (0.024)	0.206	-0.029 (0.025)	0.249	0.002 (0.004)	0.734
rs10842994	KLHDC5	C	0.031 (0.030)	0.307	0.026 (0.032)	0.410	0.002 (0.006)	0.756
rs2796441	TLE1	G	-0.007 (0.024)	0.773	-0.030 (0.025)	0.241	0.005 (0.004)	0.279
rs459193	ANKRD5	G	-0.008 (0.024)	0.719	0.003 (0.024)	0.887	0.003 (0.004)	0.472
rs10401969	CILP2	C	0.026 (0.038)	0.501	-0.003 (0.040)	0.945	-0.003 (0.007)	0.715
rs12970134	MC4R	A	-0.029 (0.032)	0.362	-0.022 (0.034)	0.515	-0.005 (0.006)	0.356
rs7202877	BCAR1	T	-0.017 (0.029)	0.561	-0.036 (0.031)	0.237	4.82×10^{-4} (0.005)	0.928
GRS-7 [d]			-0.009 (0.011)	0.399	-0.014 (0.011)	0.193	0.001 (0.002)	0.599

The results of linear regression analysis with adjustment for age, sex and BMI are presented.

[a] Information in the original report is shown.

[b] The risk allele for type 2 diabetes reported in the previous reports

[c] Values are log-transformed for the analyses

[d] The genetic risk score (GRS-7) was calculated according to the number of risk allele of 7 SNPs, and the individuals who had complete genotype data for the 7 SNPs were used for the analysis (n = 716 for HOMA-IR and HOMA-β, n = 1,008 for FPG) adjusted for age, sex and BMI.

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type 2 diabetes in European GWAS [15], but we did not replicate the original association in the Japanese population (Tables 4 and 5, S3 Table).

Genome-wide association studies for type 2 diabetes have been conducted extensively in different ethnic groups, and have successfully identified over 80 susceptibility loci thus far [1–19]. Among them, over 50 loci were identified by GWAS in European populations [1–6,9,10,15, 16], and many of the European GWAS-derived loci have been shown to be associated with type 2 diabetes in other ethnic groups, whereas others did not have significant effects in populations of non-European origin [8,11,18,20–29]. In some cases, the sample size was not

Table 7. Association of rs11063069 near *CCND2* and rs8108269 near *GIPR* with quantitative traits related to glucose metabolism in male or in female.

SNP	Nearby gene [a]	Risk allele [b]	Sex	HOMA-IR [c]		HOMA-β [c]		FPG [c]	
				Effect (SE)	p value	Effect (SE)	P value	Effect (SE)	p value
rs11063069	CCND2	G	All [d]	-0.082 (0.073)	0.265	-0.163(0.077)	0.034	-0.001 (0.014)	0.945
			Male [e]	-0.133 (0.086)	0.121	-0.256 (0.090)	4.8×10^{-3}	0.020 (0.017)	0.26
			Female [e]	0.037 (0.135)	0.782	0.055 (0.140)	0.694	-0.038 (0.024)	0.11
rs8108269	GIPR	G	All [d]	0.018 (0.024)	0.472	0.023 (0.026)	0.374	0.001 (0.004)	0.805
			Male [e]	0.024 (0.032)	0.457	0.036 (0.034)	0.281	2.6×10^{-4} (0.006)	0.965
			Female [e]	0.010 (0.037)	0.779	0.008 (0.039)	0.839	0.002 (0.007)	0.732

[a] Information in the original report is shown.

[b] Risk allele reported in the previous reports

[c] values are log-transformed for the analyses

[d] Results of logistic regression analysis with adjustment for age, sex, and BMI are shown.

[e] Results of logistic regression analysis with adjustment for age and BMI are shown.

All; n = 802 for HOMA-IR and HOMA-β, n = 1144 for FPG

Male; n = 480 for HOMA-IR and HOMA-β, n = 651 for FPG

Female; n = 322 for HOMA-IR and HOMA-β, n = 493 for FPG

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Table 8. Association of rs8090011 in *LAMA1* with quantitative traits related to glucose metabolism in obese controls (BMI ≥ 25) or in non-obese controls (BMI < 25).

SNP	Nearby gene	Risk allele[a]	BMI	HOMA-IR[b]		HOMA-β[b]		FPG[b]	
				Effect (SE)	*p* value	Effect (SE)	*p* value	Effect (SE)	*p* value
rs8090011	*LAMA1*	G	All	-0.029 (0.027)	0.284	-0.058 (0.028)	0.04	0.010 (0.005)	0.049
			BMI < 25	-0.003 (0.037)	0.945	-0.030 (0.039)	0.449	0.011 (0.008)	0.159
			BMI ≥ 25	-0.066 (0.039)	0.09	-0.076 (0.039)	0.054	0.006 (0.006)	0.286

Results of linear regression analysis with adjustment for age, sex and BMI are presented.

[a] Risk allele reported in the previous reports

[b] values are log-transformed for the analysis

BMI < 25; n = 428 for HOMA-IR and HOMA-β, n = 482 for FPG

BMI ≥ 25; n = 374 for HOMA-IR and HOMA-β, n = 662 for FPG

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sufficient, but there may be significant interethnic differences in the effect sizes at several loci; therefore, it is important to evaluate the effect of each locus in different ethnic groups [18,27].

Among 7 SNPs identified in un-stratified European GWAS meta-analyses, rs12571751 in *ZMIZ1* was significantly associated with type 2 diabetes in the present Japanese population, indicating that this locus was common susceptibility locus for type 2 diabetes across different ethnic groups. The effect size for rs12571751 in this study (OR = 1.12) was comparable with those for the previously reported type 2 diabetes loci in Japanese populations [36], but not as large as that of *TCF7L2* (OR = 1.51) or *KCNQ1* (OR = 1.42) (S6 Table). Rs12571751 is located at intron 5 of *ZMIZ1*. *ZMIZ1* has been shown to express ubiquitously in human tissues, including pancreas [37]. *ZMIZ1* encodes a member of the PIAS (protein inhibitor of activated STAT) family proteins, which interact with p53 [38], and a recent report using β-cell specific p53 deleted mice showed that p53 depletion could prevent glucotoxic β-cell death in these mice [39]; therefore, *ZMIZ1* might be involved in β-cell death through p53 activation and contribute to conferring susceptibility to type 2 diabetes.

The directions of the effects for the remaining 6 SNPs were consistent with those in the original reports [16] (p = 1.56×10^{-2}, binomial test; Table 3), and the GRS-6 constructed from the 6 SNPs was associated with type 2 diabetes (p = 7.9×10^{-3}, adjusted for sex, age and BMI; Table 3).

Since the association of GRS-7, which included the information of these 6 SNPs in addition to that of rs12571751 in *ZMIZ1* locus, with the disease was stronger than that of rs12571751 alone, it is suggested that these 6 loci have some effects on conferring susceptibility to type 2 diabetes also in the Japanese population.

With regard to rs8108269 near *GIPR* and rs11063069 near *CCND2*, the association of the *GIPR* locus with type 2 diabetes was more significant in female in the original European study, whereas the effect of *CCND2* locus was stronger in male [16]. Rs8108269 near *GIPR* was nominally associated with type 2 diabetes in female, suggesting that *GIPR* locus might be involved in the developement of type 2 diabetes in female in the Japanese population similar as that observed in the European population. Because the risk allele frequency of rs11063069 near *CCND2* was lower in the Japanese population (RAF = 1.0%) than in European populations (RAF = 20.7%), and estimated power for the present study to replicate the original finding in European GWAS was insufficient for this SNP (13% for rs11063069; S7 Table). Furthermore, the risk allele of rs11063069 was significantly associated with a decrease of HOMA-β in male (p = 0.0048 adjusted for age and BMI; Table 7); therfore, the risk allele of *CCND2* might have some effects on the decrease of insulin secretion, and on conferring susceptibility to type 2 diabetes in male, as reported in the original European GWAS.

The *LAMA1* locus has been shown to be associated with lean type 2 diabetes, but not with obese type 2 diabetes, in the European GWAS [15]. We did not replicate the original associations (p ≥ 0.05 adjusted for sex, age and BMI; Table 5, S3 Table). Because the present study had sufficient power to replicate the original findings for the *LAMA1* locus (90%; S7 Table), we considered that the effect of rs8090011-G in *LAMA1* locus was not major in the Japanese population. These 3 SNP loci need to be evaluated further in larger Japanese cohorts.

Results found in the case-control sample were not always consistent with the findings from quantitative trait analyses in this study, a sample size of quantitative traits analyses in this study was not sufficiently large enough, but it has been shown that genetic loci for glycemic traits found in non-diabetic controls were not always linked to type 2 diabetes risk [9, 40]; therefore, the biological significance of each locus need to be evaluated by alternative approaches.

In the present study, we did not observe any association with type 2 diabetes except for *ZMIZ1* and *KLHDC5* loci in a Japanese population (p ≥ 0.05 adjusted for sex, age and BMI; Table 3). Because the genotyping success rates for all of the SNPs were over 95.8%, and the genotype concordance rates were 100% (see Materials and Methods, S1 Table), the discrepancy was not considered to result from technical issues.

We also evaluated heterogeneity in terms of genotype distributions for each SNP among individual collections bacause we recruited case and control individuals from 6 and 4 distinct institutions in this study, respectively. The results indicated that there is no heterogeneity in genotype distributions among each case or control collection (S8 Table).

In this study, the control individuals were significantly younger than the type 2 diabetes patients (Table 1); therefore our control group may have included individuals who will develop the disease later, which may increase the possibility of a type 2 error, although we included age as a co-variable in the logistic regression model. Therefore, we evaluated the association of the 10 SNPs with type 2 diabetes using older control individuals (age ≥ 40 years, ≥ 50 years, or ≥ 60 years). The effect sizes of most SNP loci or the GRS-7 examined in this study increased when we used older control individuals for the association study (S9 Table). Because the effect directions of most SNPs were consistent with our original findings, we consider that our conclusions were not skewed by the inclusion of these younger control individuals, although inclusion of patients who will develop the disease later may have reduced the statistical power of our study. Because the estimated power in the present study for the non-replicated SNPs did not reach 80%, except for rs8090011 in *LAMA1* (S7 Table), insufficient sample size may be a principal explanation for the discrepancies between the present study and the original European studies.

In summary, we examined the association of 10 SNPs, previously identified in European GWAS, with type 2 diabetes in an independent Japanese population. Our results suggested that rs12571751 in *ZMIZ1* has a significant effect on conferring susceptibility to type 2 diabetes also in the Japanese. The remaining 9 SNP loci did not show significant effects in the Japanese population by themselves, although the effects of these SNP loci need to be evaluated in sufficiently powered, larger Japanese populations.

Supporting Information

S1 Table. Information of genotyping success rates for individual 10 SNPs. [a] Information in the original report is shown. [b] Risk allele reported in the previous reports.
(DOCX)

S2 Table. Association of 7 SNP loci with type 2 diabetes in a Japanese population and original reports. The results of logistic regression analysis are shown. [a] Information in the original report is shown. [b] Risk allele reported in the previous reports. [c] Adjusted for age, sex and BMI. [d] Information in the original European GWAS (Morris AP et al. *Nat Genet* 44: 981–990, 2012)

is shown. [e] The genetic risk score (GRS-7) was calculated according to the number of risk alleles by counting the 7 European genome-wide association study derived SNPs. [f] The genetic risk score (GRS-6) was calculated according to the number of risk alleles by counting the 6 European genome-wide association study derived SNPs, rs10842994, rs2796441, rs459193, rs10401969, rs12970134, rs7202877. [g] Individuals who had complete genotype data for the 7 SNPs were used for the analyses (n = 6,200).
(DOCX)

S3 Table. Association of 3 SNP loci with type 2 diabetes in a Japanese population and original reports. Results of logistic regression analysis with adjustment for age and BMI (rs11063069 and rs8108269) or age, sex and BMI (rs8090011) are shown. [a] Information in the original European GWAS (Morris AP et al. *Nat Genet* 44: 981–990, 2012, Perry JR et al *PLoS Genet* 8:e1002741, 2012). [b] Information in the present Japanese analyses. [c] Risk allele frequency in un-stratified group. [d] Association data in each stratified group.
(DOCX)

S4 Table. BMI stratified analysis for the association of *LAMA1* with type 2 diabetes. Results of logistic regression analysis with adjustment for age, sex and BMI are shown. [a] Risk allele reported in the previous reports. [b] Results of logistic regression analysis with adjustment for age, sex and BMI are shown.
(DOCX)

S5 Table. Association of 10 SNP loci with BMI in control individuals. The results of linear regression analysis with adjustment for age and sex are presented. [a] Information in the original report is shown. [b] The risk allele for type 2 diabetes reported in the previous reports. [c] Values are log-transformed for the analyses.
(DOCX)

S6 Table. Effect sizes for the 10 SNPs in this study and other known type 2 diabetes-related SNPs in Japanese populations. The 10 SNPs examined in the present study are shown in bold. *Effect sizes for known type 2 diabetes-related SNPs, which were previously shown to be associated with type 2 diabetes ($p < 0.05$) in Japanese populations. Data from a previous report (Imamura M et al. *J Clin Endocrinol Metab.* 2013 98(10)) is shown.
(DOCX)

S7 Table. Power estimation for each SNP locus to replicate the results of original European study in the present study. Power estimation was performed using CaTS power calculator, CaTS: http://www.sph.umich.edu/csg/abecasis/CaTS/). The prevalence of type 2 diabetes is assumed to be 10%, $\alpha = 0.05$. [a] Information in the original report is shown. [b] Risk allele for type 2 diabetes reported in the previous report. [c] Risk allele frequency in the Japanese population (controls) in the present study.
(DOCX)

S8 Table. Comparison of risk allele frequencies among individual areas for sample collection. [a] collection 1: BioBank Japan; collection 2: Juntendo University; collection 3: Kawasaki Medical School; collection 4: case Shiga University of Medical Science, control Keio University; collection 5: St. Marianna University; collection 6: Toyama University; collection 7: Japan SNP data base. [b] Chi square test.
(DOCX)

S9 Table. Association study of 10 SNPs with type 2 diabetes using older control (age $\geq$ 40, n = 1,928, age $\geq$ 50, n = 1,489, age $\geq$ 60 n = 845). Results of logistic regression analysis using

all type 2 diabetes participants (n = 4,280) are shown. [a] Information in the original report is shown. [b] Risk allele reported in the previous reports. [c] Adjusted for age, sex and BMI. [d] GRS-7 was calculated according to the number of risk allele of the 7 SNPs, and the individuals having complete genotype data for the 7 SNPs were used for the analysis (age $\geq$ 40, n = 5,498, age $\geq$ 50, n = 5,118, age $\geq$ 60 n = 4,545) adjusted for age, sex and BMI.
(DOCX)

Acknowledgments

Author Contributions

Conceived and designed the experiments: M. Imamura SM. Performed the experiments: RM KS M. Imamura SM. Analyzed the data: RM KS M. Imamura SM. Contributed reagents/materials/analysis tools: RM KS YT M. Iwata HH KK HM HW KT AK RK. Wrote the paper: RM M. Imamura SM. Interpretation of data for the work: YT M. Iwata HH KK HM HW KT AK RK.

References

1. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, et al. (2007) A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 445: 881–885. PMID: 17293876

2. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, et al. (2007) Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 316: 1331–1336. PMID: 17463246

3. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al (2007) A genomewide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 316: 1341–1345. PMID: 17463248

4. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, Benediktsson R, Jonsdottir T, Walters GB, et al. (2007) A variant in *CDKAL1* influences insulin response and risk of type 2 diabetes. Nat Genet 39: 770–775. PMID: 17460697

5. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, et al. (2007) Replication of genome-wide association signal in U.K. samples reveals risk loci for type 2 diabetes. Science 316: 1336–1341. PMID: 17463249

6. Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, Hu T, et al. (2008) Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 40: 638–645. doi: 10.1038/ng.120 PMID: 18372903

7. Yasuda K, Miyake K, Horikawa Y, Hara K, Osawa H, Furuta H, et al. (2008) Variants in *KCNQ1* are associated with susceptibility to type 2 diabetes mellitus. Nat Genet 40: 1092–1097. doi: 10.1038/ng.207 PMID: 18711367

8. Unoki H, Takahashi A, Kawaguchi T, Hara K, Horikoshi M, Andersen G, et al. (2008) SNPs in *KCNQ1* are associated with susceptibility to type 2 diabetes in East Asian and European populations. Nat Genet 40: 1098–1102. doi: 10.1038/ng.208 PMID: 18711366

9. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, et al. (2010) New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 42: 105–116. doi: 10.1038/ng.520 PMID: 20081858

10. Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, et al. (2010) Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet 42: 579–589. doi: 10.1038/ng.609 PMID: 20581827

11. Yamauchi T, Hara K, Maeda S, Yasuda K, Takahashi A, Horikoshi M, et al. (2010) A genome-wide association study in the Japanese population identifies susceptibility loci for type 2 diabetes at *UBE2E2* and *C2CD4A-C2CD4B*. Nat Genet 42: 864–868. doi: 10.1038/ng.660 PMID: 20818381

12. Shu XO, Long J, Cai Q, Qi L, Xiang YB, Cho YS, et al. (2010) Identification of new genetic risk variants for type 2 diabetes. PLOS Genet 6: e1001127. doi: 10.1371/journal.pgen.1001127 PMID: 20862305

13. Kooner JS, Saleheen D, Sim X, Sehmi J, Zhang W, Frossard P, et al. (2011) Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci. Nat Genet 43: 984–989. doi: 10.1038/ng.921 PMID: 21874001

14. Cho YS, Chen CH, Hu C, Long J, Ong RT, Sim X, et al. (2011) Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. Nat Genet 44: 67–72. doi: 10.1038/ng.1019 PMID: 22158537

15. Perry JR, Voight BF, Yengo L, Amin N, Dupuis J, Ganser M, et al. (2012) Stratifying type 2 diabetes cases by BMI identifies genetic risk variants in LAMA1 and enrichment for risk variants in lean compared to obese cases. PLoS Genet 8(5):e1002741. doi: 10.1371/journal.pgen.1002741 PMID: 22693455

16. Morris AP, Voight BF, Teslovich TM, Ferreira T, Segrè AV, Steinthorsdottir V, et al. (2012) Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet 44: 981–990. doi: 10.1038/ng.2383 PMID: 22885922

17. Imamura M, Maeda S, Yamauchi T, Hara K, Yasuda K, Morizono T, et al. (2012) A singlenucleotide polymorphism in ANK1 is associated with susceptibility to type 2 diabetes in Japanese populations. Hum Mol Genet 10: 1093–1113.

18. Hara K, Fujita H, Johnson TA, Yamauchi T, Yasuda K, Horikoshi M, et al. (2014) Genome-wide association study identifies three novel loci for type 2 diabetes. Human Molecular Genetics 23: 239–246. doi: 10.1093/hmg/ddt399 PMID: 23945395

19. Mahajan A, Go MJ, Zhang W, Below JE, Gaulton KJ, Ferreira T, et al. (2014). Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. Nat Genet 46(3):234–244. doi: 10.1038/ng.2897 PMID: 24509480

20. Horikoshi M, Hara K, Ito C, Nagai R, Froguel P, Kadowaki T. (2007) A genetic variation of the transcription factor 7-like 2 gene is associated with the risk of type 2 diabetes in the Japanese population. Diabetologia 50: 747–751. PMID: 17245589

21. Hayashi T, Iwamoto Y, Kaku K, Hirose H, Maeda S. (2007) Replication study for the association of TCF7L2 with susceptibility to type 2 diabetes in a Japanese population. Diabetologia 50: 980–984. PMID: 17340123

22. Omori S, Tanaka Y, Takahashi A, Hirose H, Kashiwagi A, Kaku K, et al. (2008) Association of CDKAL1, IGF2BP2, CDKN2A/B, HHEX, SLC30A8, and KCNJ11 with susceptibility to type 2 diabetes in a Japanese population. Diabetes 57: 791–795. PMID: 18162508

23. Omori S, Tanaka Y, Horikoshi M, Takahashi A, Hara K, Hirose H, et al. (2009) Replication study for the association of new meta-analysis-derived risk loci with susceptibility to type 2 diabetes in 6,244 Japanese individuals. Diabetologia 52:1554–60 doi: 10.1007/s00125-009-1397-5 PMID: 19455301

24. Ohshige T, Iwata M, Omori S, Tanaka Y, Hirose H, Kaku K, et al. (2011) Association of New Loci Identified in European Genome-wide Association Studies with Susceptibility to Type 2 Diabetes in the Japanese. PLoS One 6: e26911. doi: 10.1371/journal.pone.0026911 PMID: 22046406

25. Imamura M, Iwata M, Maegawa H, Watada H, Hirose H, Tanaka Y, et al. (2011) Genetic variants at CDC123/CAMK1D and SPRY2 are associated with susceptibility to type 2 diabetes in Japanese population. Diabetologia 54: 3071–3077. doi: 10.1007/s00125-011-2293-3 PMID: 21909839

26. Fukuda H, Imamura M, Tanaka Y, Iwata M, Hirose H, Kaku K, et al. (2012) A single nucleotide polymorphism within DUSP9 is associated with susceptibility to type 2 diabetes in a Japanese population. PLOS ONE 7: e46263. doi: 10.1371/journal.pone.0046263 PMID: 23029454

27. Imamura M, Maeda S. (2011) Genetics of type 2 diabetes: the GWAS era and future perspectives. Endocr J 58: 723–739. PMID: 21778616

28. Iwata M, Maeda S, Kamura Y, Takano A, Kato H, Murakami S, et al. (2012) 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. Diabetes Care 35:1763–1770. doi: 10.2337/dc11-2006 PMID: 22688542

29. Sakai K, Imamura M, Tanaka Y, Iwata M, Hirose H, Kaku K, et al. (2013) Replication study for the association of 9 East Asian GWAS-derived loci with susceptibility to type 2 diabetes in a Japanese population. PLOS ONE 8: e76317. doi: 10.1371/journal.pone.0076317 PMID: 24086726

30. Alberti KG, Zimmet PZ. (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 15: 539–553. PMID: 9686693

31. Examination Committee of Criteria for 'Obesity Disease' in Japan; Japan Society for the Study of Obesity (2002) New criteria for 'obesity disease' in Japan. Circ J 66: 987–992. PMID: 12419927

32. Maeda S, Tsukada S, Kanazawa A, Sekine A, Tsunoda T, Koya D, et al. (2005) Genetic variations in the gene encoding *TFAP2B* are associated with type 2 diabetes mellitus. J Hum Genet 50: 283–292. PMID: 15940393

33. Nielsen DM, Ehm MG, Weir BS. (1998) Detecting marker-disease association by testing for Hardy-Weinberg disequilibrium at a marker locus. Am J Hum Genet 63: 1531–1540. PMID: 9867708

34. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412–419. PMID: 3899825

35. Wallace TM, Levy JC, Matthews DR. (2004) Use and abuse of HOMA modeling. Diabetes Care 27: 1487–1495. PMID: 15161807

36. Imamura M, Shigemizu D, Tsunoda T, Iwata M, Maegawa H, Watada H, et al. (2013) Assessing the clinical utility of a genetic risk score constructed using 49 susceptibility alleles for type 2 diabetes in a Japanese population. J Clin Endocrinol Metab. 98:E1667–1673. doi: 10.1210/jc.2013-1642 PMID: 23956346

37. Nagase T, Ishikawa K, Kikuno R, Hirosawa M, Nomura N, Ohara O, et al. (1999) Prediction of the coding sequences of unidentified human genes. XV. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro. DNA Research 6:337–345. PMID: 10574462

38. Lee J, Beliakoff J, Sun Z (2007) The novel PIAS-like protein hZimp10 is a transcriptional co-activator of the p53 tumor suppressor. Nucleic Acids Res 35: 4523–4534. PMID: 17584785

39. Tornovsky-Babeay S, Dadon D, Ziv O, Tzipilevich E, Kadosh T, Schyr-Ben Haroush R, et al. (2014) Type 2 Diabetes and Congenital Hyperinsulinism Cause DNA Double-Strand Breaks and p53 Activity in β Cells. Cell Metabolism 19:109–121. doi: 10.1016/j.cmet.2013.11.007 PMID: 24332968

40. Scott RA, Lagou V, Welch RP, Wheeler E, Montasser ME, Luan J, et al. (2012) Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. Nat Genet. 44:991–1005 doi: 10.1038/ng.2385 PMID: 22885924