Replication Study in a Japanese Population of Six Susceptibility Loci for Type 2 Diabetes Originally Identified by a Transethnic Meta-Analysis of Genome-Wide Association Studies

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Abstract

Aim
We performed a replication study in a Japanese population to evaluate the association between type 2 diabetes and six susceptibility loci (TMEM154, SSR1, FAF1, POU5F1, ARL15, and MPHOSPH9) originally identified by a transethnic meta-analysis of genome-wide association studies (GWAS) in 2014.

Methods
We genotyped 7,620 Japanese participants (5,817 type 2 diabetes patients and 1,803 controls) for each of the single nucleotide polymorphisms (SNPs) using a multiplex polymerase chain reaction invader assay. The association of each SNP locus with the disease was evaluated using logistic regression analysis.

Results
Of the six SNPs examined in this study, four (rs6813195 near TMEM154, rs17106184 in FAF1, rs3130501 in POU5F1, and rs4275659 near MPHOSPH9) had the same direction of effect as in the original reports, but two (rs9505118 in SSR1 and rs702634 in ARL15) had the opposite direction of effect. Among these loci, rs3130501 and rs4275659 were nominally associated with type 2 diabetes (rs3130501; p = 0.017, odds ratio [OR] = 1.113, 95%
Competing Interests: The authors have read the journal’s policy and the authors of this manuscript have the following competing interests. HW is a member of advisory panel of Novo Nordisk Pharma, Sanofi, Dainippon Sumitomo Pharma, Mochida Pharmaceutical Co, MSD, Takeda Pharmaceutical Co, Boehringer Ingelheim, Ono Pharmaceutical Co., Novartis Pharmaceuticals, Mitsubishi-Tanabe Pharma, AstraZeneca, Kowa Co. Astellas Pharma Inc., Pfizer Inc., has received lecture fees from Novo Nordisk Pharma, Eli Lilly Japan, Sanofi, Dainippon Sumitomo Pharma, Fujifilm, Bayer, Kissei Pharmaceutical Co., Mochida Pharmaceutical Co, MSD, Takeda Pharmaceutical Co, Boehringer Ingelheim, Daiichi Sankyo, Inc, Ono Pharmaceutical Co., Novartis Pharmaceuticals, Boehringer Ingelheim, Mitsubishi-Tanabe Pharma, AstraZeneca, Kyowa Hakko Kirin Co, Sanwa Kagaku Kenkyusho Co, Kowa Co. Astellas Pharma Inc., Pfizer Inc., and received research funds from Johnson & Johnson, Kyowa Hakko Kirin Co., Kissei Pharmaceutical Co., Bristol-Myers Squibb, Novo Nordisk Pharma, Astellas Pharma Inc., MSD, Dainippon Sumitomo Pharma., AstraZeneca, Teijin Pharma, Mochida Pharmaceutical Co., Sanofi, Sanwa Kagaku Kenkyusho Co., Astellas Pharma Inc., Pfizer Inc., Novartis Pharmaceuticals, Ono Pharmaceutical Co., Mitsubishi-Tanabe Pharma, Daiichi Sankyo, Inc, Takeda Pharmaceutical Co., Eli Lilly Japan, Taisho Toyama Pharmaceutical Co. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

Introduction

Diabetes mellitus affects nearly 400 million individuals worldwide, and its prevalence is progressively increasing in both developing and developed countries, including Japan [1]. Although the pathogenesis of type 2 diabetes, which is the most common form of the disease, has not yet been fully elucidated, the combination of insulin resistance in peripheral tissues and impairment of insulin secretion from pancreatic islets is considered a principal cause. Cumulative evidence has suggested that genetic factors play an important role in the pathogenesis of type 2 diabetes, and extensive efforts are being made to identify genetic loci conferring susceptibility to type 2 diabetes [2].

In 2007, five independent groups performed genome-wide association studies (GWAS) for type 2 diabetes using populations of European descent; they identified TCF7L2, which had previously been discovered via positional cloning in 2006 [3], as well as eight other genetic loci that affect susceptibility to type 2 diabetes, namely SLC30A8, HHEX, CDKN2A/B, CDKAL1, IGF2BP2, KCNJ11, PPARG, and FTO [4–8]. Subsequently, these study groups have been attempting to identify additional susceptibility loci with smaller effect sizes by increasing sample sizes for European GWAS, and nearly 50 susceptibility loci to type 2 diabetes have been confirmed through these studies [9–12]. Most of these loci have been consistent in their effects across non-European populations including the Japanese [13–20], but associations of some loci with type 2 diabetes were not replicated, suggesting differences of genetic background among different ethnic groups.

In 2008, two Japanese GWAS simultaneously identified the KCNQ1 as a strong susceptibility locus for type 2 diabetes [21, 22]. Although large-scale European GWAS meta-analyses had not detected it, the KCNQ1 locus was shown to be a common susceptibility locus to type 2 diabetes in several ethnic groups including European populations [21, 22], indicating the importance of performing GWAS in various ethnic groups. Larger-scale Japanese GWAS have been conducted, and six additional susceptibility loci to type 2 diabetes have been identified: UBE2E2, C2CD4A-C2CD4B, ANK1, SLC16A13, LEP-MIR129 and GPSM1 [23–25]. The former three loci were significantly associated with European type 2 diabetes, but the latter three were not [25], highlighting the possibility of genetic heterogeneity between Japanese and European populations with regard to type 2 diabetes.

In 2014, a transethnic GWAS meta-analysis which combined GWAS data from multiple ethnic groups, including European, East Asian, South Asian and Mexican/Mexican American, identified seven novel loci for type 2 diabetes [26]: rs6813195 near TMEM154, rs9505118 in SSR1, rs17106184 in FAF1, rs3130501 in POUSF1, rs6808574 near LPP, rs702634 in ARL15, and rs4275659 near MPHOSPH9. Since this study addressed problems of sample size and
genetic heterogeneity by performing a large-scale meta-analysis over a variety of ethnic groups, these seven SNP loci are likely to be common susceptibility loci for type 2 diabetes across all these groups (European, East Asian, South Asian and Mexican/Mexican American). However, the 18,820 East Asian individuals, 6,954 type 2 diabetes cases and 11,866 controls, used in the transethnic GWAS meta-analysis consisted of several different subpopulations, including Han Chinese (n = 10,717), Korean (n = 3,985), Filipino (n = 1,783), and Japanese (n = 2,335), and it has been suggested that genetic heterogeneity may also exist to some degree among subpopulations of East Asian descent [27]. Therefore, the contribution of the seven loci for susceptibility to type 2 diabetes needs to be evaluated independently in each subpopulation.

In this study, we evaluated the effects of the seven loci identified by the transethnic GWAS meta-analysis in an independent Japanese population by examining the association of these loci with type 2 diabetes.

**Materials and Methods**

**Participants and DNA Preparation**

We enrolled 5,817 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 with BioBank Japan [23]. Diabetes mellitus was diagnosed according to the World Health Organization (WHO) criteria [28], and type 2 diabetes was clinically defined as 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 1,803 controls who underwent annual health checks at Keio University, St. Marianna University, or Toyama University Hospital. Genomic DNA was extracted from peripheral blood using a standard phenol-chloroform procedure.

**Ethics Statements**

All participants agreed to the protocol of this study and provided written informed consent. This protocol was approved by the ethics committees of the 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 seven autosomal single nucleotide polymorphisms (SNPs) identified by transethnic GWAS meta-analysis in 2014, including rs6813195 near TMEM154, rs9505118 in SSR1, rs17106184 in FAF1, rs3130501 in POU5F1, rs6808574 near LPP, rs702634 in ARL15, and rs4275659 near MPHOSPH9 [26]. Out of these seven SNPs, rs6808574 near LPP was excluded from this analysis because it was monoallelic in the Japanese population (rs6808574-C 100% in Hapmap phase 3 JPT: http://hapmap.ncbi.nlm.nih.gov/ and 1000 genomes project phase 3 JPT: http://www.1000genomes.org/).

Genotyping was performed using a multiplex-polymerase chain reaction (PCR) invader assay as reported previously [29, 30]. The genotyping success rates for all of the SNPs were over 98.0% (S1 Table). The genotype concordance rates in the 64 duplicated samples were 100%.
Statistical Analysis

Statistical analyses were performed using methods described in our previous study [30]. We applied Hardy-Weinberg equilibrium (HWE) tests according to the protocol described by Nielsen et al. [31]. Differences in the genotype distribution of each SNP between cases and controls were evaluated using logistic regression analyses with and without adjustments for age, sex, and body mass index (BMI). The association of each SNP with quantitative traits, including fasting plasma glucose (FPG), insulin (IRI), the homeostasis model assessment of beta-cell function (HOMA-β), and HOMA of insulin resistance (HOMA-IR) [32, 33], was evaluated using multiple linear regression analysis. Because the values of these traits in the experimental Japanese population displayed skewed distribution, we used log-transformed values for 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; \( p < 0.0083 \) (0.05 divided by 6) was therefore considered statistically significant. We estimated the statistical power of our study at different expected odds ratios based on the sample size of our study (n = 7,620) and allele frequencies obtained from a public database (1000 genomes project, phase 3 JPT http://browser.1000genomes.org/ind3x.html). The sample size in this study (n = 7,620) meets the 80% statistical power (\( \alpha = 0.05/6 = 0.0083 \)) for the six SNP loci assuming each of their effect sizes (odds ratios) was 1.2, and for the four SNP loci (rs6813195, rs9505118, rs3130501 and rs4275659) assuming their odds ratio was 1.1. However, if the effect sizes of individual SNP loci had been assumed to be the same as those in the original study [26], the estimated statistical power of this study would have been between 23% and 58% (\( \alpha = 0.05 \), S2 Table).

Results

Clinical characteristics of the participants are shown in Table 1. The genotype distributions of all SNPs were in accordance with Hardy-Weinberg equilibrium proportions (S3 Table). Of the six SNPs examined, four (rs6813195 near TMEM154, rs17106184 in FAF1, rs3130501 in POUSFI, and rs4275659 near MPHOSPH9) had the same direction of effect (odds ratio > 1.0, adjusted for sex, age, and BMI; Table 2, S4 Table) as previously reported, but two (rs9505118 in SSR1 and rs702634 in ARL15) had the opposite direction of effect (odds

### Table 1. Clinical characteristics of participants.

|                      | Sample size (case/control) | Type 2 diabetes | Controls | \( p \) value |
|----------------------|---------------------------|----------------|----------|---------------|
| Sex (M:F)            |                           |                |          | < 0.0001*     |
| Age (year)*          | 5,817/1,803               | 63.3 ± 11.3    | 49.7 ± 17.0 | < 0.0001*     |
| BMI (kg/m²)*         | 5,817/1,803               | 24.2 ± 4.0     | 22.4 ± 3.2 | < 0.0001*     |
| HbA1c (%)            | 4,624/570                 | 8.1 ± 2.6      | 5.3 ± 0.3 | < 0.0001*     |
| FPG (mmol/L)*        | 1,938/1,144               | 8.3 ± 2.9      | 5.3 ± 0.6 | < 0.0001*     |

* Data are mean ± standard deviation.

* Student’s unpaired t-test

* Mann-Whitney U test

BMI: body mass index, HbA1c: Glycated hemoglobin, FPG: fasting plasma glucose
ratio < 1.0; p = 0.2344, binomial test; Table 2, S4 Table). Among them, rs3130501 and rs4275659 were nominally associated with type 2 diabetes (rs3130501, p = 0.017, odds ratio [OR] = 1.113, 95% confidence interval [CI] 1.019–1.215; and rs4275659, p = 0.012, OR = 1.127, 95% CI 1.026–1.238; both adjusted for sex, age, and body mass index (BMI); Table 2). The remaining 4 SNPs were not associated with type 2 diabetes in this study (p > 0.05, adjusted for sex, age and BMI; Table 2).

We further examined the association of the six SNPs with the quantitative glycemic traits, HOMA-IR, HOMA-β, IRI, and FPG using control participants. The risk allele for type 2 diabetes of rs17106184 in FAF1 was nominally associated with an increase in the HOMA-IR value (0.0083, p < 0.05 adjusted for sex, age and BMI; Table 3). The remaining five SNP loci were not associated with any glycemic traits in this study (p > 0.05, adjusted for sex, age and BMI; Table 3).

### Discussion

We examined the association of six loci, rs6813195 near TMEM154, rs9505118 in SSR1, rs17106184 in FAF1, rs3130501 in POU5F1, rs702634 in ARL15, and rs4275659 near

### Table 2. Association of 6 SNP loci with type 2 diabetes in a Japanese population.

| SNP         | Nearby gene | Risk Allele | RAF | Unadjusted | Adjusted |
|-------------|-------------|-------------|-----|------------|----------|
|             |             |             |     | p value    | OR(95%CI) | p value   | OR (95%CI) |
| rs6813195   | TMEM154     | C           | 0.47| 0.062      | 1.075(0.996–1.159) | 0.096     | 1.077(0.987–1.174) |
| rs9505118   | SSR1        | A           | 0.56| 0.935      | 0.997(0.924–1.075) | 0.513     | 0.971(0.890–1.060) |
| rs17106184  | FAF1        | G           | 0.90| 0.589      | 1.037(0.909–1.183) | 0.616     | 1.040(0.893–1.210) |
| rs3130501   | POU5F1      | G           | 0.57| 0.012      | 1.103(1.021–1.191) | 0.017     | 1.113(1.019–1.215) |
| rs702634    | ARL15       | A           | 0.82| 0.962      | 1.002(0.908–1.107) | 0.848     | 0.989(0.883–1.108) |
| rs4275659   | MPHOSPH9    | C           | 0.67| 0.010      | 1.114(1.027–1.209) | 0.012     | 1.127(1.026–1.238) |
| GRSc,d     |             |             |     | 2.4×10⁻³   | 1.056(1.020–1.094) | 0.014     | 1.052(1.010–1.095) |

The results of logistic regression analysis are shown.

### Table 3. Association of 6 SNP loci with quantitative traits related to glucose metabolism in control individuals.

| SNP         | Nearby gene | Risk Allele | HOMA-IRb (n = 802) | HOMA-βb (n = 802) | FPGb (n = 1,148) | IRIb (n = 867) |
|-------------|-------------|-------------|-------------------|------------------|-----------------|----------------|
|             |             |             | p value           | p value           | p value         | p value         |
| rs6813195   | TMEM154     | C           | -0.032 (0.024)    | 0.175            | -0.018 (0.025)  | 0.466           | -0.002 (0.004)  | 0.705           |
| rs9505118   | SSR1        | A           | -0.020 (0.024)    | 0.405            | -0.001 (0.025)  | 0.982           | -0.004 (0.004)  | 0.312           |
| rs17106184  | FAF1        | G           | 0.083 (0.041)     | 0.043            | 0.044 (0.043)   | 0.308           | 0.004 (0.008)   | 0.578           |
| rs3130501   | POU5F1      | G           | 3.8E-4 (0.024)    | 0.987            | -0.019 (0.025)  | 0.450           | 0.004 (0.004)   | 0.406           |
| rs702634    | ARL15       | A           | -0.007 (0.032)    | 0.827            | -0.016 (0.033)  | 0.639           | -0.006 (0.006)  | 0.283           |
| rs4275659   | MPHOSPH9    | C           | 0.033 (0.025)     | 0.192            | 0.031 (0.027)   | 0.244           | -0.008 (0.005)  | 0.081           |

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

a Risk allele reported in the original trans-ethnic GWAS
b log-transformed values were applied for the analyses
MPHOSPH9, identified by transethnic GWAS meta-analysis with susceptibility to type 2 diabetes in a Japanese population. While rs3130501 in POU5F1 and rs4275659 near MPHOSPH9 were nominally associated with type 2 diabetes, none of the six SNPs was significantly associated with the disease in this population (Table 2).

The transethnic GWAS meta-analysis from the original report had the largest sample size of 110,455 participants (26,490 cases and 83,965 controls), including European (n = 69,033), East Asian (n = 18,820), South Asian (n = 20,019) and Mexican/Mexican American (n = 2,583) populations, and the identified seven loci were found to be common susceptibility loci for type 2 diabetes across all these ethnic groups [26]. However, only 2,335 Japanese individuals were included in that meta-analysis. In addition, it has been suggested that East Asian subpopulations differ to some degree with regards to the genetic component of predisposition to type 2 diabetes [27]; therefore, the association of the seven loci with type 2 diabetes needs to be evaluated in a larger, solely Japanese population sample. Indeed, risk allele frequencies for rs6808574 near LPP and rs3130501 in POU5F1 did differ between the Japanese and the Han Chinese populations (for rs6808574-C, 1.0 in Japanese vs. 0.985–1.0 in Han Chinese; and for rs3130501-G, 0.543 in Japanese vs. 0.662–0.680 in Han Chinese; 1000 genomes project data from http://www.1000genomes.org/; rs6808574 was excluded from the present Japanese study).

We did not observe any significant association of the six SNPs with susceptibility to type 2 diabetes in our Japanese population. Among these six SNPs, the effect direction of four of them (rs3130501 in POU5F1, rs4275659 near MPHOSPH9, rs6813195 near TMEM154 and rs17106184 in FAF1) was consistent with that of the original report (OR > 1.0 adjusted for sex, age, and BMI; S4 Table), but rs9505118 in SSR1 and rs702634 in ARL15 showed inconsistent direction of effect with the prior study (OR < 1.0 adjusted for sex, age, and BMI; S4 Table).

The genotyping success rates for all SNPs were over 98.0% (S1 Table), and while the geno-type concordance rates were 100% in duplicated samples (n = 64, see Materials and Methods), this discrepancy was not considered to be the consequence of technical error.

In our study, control individuals were younger than type 2 diabetes subjects; therefore our control group may have included individuals who will develop the disease later in life, which may increase the possibility of a type II error, although we included age as a co-variable in our logistic regression model. Then, we evaluated the association of the six SNPs with type 2 diabetes using older control individuals (age ≥ 40 years, ≥ 50 years, or ≥ 60 years). However, in these analyses, the effect sizes of the six SNP loci were almost the same as those in our original finding (S5 Table). We thereby deduced that our conclusions were not significantly skewed by the inclusion of the younger control individuals.

Because it has been shown that a genetic risk score (GRS) constructed from the sum of the number of risk alleles is a useful measure for evaluating effects of multiple candidate loci of interest in the underpowered sample [19, 20, 30], we constructed the genetic risk scores of our experimental subjects by summing up their number of risk alleles for the six SNPs. However, the association of GRS with type 2 diabetes was not statistically significant (p = 0.014, OR = 1.052, 95% CI 1.010–1.095; Table 2). The association of the six-loci GRS with type 2 diabetes is therefore probably not stronger than that of rs3130501 in POU5F1 (p = 0.017) or rs4275659 near MPHOSPH9 (p = 0.012) alone, suggesting that although the POU5F1 and MPHOSPH9 loci may individually have some effect on susceptibility to type 2 diabetes, the overall effect of the six SNP loci is not major in the Japanese.

The reasons for why the overall effect of the six SNP loci was not major in this Japanese population were not clear, but effect sizes of novel type 2 diabetes susceptibility loci in the transethnic GWAS were shown to be smaller (odds ratio = 1.06–1.10) in multiethnic populations [26] compared with those for type 2 diabetes loci identified in previous GWAS [3–12, 21–25]. Moreover, in the original transethnic GWAS meta-analysis, the Japanese sample size...
(n = 2,335) was much smaller than the European sample size (n = 69,033) [26], indicating the
effect sizes in the original report might preferentially reflect effect sizes in European populations. Thus, the effects of these six SNP loci might be smaller among the Japanese, although heterogeneity in the effect sizes was not observed in the transethnic GWAS meta-analysis [26].

Estimated study power for this study to replicate the original association of each SNP with type 2 diabetes was between 23% and 58% when we set $\alpha = 0.05$, and the prevalence of the disease was assumed to be 10% (S2 Table). Therefore, insufficient study power might explain the lack of statistically significant association of individual SNPs with the disease, especially for rs3130501, rs4275659, and rs6813195, whose effect sizes in this study were comparable to those in the original report (S4 Table); this could be a limitation of our present study.

In conclusion, we performed a replication study in a Japanese population for the association of six SNPs with type 2 diabetes, which were previously identified in a transethnic GWAS meta-analysis. Our results suggest that the six SNP loci derived from the transethnic GWAS meta-analysis do not have a significant effect on susceptibility to type 2 diabetes in the Japanese, although further evaluation for the association of the six loci using a larger Japanese population is required.

**Supporting Information**

S1 Table. Information of genotyping success rates for individual 6 SNPs.

S2 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/](http://www.sph.umich.edu/csg/abecasis/CaTS/). The prevalence of type 2 diabetes is assumed to be 10%, $\alpha = 0.05$. a Risk allele for type 2 diabetes reported in the original trans-ethnic GWAS. b Risk allele frequency in the Japanese population (controls) in the present study. c Information in the original trans-ethnic GWAS is shown.

S3 Table. Genotype distributions of 6 SNPs in case and control groups. HWE: Hardy-Weinberg equilibrium. a Risk alleles reported in the original trans-ethnic GWAS.

S4 Table. Association of 6 SNP loci with type 2 diabetes in a Japanese population and the original report. The results of logistic regression analysis are shown. a Risk allele reported in the original trans-ethnic GWAS. b Adjusted for age, sex and BMI. c Information in the original trans-ethnic GWAS is shown. RAF: Risk allele frequency.

S5 Table. Association study of 6 SNPs with type 2 diabetes using older control (age $\geq 40$, n = 1,204, age $\geq 50$, n = 906, age $\geq 60$ n = 543). Results of logistic regression analysis using all type 2 diabetes participants (n = 5,817) are shown. a Risk allele reported in the original trans-ethnic GWAS. b Adjusted for age, sex and BMI.

**Acknowledgments**

We wish to thank the technical staff at the Laboratory for Endocrinology, Metabolism and Kidney Diseases, RIKEN Center for Integrative Medical Sciences, for their technical assistance.
Author Contributions
Conceived and designed the experiments: M. Imamura SM. Performed the experiments: RM M. Imamura SM. Analyzed the data: RM M. Imamura SM. Contributed reagents/materials/analysis tools: YT M. Iwata HH KK HM HW KT AK RK. Wrote the paper: RM M. Imamura SM. Contributed to the interpretation of the data: YT M. Iwata HH KK HM HW KT AK RK.

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