for acute-onset type 1 diabetes in a white population [19]. A similar tendency of association was also suggested for several SNPs located in the most centromeric region (rs45261, rs1059288, rs2071888, rs2073525) (Table 4).

Although extensive effort has been put into collecting samples, particularly for fulminant type 1 diabetes, the number of samples in the present study is still modest for this kind of study. Further studies with dense SNPs and a larger number of participants are necessary to clarify whether or not the association of these SNPs with each subtype is real and independent of other nearby genes. In addition, the contribution of genes outside the extended HLA, as well as of those on different chromosomes to the disease, should also be studied in these three subtypes of type 1 diabetes.

In conclusion, the present study demonstrates that class II HLA is associated with three subtypes of type 1 diabetes, fulminant, acute-onset and slowly progressive forms, but the alleles, haplotypes and genotypes associated with the disease differ among the three subtypes. The association with HLA in fulminant type 1 diabetes is qualitatively different from that in other subtypes of type 1 diabetes, which may reflect the difference in aetiology between fulminant and other subtypes of type 1 diabetes. In contrast, the association with HLA in slowly progressive type 1 diabetes is qualitatively similar to, but quantitatively different from that in acute-onset type 1 diabetes. Given that in this study a substantial number of patients with fulminant type 1 diabetes or with well characterised slowly progressive type 1 diabetes were recruited only in Japan, further studies with dense genetic markers, including whole-genome association studies comparing these three subtypes of type 1 diabetes, are necessary. Such studies are now underway.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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Confirmation of Multiple Risk Loci and Genetic Impacts by a Genome-Wide Association Study of Type 2 Diabetes in the Japanese Population

Fumihiko Takeuchi,[1,2] Masakuni Serizawa,[3] Ken Yamamoto,[4] Tomomi Fujisawa,[5] Eitaro Nakashima,[6,7] Keizo Ohnaka,[8] Hiroshi Ikegami,[9] Takao Sugiyama,[10] Tomohiro Katsuya,[5] Makoto Miyagishi,[3] Naoki Nakashima,[11] Hajime Nawata,[12] Jiro Nakamura,[6] Suminori Kono,[13] Ryoichi Takayanagi,[14] and Norihiro Kato[3]

OBJECTIVE—To identify novel type 2 diabetes gene variants and confirm previously identified ones, a three-staged genome-wide association study was performed in the Japanese population.

RESEARCH DESIGN AND METHODS—In the stage 1 scan, we genotyped 519 case and 503 control subjects with 482,625 single nucleotide polymorphism (SNP) markers; in the stage 2 panel comprising 1,110 case subjects and 1,014 control subjects, we assessed 1,456 SNPs ($P < 0.0025$, stage 1); additionally to direct genotyping, 964 healthy control subjects formed the in silico control panel. Along with genome-wide exploration, we aimed to replicate the disease association of 17 SNPs from 16 candidate loci previously identified in Europeans. The associated and/or replicated loci (23 SNPs; $P < 7 \times 10^{-5}$ for genome-wide exploration and $P < 0.05$ for replication) were examined in the stage 3 panel comprising 4,000 case subjects and 12,569 population-based samples, from which 4,889 nondiabetic control subjects were preselected. The 12,569 subjects were used for overall risk assessment in the general population.

RESULTS—Four loci—1 novel with suggestive evidence (*PEPD* on 19q13, $P = 1.4 \times 10^{-5}$) and three previously reported—were identified; the association of *CDKAL1*, *CDKN2A/CDKN2B*, and *KCNQ1* were confirmed ($P < 10^{-19}$). Moreover, significant associations were replicated in five other candidate loci: *TCF7L2*, *IGF2BP2*, *SLC30A8*, *IIIIEX*, and *KCNJ11*. There was substantial overlap of type 2 diabetes susceptibility genes between the two populations, whereas effect size and explained variance tended to be higher in the Japanese population.

CONCLUSIONS—The strength of association was more prominent in the Japanese population than in Europeans for more than half of the confirmed type 2 diabetes loci. ***Diabetes* 58: 1690–1699, 2009**

The predisposition to and the course of type 2 diabetes vary according to ethnic group (1–3). In Japan, the incidence of type 2 diabetes has increased recently and is now comparable to that of other countries; this is supposedly attributable to the gradual spread of Western habits, such as consuming a high-fat diet, and the lower insulin secretory capacity of Japanese subjects (4,5). Recent technological developments have allowed the successful identification of gene regions involved in the development of type 2 diabetes in genome-wide association (GWA) studies (6–17). Several susceptibility gene loci identified by GWA studies to date have been used to obtain reproducible evidence of disease association in different populations of European descent and Asians, but not necessarily in African Americans (18–24). A number of GWA studies on type 2 diabetes have been conducted on populations of European descent (6–12). Two GWA scans in the Japanese population simultaneously reported the discovery of type 2 diabetes susceptibility gene (*KCNQ1*) variants in non-European populations; this result was also obtained in Scandinavian samples (25,26). Thus far, the replicated associations for a limited number of candidate genes have broadly indicated the tendency of interethnic similarity. Even though the common (or cosmopolitan) effect of type 2 diabetes risk variants is known, the extent to which the causation of this disease differs or overlaps between populations remains unknown. Here, besides comparing the genetic associations between European-descent and Japanese populations, we aimed to identify new genetic variants using a three-staged GWA study design.

RESEARCH DESIGN AND METHODS

Detailed characteristics of the subjects enrolled in each stage are described in the supplementary information and in supplementary Table S1, which is available in an online appendix at http://diabetes.diabetesjournals.org/cgi/content/full//db08-1494/DC1. Briefly, patients and unaffected control subjects analyzed in stages 1 and 2 were enrolled depending on whether they met certain uniform criteria. Type 2 diabetes was diagnosed according to 1999 World Health Organization criteria. All stage 1 and 2 control subjects (≥55

From the [1]Department of Medical Ecology and Informatics, Research Institute, International Medical Center of Japan, Tokyo, Japan; the [2]Wellcome Trust Sanger Institute, Cambridge, U.K; the [3]Department of Gene Diagnostics and Therapeutics, Research Institute, International Medical Center of Japan, Tokyo, Japan; the [4]Department of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; the [5]Department of Geriatric Medicine, Osaka University Graduate School of Medicine, Osaka, Japan; the [6]Division of Endocrinology and Diabetes, Department of Internal Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan; the [7]Department of Metabolism and Endocrine Internal Medicine, Chubu Rosai Hospital, Nagoya, Japan; the [8]Department of Geriatric Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; the [9]Department of Endocrinology, Metabolism and Diabetes, Kinki University School of Medicine, Osaka, Japan; the [10]Institute for Adult Diseases, Asahi Life Foundation, Tokyo, Japan; the [11]Department of Medical Informatics, Kyushu University Hospital, Fukuoka, Japan; [12]Fukuoka Prefectural University, Fukuoka, Tokyo, Japan; the [13]Department of Preventive Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; and the [14]Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Corresponding author: Norihiro Kato, nokato@ri.imcj.go.jp.

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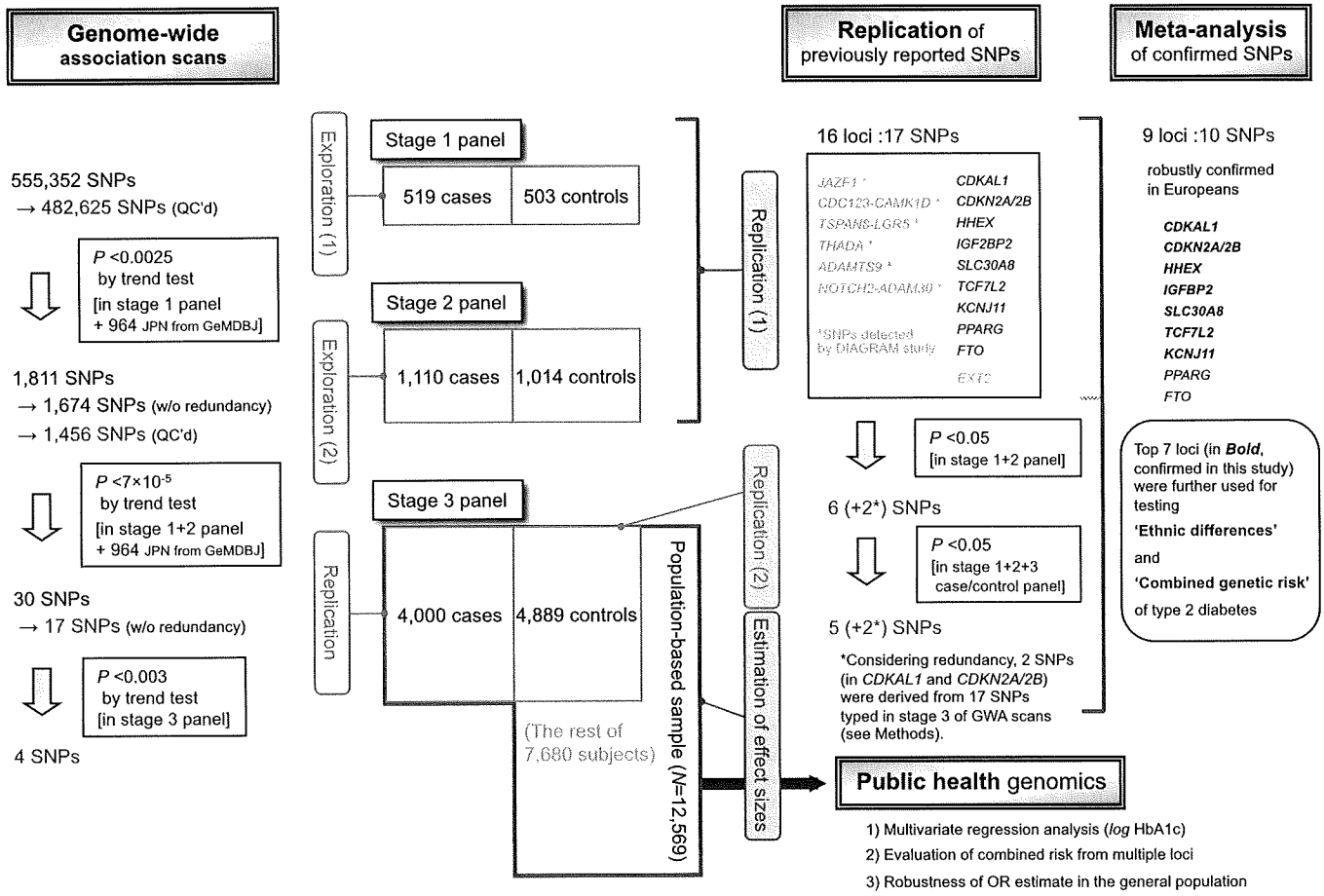

FIG. 1. Flow chart summarizing the multistage design and study aims. (A high-quality digital representation of this figure is available in the online issue.)

years of age at examination) had normal glucose tolerance. The stage 3 samples comprised 4,000 type 2 diabetes case subjects derived from the Biobank Japan project (http://biobankjp.org/) (27) and 12,569 subjects randomly selected from residents aged 50–74 years in the general population. The 12,569 subjects were used as a population panel; this panel contained 4,889 nondiabetic subjects who met the following conditions: age >55 years, A1C ≤5.0%, no previous and/or current treatment for diabetes, and absence of renal failure (serum creatinine <3.0 mg/dl). In stage 3, these 4,889 control subjects were used in a replication study wherein their genotypes were compared with those of 4,000 patients. In addition to the samples genotyped here, to boost the power of the GWA scan, we incorporated genotype frequencies in the general Japanese population (n = 964) derived from the Genome Medicine Database of Japan (GeMDBJ; http://gemdbj.nibio.go.jp), which was used as an in silico control panel. A flowchart summarizing the multistage design and study aims is shown in Fig. 1.

Stage 1 genome-wide scan and quality control. Genotyping was performed with the Infinium HumanHap550 BeadArray (Illumina), which interrogated 555,352 SNPs (supplementary information). The average call rate was 96.9% for the case and control subjects. Data cleaning and analysis were performed using PLINK software (28). Samples with a genotype call rate of <90% were excluded, as were outliers with respect to the number of heterozygous SNPs, duplicates or relatives of another sample, or ethnic outliers. We excluded SNPs for the following reasons: *1*) GenTrain genotype quality score <0.53, *2*) genotype call rate <0.95, *3*) genotype call rate <0.99 and minor allele frequency (MAF) <0.05, *4*) significant ($P < 10^{-6}$) deviation from the Hardy-Weinberg equilibrium in the control subjects, or *5*) MAF <0.001 (supplementary Table S2); the remaining 482,625 SNPs were analyzed.

Analysis of stage 1 genotype data. Ethnicity was verified for 1,022 samples (519 case and 503 control subjects) in the stage 1 panel with reference to data from HapMap populations (29) (see supplementary information). Type 2 diabetes association was tested with the Cochran-Armitage trend test in the stage 1 panel and an additional panel of 964 random control subjects. We pooled the genotype counts for combining multiple panels. To detect and correct population stratification and unnoticed differences in data processing between facilities, the test statistic was adjusted using Eigenstrat software (30) and the genomic-control method (31). The significance level for the first-stage scan was set to $P < 0.0025$; significant SNPs were additionally chosen using Fisher's χ^2 test ($P < 0.0025$) to combine the association results with the P value at the same locus in our previous affected sib-pair scan (32). A total of 1,811 SNPs surpassed the stage 1 threshold, and we removed redundant SNPs that were in mutual strong linkage disequilibrium (r^2 >0.9) before proceeding to stage 2 (see supplementary information and supplementary Fig. S1 and Table S2 for detailed analysis).

Stage 2 genotyping and analysis. Stage 2 genotyping was performed with iPLEX (Sequenom) and GoldenGate (Illumina) assays. Quality control was conducted as described in stage 1, and 1,456 SNPs were successfully genotyped. We calculated P values with the trend test by combining 1,517 nondiabetic control subjects with 964 random control subjects similar to stage 1. The significance level for the second-stage scan was set to $P < 7 \times 10^{-5}$ in the comparison between 1,629 case subjects and 2,481 control subjects (i.e., the stage 1 + 2 panels and the 964 random control subjects). A total of 30 SNPs representing 17 unique loci remained significant.

Replication of previously reported SNPs. Along with genome-wide exploration, type 2 diabetes association was tested in the stage 1 and 2 panels using the HumanHap550 BeadArray, iPLEX assay, or TaqMan method (Applied Biosystems) for 17 SNPs from 16 candidate loci previously identified by GWA studies in populations of European descent (6–17). These included *IGF2BP2* (rs4402960), *PPARG* (rs1801282), *CDKAL1* (rs7754840 and rs7756992), *SLC30A8* (rs13266634), *CDKN2A/CDKN2B* (rs10811661), *HHEX* (rs1111875), *TCF7L2* (rs7903146), *EXT2* (rs3740878), *KCNJ11* (rs5219), *FTO* (rs8050136), *JAZF1* (rs864745), *CDC123-CAMK1D* (rs12779790), *TSPAN8-LGR5* (rs7961581), *THADA* (rs7578597), *ADAMTS9* (rs4607103), and *NOTCH2-ADAM30* (rs10923931). The significant SNPs (trend test, P < 0.05) were further analyzed in the stage 3 panel with the TaqMan method. Despite finding significant association for *CDKAL1* and *CDKN2A/CDKN2B* in the stage 1 and 2 panels, we proceeded with rs4712523 instead of rs7754840 and rs7756992 (*CDKAL1*) and with rs2383208 instead of rs10811661 (*CDKN2A/CDKN2B*) in the GWA scans from stage 1 to stage 3; this decision was made considering the

strong linkage disequilibrium between the SNPs in each of the corresponding loci.

Stage 3 genotyping and analysis. The stage 3 design involved the replication of association and the estimation of effect sizes in the GWA scan and/or replication study of previously reported SNPs. For an association to be considered significant in the case-control comparison (4,000 case vs. 4,889 nondiabetic control subjects), it had to involve the same risk allele as that in the previous stages, and it was accordingly assessed with a one-tailed test. For each SNP locus confirmed in stage 3, the association of additional independent SNPs or haplotypes in the locus was also tested (supplementary information). Moreover, to assess the risk of diabetes and pre-diabetes in the general population from the combination of SNPs robustly confirmed both in populations of European descent and in our panel, multiple regression analysis was performed with the logarithm of A1C (log A1C) as a response variable (supplementary information), using the entire 12,569-subject population-based sample.

Meta-analysis of other type 2 diabetes case-control studies in the Japanese population. In addition, for SNPs with robustly confirmed association in populations of European descent, we performed a meta-analysis by combining our stage 1 + 2 (rs1801282, rs7756992, and rs8050136) or stage 1 + 2 + 3 results (the remaining seven SNPs shown in supplementary Figure S2) with those of previous Japanese studies conducted by three other groups (19–21,33–36). According to Woolf's test (37), the heterogeneity among the studies in the Japanese population was insignificant ($P > 0.05$), with the exception of *PPARG* rs1801282 ($P = 0.0012$), for which the observed heterogeneity is supposedly attributable to low allele frequency. Thus, we pooled genotype counts across the studies to form a combined dataset for the Japanese population, and we estimated the effect sizes of individual loci. We used the rmeta package for R software (http://www.r-project.org) for the analysis.

Moreover, to compare the explained variance between the Japanese population and populations of European descent, we calculated the coefficient of determination R^2 for the loci confirmed in our replication study. Here, R^2 is the square of the correlation between the genotypes of an SNP coded by the number of risk alleles (0, 1, and 2) and the disease status (0 and 1) (supplementary information).

RESULTS

GWA scans. Of 482,625 SNPs that passed quality control in stage 1, genotypes were obtained for an average of 99.8% markers for each subject. The subjects were enrolled from regions of Japan with no strong population stratification (38), and although some variance inflation partly attributable to the subtle subpopulation structure was apparent, such confounding influences could be sufficiently removed using Eigenstrat (30) and genomic-control adjustment (31). A total of 1,456 markers were assessed in the stage 2 panel (Fig. 1 and supplementary Fig. S1 and Table S2).

After the second-stage scan, 30 SNPs representing 17 unique loci attained the arbitrarily defined statistical significance ($P < 7 \times 10^{-5}$) (supplementary Table S3). We used one SNP each from these 17 loci in the third-stage scan. Of 17 SNPs, 4 reached the significance threshold of $P < 0.003$ (= 0.05/17) with Bonferroni correction.

The current GWA study showed strong and highly consistent evidence for disease association of SNPs from *CDKAL1*, *CDKN2A/CDKN2B*, and *KCNQ1* (Fig. 2 and Table 1 and supplementary Tables S4 and S5). Although these three loci had already been reported in previous GWA studies (8,11,25,26), here they were identified as part of our genome-wide exploration. *CDKAL1* is among the best-replicated susceptibility loci. Significant association has also been detected in a region on chromosome 9p, near *CDKN2A/CDKN2B*. Moreover, strong association signals were observed in the intron of *KCNQ1* on chromosome 11p15.5, which is in agreement with the results of two previous GWA scans in the Japanese population (25,26).

Stage 2 genotyping provided evidence suggestive of a new association on chromosome 19q13. Several SNPs located in the vicinity of the *PEPD* (peptidase D) gene showed the tendency of replicated association in stages 1 and 2 (supplementary Table S3). Significant association was further replicated in a relatively large case-control study on the stage 3 panel (rs10425678, $P = 0.002$), but it did not attain genome-wide significance (i.e., $P = 1.4 \times 10^{-5}$ for all stages and $P = 2.1 \times 10^{-6}$ when the number of control subjects was increased by adding 964 random control subjects) (supplementary Table S4). Given the modest strength of association ($R^2 = 0.0017$, see below) assumed for this locus, the association still needs to be established.

Replication of previously reported SNPs. Of 16 candidate loci tested for replication in the Japanese population, 7 were found to be associated with type 2 diabetes (Table 1). However, no significant association was observed for SNPs from the remaining nine loci (*FTO*, *PPARG*, *EXT2*, *JAZF1*, *CDC123-CAMK1D*, *TSPAN8-LGR5*, *ADAMTS9*, and *NOTCH2-ADAM30* in the stage 1 + 2 panel and *THADA* in the stage 1 + 2 + 3 panel). Notably, the originally reported SNPs or those in complete linkage disequilibrium showed the strongest statistical evidence of association in the seven confirmed loci, where the linkage disequilibrium relations and haplotype patterns appear to be similar but not identical between European-descent and Japanese populations (supplementary Figs. S3 and S4).

Besides *KCNQ1* and the 16 candidate loci prioritized here, we investigated the disease association of two candidate gene SNPs—rs734312 in *WFS1* (39) and rs7501939 in *TCF2* (40)—based on the genotype data of our stage 1 panel ($n = 1,022$) and 964 random control subjects (supplementary Table S6). In some instances, it appeared that the sample size was not sufficient to detect the presumed odds ratio (OR) (supplementary Table S7). Nevertheless, except for rs12779790 in *CDC123-CAMK1D* and rs3740878 in *EXT2*, in the majority of instances, the ORs were consistent with those previously reported. Furthermore, we analyzed seven previously reported SNPs with suggestive evidence of an association in the Japanese population (25), but none attained nominal significance in our first-stage scan (supplementary Table S8).

Ethnic differences in genetic effects on type 2 diabetes. With regard to the candidate gene SNPs robustly confirmed in GWA studies conducted on Japanese and European-descent populations, we compared the risk allele frequency and OR between the meta-analysis dataset of the Japanese population and that of populations of European descent (8–10) (Fig. 3). The OR was consistently higher in the Japanese population for all SNPs except rs5219 in *KCNJ11*. Among the confirmed loci, *CDKAL1*, *CDKN2A/CDKN2B*, *SLC30A8*, and *HHEX* showed a significant difference in the ORs between European-descent and Japanese populations ($P < 0.05$, Woolf's test) (supplementary Table S6). However, the risk allele frequency fluctuated between the two ethnic groups, and the strength of association differed accordingly; this is because an SNP with an risk allele frequency of ~0.5 and a higher OR can give rise to stronger association signals. Thus, whereas *TCF7L2* was shown as the strongest susceptibility locus in populations of European descent (41), its association is estimated to be much weaker in the Japanese population because of the low risk allele frequency. In contrast, the results of the meta-analysis showed that the *CDKN2A/CDKN2B* and *CDKAL1* loci had the strongest associations in the Japanese population;

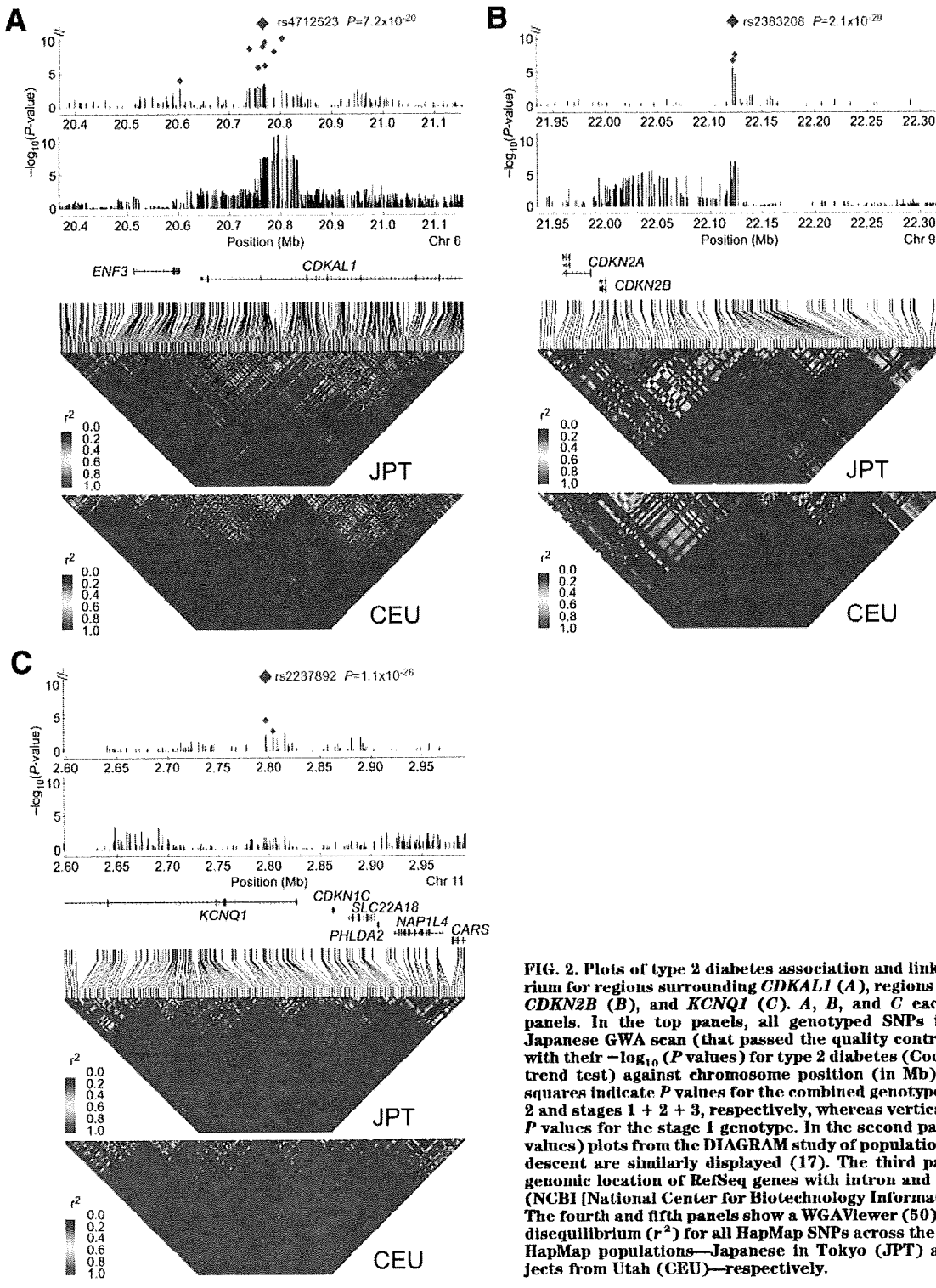

FIG. 2. Plots of type 2 diabetes association and linkage disequilibrium for regions surrounding *CDKAL1* (*A*), regions near *CDKN2A/CDKN2B* (*B*), and *KCNQ1* (*C*). *A*, *B*, and *C* each contain five panels. In the top panels, all genotyped SNPs in the current Japanese GWA scan (that passed the quality control) are plotted with their $-\log_{10}$ (*P* values) for type 2 diabetes (Cochran-Armitage trend test) against chromosome position (in Mb). Blue and red squares indicate *P* values for the combined genotypes of stages 1 + 2 and stages 1 + 2 + 3, respectively, whereas vertical bars indicate *P* values for the stage 1 genotype. In the second panels, $-\log_{10}$ (*P* values) plots from the DIAGRAM study of populations of European descent are similarly displayed (17). The third panels show the genomic location of RefSeq genes with intron and exon structure (NCBI [National Center for Biotechnology Information] Build 35). The fourth and fifth panels show a WGAViewer (50) plot of linkage disequilibrium (r^2) for all HapMap SNPs across the regions for the HapMap populations—Japanese in Tokyo (JPT) and CEPH subjects from Utah (CEU)—respectively.

indeed, we obtained the highest number of hits for these loci.

Next, we compared the strength of association for the seven confirmed loci between Japanese and European-descent populations and calculated R^2 as the proportion of phenotypic variance explained by an SNP (see RESEARCH DESIGN AND METHODS). In Fig. 3, we illustrate the curves corresponding to $R^2 = 0.008$, 0.004, and 0.002, for which the total sample size of case and control subjects required to attain 80% power is $n = 4{,}300$, 8,600, and 17,200 at a significance level of $P = 5 \times 10^{-7}$ (which is the significance threshold generally required in GWA tests), and $n = 1{,}000$, 2,000, and 3,900 at a level of $P = 0.05$. Based on R^2 measurements using the meta-analysis data, the associations of five of seven replicated loci are stronger in the Japanese population than in populations of European descent. For the *CDKAL1* locus, for example, one-fourth of the sample size necessary in populations of European descent is

TABLE 1
Type 2 diabetes susceptibility loci identified or tested for replication in the current Japanese study

rs no.*	Chromosome	Position (bp)	Region	Risk allele/ nonrisk allele	Control risk allele proportion	Stage 1 + 2 (1,629 case subjects/1,517 control subjects) OR (95% CI)	*P* trend
Identified in this GWA scan							
rs1712523	6	20,765,543	*CDKAL1*	G/A	0.407	1.38 (1.25–1.52)	8.0E-10
rs2383208	9	22,122,076	*CDKN2A/B*	A/G	0.553	1.31 (1.18–1.45)	1.6E-07
rs2237892	11	2,796,327	*KCNQ1*	C/T	0.594	1.25 (1.13–1.39)	2.3E-05
rs10425678	19	38,669,236	*PEPD*	C/T	0.261	1.23 (1.10–1.37)	3.6E-04
Replication of previously-reported SNPs							
rs10923931	1	120,230,001	*NOTCH2-ADAM30*	T/G	0.020	1.17 (0.83–1.65)	0.3821
rs7578597	2	43,644,474	*THADA*	T/C	0.990	1.95 (1.03–3.67)	0.0392
rs1801282	3	12,368,125	*PPARG*	C/G	0.969	1.00 (0.75–1.34)	0.9741
rs4607103	3	64,686,944	*ADAMTS9*	C/T	0.594	1.09 (0.99–1.21)	0.0902
rs4402960	3	186,994,389	*IGF2BP2*	T/G	0.310	1.15 (1.04–1.28)	0.0098
rs7754840	6	20,769,229	*CDKAL1*	C/G	0.392	1.42 (1.28–1.57)	1.7E-10
rs7756992	6	20,787,688	*CDKAL1*	G/A	0.448	1.35 (1.23–1.50)	4.6E-09
rs864745	7	27,953,796	*JAZF1*	T/C	0.789	1.08 (0.95–1.22)	0.2456
rs13266634	8	118,253,964	*SLC30A8*	C/T	0.570	1.18 (1.06–1.30)	0.0015
rs10811661	9	22,124,094	*CDKN2A/B*	T/C	0.555	1.35 (1.21–1.49)	2.2E-08
rs12779790	10	12,368,016	*CDC123-CAMK1D*	G/A	0.151	0.98 (0.85–1.13)	0.7984
rs1111875	10	94,452,862	*HHEX*	C/T	0.275	1.19 (1.07–1.33)	0.0011
rs7903146	10	114,748,339	*TCF7L2*	T/C	0.035	1.42 (1.10–1.84)	0.0073
rs5219	11	17,366,148	*KCNJ11*	T/C	0.355	1.22 (1.09–1.35)	2.5E-04
rs3740878	11	44,214,378	*EXT2*	A/G	0.633	1.01 (0.91–1.12)	0.8849
rs7961581	12	69,949,369	*TSPAN8-LGR5*	C/T	0.202	1.12 (0.99–1.27)	0.0751
rs8050136	16	52,373,776	*FTO*	A/C	0.203	1.11 (0.98–1.26)	0.0915

Continued on following page

sufficient to obtain the same level of statistical significance in the Japanese population. This is true for *CDKN2A/CDKN2B*, *HHEX*, and *SLC30A8*, in which <50% of the sample size seems to be sufficient for significance in the Japanese population. However, *TCF7L2* shows an opposite trend in this regard.

Combined genetic risk of type 2 diabetes. Despite the small value of explained variance (R^2) at each risk locus, it is assumed that knowledge about multiple-risk loci could allow us to identify individuals with accumulated genetic risk (42). To this end, a GWA study in Finns (10) investigated the combined risk of type 2 diabetes based on 10 associated loci by logistic regression analysis of the resampled dataset. The total variance explained by 10 loci in Finns is $R^2 = 0.030$, which is equivalent to the value for 7 loci obtained here (see DISCUSSION). Likewise, in a simulated population, we arranged the individuals in the order of the risk estimated by logistic regression, sorted them into 20 equal-sized groups (5% in each), and calculated the actual proportion of affected individuals in each group. We found a 3.7-fold variation in type 2 diabetes prevalence from the lowest to highest estimated risk groups for the combination of seven associated loci in our study (Fig. 4). The receiver operating characteristic curve was also depicted for the combined SNPs as a measure of sensitivity and specificity (supplementary Fig. S5).

Moreover, for risk assessment in the general population, we performed multiple regression analysis using A1C as a surrogate quantitative phenotype to estimate the unbiased effect size of individual loci (supplementary Table S9) and evaluated the combined risk from multiple loci in 12,569 population-based samples (Table 2 and supplementary information). Then, the estimated risk was compared with the actual A1C value and the disease classification of diabetes or pre-diabetes (supplementary information). In the multiple regression analysis, significant association ($P < 0.005$) was observed for all seven loci tested in accordance with the results for the case-control study (Table 1 and supplementary Table S4). As shown in Fig. 4, 5% of male subjects with the highest estimated risk are 2.3 times more likely to suffer from diabetes than those with the lowest estimated risk; the risk is 5.2 times in female subjects, indicating the potential existence of sex difference in the genetic risk of type 2 diabetes (supplementary Fig. S6). Moreover, notably, SNP genotypes alone exerted more exaggerated effects on the increase in genetic risk in diabetes compared with pre-diabetes (Table 2).

DISCUSSION

Conducting GWA studies on a wider range of populations, including East Asians, has recently gained importance because of the discovery of new type 2 diabetes susceptibility variants mapping to the *KCNQ1* gene simultaneously reported in two Japanese studies (25,26). Both studies were, however, initiated some years ago, and they are, by current standards, considered to be modest with regard to the coverage of common SNPs (21 and 56% in HapMap) and number of case subjects (187 and 194 subjects, respectively) in the first-stage scan. Therefore, we conducted another GWA study on the Japanese population with greater coverage of common SNPs (87% of all phase

TABLE 1
Continued

Stage 3 (4,000 case subjects/4,889 control subjects)†		All combined (5,629 case subjects/6,406 control subjects)†		OR (95% CI) reported in Europeans (14,586 case subjects/17,968 control subjects)
OR (95% CI)	*P* trend‡	OR (95% CI)	*P* trend	
1.23 (1.16–1.30)	4.0E-12	1.27 (1.21–1.33)	7.2E-20	1.12 (1.08–1.16)
1.33 (1.26–1.42)	4.8E-22	1.31 (1.27–1.41)	2.1E-29	1.20 (1.14–1.25)
1.36 (1.28–1.45)	8.0E-23	1.33 (1.27–1.41)	1.1E-26	1.18 (1.03–1.33)§
1.10 (1.03–1.18)	0.0020	1.14 (1.07–1.20)	1.4E-05	1.03 (0.97–1.09)§
—	—	—	—	1.13 (1.08–1.17)‖
0.98 (0.73–1.31)	0.55	1.13 (0.87–1.47)	0.35	1.15 (1.10–1.20)‖
—	—	—	—	1.14 (1.08–1.20)
—	—	—	—	1.09 (1.06–1.12)‖
1.14 (1.07–1.21)	2.5E-05	1.14 (1.08–1.21)	1.0E-06	1.14 (1.11–1.18)
—	—	—	—	1.12 (1.08–1.16)
—	—	—	—	1.26 (1.18–1.34)§
—	—	—	—	1.10 (1.07–1.13)‖
1.24 (1.17–1.31)	5.8E-13	1.22 (1.16–1.28)	1.8E-14	1.12 (1.07–1.16)
—	—	—	—	1.2 (1.14–1.25)
—	—	—	—	1.11 (1.07–1.14)‖
1.21 (1.13–1.29)	2.6E-09	1.21 (1.15–1.28)	6.7E-12	1.13 (1.09–1.17)
1.59 (1.38–1.83)	5.3E-11	1.54 (1.36–1.74)	7.6E-12	1.37 (1.31–1.43)
1.02 (0.96–1.08)	0.3008	1.07 (1.01–1.13)	0.0149	1.14 (1.10–1.19)
—	—	—	—	1.20 (1.11–1.30)¶
—	—	—	—	1.09 (1.06–1.12)‖
—	—	—	—	1.17 (1.12–1.22)

Results for one SNP each selected from the individual chromosomal regions in the GWA scans are shown in the table (see supplementary Table S4 for details and supplementary Table S5 for the results of logistic regression adjusted for BMI). The final *P* value was assessed by pooling genotype counts for each SNP from all stages tested (without including 964 random control subjects from GeMBDJ). In two regions, chromosome 6p22.3 (*CDKAL1*) and 19p13 (*PEPD*), the haplotype class showed more significant association than the individual SNP (see supplementary Information). *In the stage 3 panel, we genotyped rs4712523 instead of rs7754840 (r^2 = 0.96) or rs7756992 (r^2 = 0.65) in CDKAL1, and rs2383208 instead of rs10811661 (r^2 = 0.89) near CDKN2A/B, with the aim of determining the SNP(s) with the strongest association in the Japanese population. †In stage 3 of the replication study on previously reported SNPs, after the confirmation of significant association in 4,000 case subjects and 4,889 preselected control subjects, we further characterized 7,680 subjects (who comprised the rest of the 12,569 population-based samples) (see RESEARCH DESIGN AND METHODS and Fig. 1). Thus, for the corresponding SNPs, 5,395 control subjects were reselected from the entire population-based samples and used for the final association analysis in stage 3, which increased the total number of control subjects across the three stages to 6,912. ‡One-tailed test for association was performed in the direction consistent with stage 1 + 2 data; §for 4,549 case and 5,579 control subjects derived from the DIAGRAM consortium of Zeggini et al. (17); ‖for ~60,000 total samples from Zeggini et al. (17); ¶for 3,278 case and 3,508 control subjects from Sladek et al. (6).

1 + 2 HapMap variants [MAF ≥0.05] in CHB (Chinese in Beijing) + JPT (Japanese in Tokyo) and a larger number of case subjects (519 subjects) and unaffected control subjects (503 subjects) in addition to random control subjects in the first-stage scan. Four loci (three previously reported and one novel) were identified via the multistage scans. For the top three loci (*KCNQ1*, *CDKN2A/CDKN2B*, and *CDKAL1*) the OR (>1.25) and MAF (0.41–0.45 in the control subjects) were higher in the Japanese population than in populations of European descent. In addition to the nomination of four susceptibility loci (*KCNQ1*, *CDKN2A/CDKN2B*, *CDKAL1*, and *PEPD*), the current study replicated the significant association of five other loci (*TCF7L2*, *IGF2BP2*, *SLC30A8*, *HHEX*, and *KCNJ11*) previously reported in populations of European descent (6–17) and provided an unbiased estimate of the risk from the confirmed disease genotype.

Empirical studies suggest that the genetic effects of individual causal risk alleles underlying complex genetic diseases such as type 2 diabetes are modest, with most genotype relative risks in the range of 1.1–2.0 (43). Indeed, we observed this to be true for loci that were robustly implicated in the development of type 2 diabetes by GWA scans and/or extensive candidate gene approaches in populations of European descent. Currently, the number of loci has increased to almost 20 (as listed in supplementary Table S6), and in most cases, except for *TCF7L2* and *KCNQ1*, the OR is estimated to be between 1.09 and 1.20.

The current study provides, via genome-wide exploration and replication analysis of some a priori selected loci, significant evidence for the overall tendency toward a stronger association in Japanese rather than European-descent populations at least for alleles with a cosmopolitan effect. The tendency for higher OR in Asians than in Europeans was previously reported for the *CDKAL1* locus (22). Currently, it remains unknown whether the penetrance for a genotype of interest differs considerably between Japanese and European-descent populations. With regard to genetic effects, four of seven confirmed loci have demonstrated significantly higher OR in the Japanese population ($P = 4.1 \times 10^{-5}$ to 0.024) (supplementary Table S6). To simplify the situation, we have further assessed the strength of association for individual SNPs by measuring R^2, which is scaled against OR and risk allele frequency in

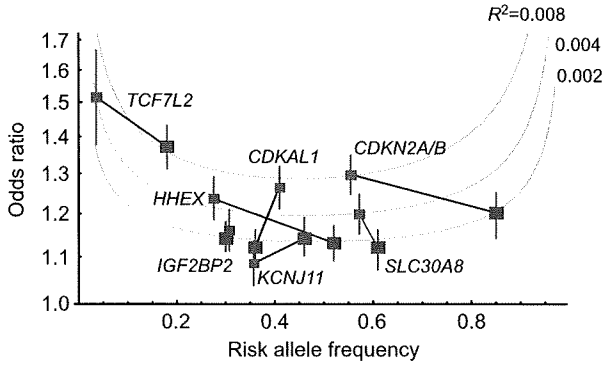

FIG. 3. Comparison of the strength of association for seven confirmed type 2 diabetes loci between Japanese and European-descent populations. For the Japanese population, we estimated ORs and their 95% CIs (red solid squares and vertical lines, respectively) for each locus based on our meta-analysis involving four Japanese case-control studies (supplementary Fig. S2). For populations of European descent, on the other hand, the corresponding values (blue solid squares and vertical lines) were derived from the published data (8–10). The association of an SNP with type 2 diabetes is measured by the coefficient of determination (R^2), which represents the ability to detect association signals using the Cochran-Armitage trend test.

Fig. 3. We found that despite the limited number of SNPs tested here, the same level of statistical significance is often detectable in the Japanese population with a much smaller sample size than that in populations of European descent (supplementary Table S7). Theoretically, the stringency of ascertaining control subjects could lead to some bias in effect size (44). In this respect, in addition to the multistage case-control study, an extensive analysis of associated loci in the general population was conducted, which is the strength of the current study. We used the population-based samples (n = 12,569) in stage 3 to

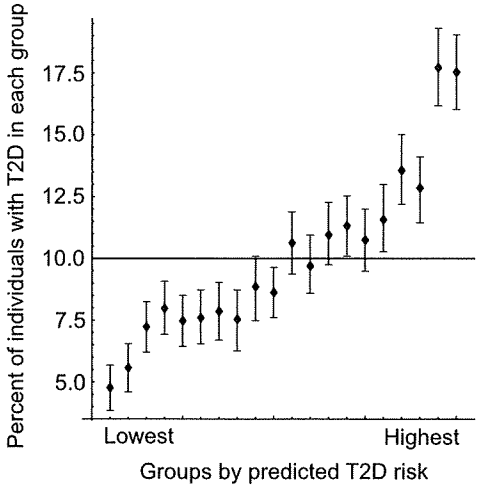

FIG. 4. Estimation of the increase in type 2 diabetes risk from the combination of seven susceptibility variants previously identified and robustly replicated in the current study. We used case and control subjects with complete data from all stages of our study (n = 12,105). First, the risk for the genotypes of an SNP was estimated by logistic regression. Then, the multilocus risk for an individual was assessed as the sum of the risks for his/her genotype at seven SNPs. We simulated a population with 10% prevalence by bootstrap sampling. In the simulated population, we arranged the individuals in the order of their multilocus risk, sorted them into 20 equal-sized groups, and calculated the actual prevalence in each group. Means and 95% CIs of the groupwise prevalence were estimated based on 1,000 bootstrap sampling trials and are plotted in the figure. No significant gene-gene interaction was observed between the seven SNPs by multiple logistic regression analysis. T2D, type 2 diabetes.

investigate the effect of control selection criteria on OR in a case-control comparison and found that the ORs in our meta-analysis were almost comparable to those estimated in the general Japanese population (supplementary Table S10). Moreover, with regard to ethnic diversity, linkage disequilibrium in *CDKAL1* and *KCNJ11* is stronger in East Asians (JPT + CHB), whereas linkage disequilibrium in *IGFBP2* and *HHEX* tends to be stronger in Europeans (CEU [Centre d'Etude du Polymorphisme Humain (CEPH) subjectsfrom Utah]) (Supplementary Figure S4); thus, besides the issue of power, the results of the GWA scans in the Japanese population (or East Asians) seem to be useful in terms of interethnic comparison of association signals, which may enhance the power of fine-mapping efforts designed to identify the causal variants (45).

The tendency of stronger genetic association in the Japanese population is also supported by the concomitant evaluation of multilocus effects. When assuming an additive model, the combined risk of type 2 diabetes can be measured by the sum of the R^2 values of individual loci. For example, the total variance explained by the seven loci depicted in Fig. 3 is 0.030 in the Japanese population and 0.018 in populations of European descent. It remains unknown whether these findings reflect higher heritability of type 2 diabetes in Japanese than in European-descent populations. Because little data are available on the estimation of heritability in the Japanese or East Asian populations, further studies are required to obtain the standardized measures of heritability across different populations by taking into account potential sources of heterogeneity, such as the degree of westernization of lifestyle.

Suggestive evidence of association was identified for SNPs in the *PEPD* gene. *PEPD* plays an important role in collagen metabolism, and some extracellular matrix constituents such as collagen IV have been shown to have a profound impact on insulin secretion (46). Moreover, enhanced collagen degradation via *PEPD* activity has been reported in diabetic patients (47). Although there is evidence suggestive of association at *PEPD* in all three stages, the current GWA study by itself could not confirm or refute the evidence; no significant association was found in the previously reported Diabetes Genetics Replication and Meta-Analysis (DIAGRAM) data from Europeans (risk allele frequency = 0.52, OR = 1.03) (Table 1) and in the initial screening data of the JSNP (Japanese Single Nucleotide Polymorphisms) scan in the Japanese population (187 casevs. 752 random control subjects; P = 0.18 at rs2241380, which is in complete linkage disequilibrium with rs10425678 in *PEPD*; r^2 = 1.0) (25).

The number of genes that could account for an appreciable population-attributable fraction of common diseases is under debate (48). Although the current study detected and/or replicated a total of nine susceptibility loci, including *PEPD* in the Japanese population, a substantial number of SNPs showing some extent of association signals in the first-stage scan remain to be investigated, as reflected by the wide distribution of replicated SNPs with unexamined "gaps" in the lower-left part of the Q-Q plot (supplementary Fig. S7). The ORs corresponding to such unexamined SNPs mostly fall in the range of 1.10–1.25. To assess the statistical power in our GWA scan, we simulated the frequency at which a disease-associated SNP could surpass the cutoff level of the first two stages (stages 1 and 2) (supplementary information and supplementary Table S11). In the current experimental setting, it is likely that >50% of the susceptibility loci

TABLE 2
Combined risk of diabetes and pre-diabetic status based on seven confirmed loci, age, BMI, and sex in the general Japanese population

		Diabetes		Pre-diabetes		Diabetes + Pre-diabetes	
	A1C (%)	RR versus population average (95% CI)	Prevalence	RR versus population average (95% CI)	Prevalence	RR versus population average (95% CI)	Prevalence
Male							
Whole population	5.29 ± 0.88	1.00	0.16	1.00	0.07	1.00	0.23
Highest risk group (5%) assessed by							
All predictors	5.48 ± 0.87	1.65 (1.29–1.97)	0.27	1.34 (0.73–1.83)	0.09	1.56 (1.26–1.78)	0.36
SNP genotypes	5.57 ± 1.12	1.67 (1.32–2.06)	0.27	0.92 (0.44–1.40)	0.07	1.45 (1.16–1.73)	0.34
Age and BMI*	5.44 ± 0.78	1.16 (0.87–1.46)	0.19	1.95 (1.39–2.60)	0.14	1.40 (1.16–1.65)	0.33
Lowest risk group (5%) assessed by							
All predictors	4.98 ± 0.73	0.46 (0.26–0.74)	0.08	0.50 (0.20–0.90)	0.04	0.47 (0.33–0.70)	0.11
SNP genotypes	5.11 ± 0.74	0.72 (0.39–0.92)	0.12	0.71 (0.30–1.10)	0.05	0.72 (0.46–0.86)	0.17
Age and BMI*	4.98 ± 0.77	0.46 (0.30–0.73)	0.08	0.40 (0.10–0.60)	0.03	0.44 (0.27–0.63)	0.10
Female							
Whole population	5.17 ± 0.60	1.00	0.07	1.00	0.06	1.00	0.13
Highest risk group (5%) assessed by							
All predictors	5.55 ± 0.96	3.09 (2.36–3.73)	0.22	2.05 (1.37–2.60)	0.13	2.61 (2.10–2.96)	0.35
SNP genotypes	5.37 ± 0.88	2.30 (1.60–2.78)	0.17	1.17 (0.73–1.80)	0.07	1.78 (1.41–2.10)	0.24
Age and BMI*	5.42 ± 0.78	2.26 (1.71–2.78)	0.16	1.95 (1.34–2.53)	0.12	2.12 (1.73–2.46)	0.28
Lowest risk group (5%) assessed by							
All predictors	4.91 ± 0.43	0.16 (0.00–0.32)	0.01	0.14 (0.00–0.28)	0.01	0.15 (0.04–0.26)	0.02
SNP genotypes	5.02 ± 0.45	0.45 (0.16–0.73)	0.03	0.80 (0.38–1.22)	0.05	0.61 (0.35–0.83)	0.08
Age and BMI*	4.94 ± 0.36	0.24 (0.08–0.64)	0.02	0.19 (0.00–0.37)	0.01	0.22 (0.09–0.47)	0.03

Data are the means ± SD, unless otherwise indicated. Relative risk (RR) is calculated as the ratio of the prevalence in 5% of people with the highest or lowest risk to the prevalence in the whole population. In this study, the combined disease risk for each individual was assessed using the regression for A1C (see supplementary information). Subjects with self-reported diabetes or with A1C ≥6.1 were classified as diabetic, and those who were not under antidiabetic medication and with 5.6 ≤ A1C < 6.1 were classified as pre-diabetic. The actual A1C level and the distribution by diabetic status for each 5% subgroup of the risk group are illustrated in supplementary Fig. S6. *For reference, diabetes and/or pre-diabetes risk was assessed using the participant's age and BMI alone as predictors.

with modest but substantial effects (OR = 1.2–1.3) were unidentified. For example, though not statistically significant, the association of *PPARG* in the Japanese population showed an OR (P = 0.06, OR = 1.18 at rs1801282) similar to that in populations of European descent in a meta-analysis, including the current study (supplementary Table S4). Increasing the sample size of the stage 1 panel and/or the number of SNPs genotyped in the second-stage scan would allow us to discover more susceptibility variants, including new population-specific loci, in the Japanese population.

The incidence of type 2 diabetes is escalating to epidemic proportions globally, with a higher acceleration rate in non-European populations (49). The integration of GWA study results, i.e., a meta-analysis (17), for both European-descent and non-European populations is necessary for a comprehensive understanding of the genetics of type 2 diabetes, and it will lead to the efficient use of genomic information based on ethnic diversity in clinical research.

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ARTICLE

Common variants at the *GCK*, *GCKR*, *G6PC2–ABCB11* and *MTNR1B* loci are associated with fasting glucose in two Asian populations

F. Takeuchi · T. Katsuya · S. Chakrewarthy · K. Yamamoto · A. Fujioka · M. Serizawa · T. Fujisawa · E. Nakashima · K. Ohnaka · H. Ikegami · T. Sugiyama · T. Nabika · A. Kasturiratne · S. Yamaguchi · S. Kono · R. Takayanagi · Y. Yamori · S. Kobayashi · T. Ogihara · A. de Silva · R. Wickremasinghe · N. Kato

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Abstract
Aims/hypothesis To test fasting glucose association at four loci recently identified or verified by genome-wide association (GWA) studies of European populations, we performed a replication study in two Asian populations.

Methods We genotyped five common variants previously reported in Europeans: rs1799884 (*GCK*), rs780094 (*GCKR*), rs560887 (*G6PC2–ABCB11*) and both rs1387153 and rs10830963 (*MTNR1B*) in the general Japanese (n=4,813) and Sri Lankan (n=2,319) populations. To identify novel

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F. Takeuchi · M. Serizawa · N. Kato (✉)
Department of Gene Diagnostics and Therapeutics,
Research Institute, International Medical Center of Japan,
1-21-1 Toyama, Shinjuku-ku,
Tokyo 162-8655, Japan
e-mail: nokato@ri.imcj.go.jp

T. Katsuya
Department of Clinical Gene Therapy,
Osaka University Graduate School of Medicine,
Suita, Japan

S. Chakrewarthy · A. Kasturiratne · A. de Silva · R. Wickremasinghe
Faculty of Medicine, University of Kelaniya,
Kelaniya, Sri Lanka

K. Yamamoto
Department of Molecular Genetics,
Medical Institute of Bioregulation, Kyushu University,
Fukuoka, Japan

A. Fujioka
Amagasaki Health Medical Foundation,
Amagasaki, Japan

T. Fujisawa
Department of Geriatric Medicine,
Osaka University Graduate School of Medicine,
Suita, Japan

E. Nakashima
Division of Endocrinology and Diabetes,
Department of Internal Medicine,
Nagoya University Graduate School of Medicine,
Nagoya, Japan

E. Nakashima
Department of Metabolism and Endocrine Internal Medicine,
Chubu Rosai Hospital,
Nagoya, Japan

K. Ohnaka
Department of Geriatric Medicine,
Graduate School of Medical Sciences, Kyushu University,
Fukuoka, Japan

H. Ikegami
Department of Endocrinology, Metabolism and Diabetes,
Kinki University School of Medicine,
Osaka-Sayama, Japan

T. Sugiyama
Institute for Adult Diseases, Asahi Life Foundation,
Tokyo, Japan

T. Nabika
Department of Functional Pathology,
Shimane University School of Medicine,
Izumo, Japan

variants, we further examined genetic associations near each locus by using GWA scan data on 776 non-diabetic Japanese samples.

Results Fasting glucose association was replicated for the five single nucleotide polymorphisms (SNPs) at $p<0.05$ (one-tailed test) in South Asians (Sri Lankan) as well as in East Asians (Japanese). In fine-mapping by GWA scan data, we identified in the *G6PC2–ABCB11* region a novel SNP, rs3755157, with significant association in Japanese ($p=2.6\times10^{-8}$) and Sri Lankan ($p=0.001$) populations. The strength of association was more prominent at rs3755157 than that of the original SNP rs560887, with allelic heterogeneity detected between the SNPs. On analysing the cumulative effect of associated SNPs, we found the per-allele gradients ($\beta=0.055$ and 0.069 mmol/l in Japanese and Sri Lankans, respectively) to be almost equivalent to those reported in Europeans.

Conclusions/interpretation Fasting glucose association at four tested loci was proven to be replicable across ethnic groups. Despite this overall consistency, ethnic diversity in the pattern and strength of linkage disequilibrium certainly exists and can help to appreciably reduce potential causal variants after GWA studies.

Keywords Asians · Association study · Ethnicity · Fasting plasma glucose · Polymorphisms

Abbreviations

CEU	Utah residents with northern and western European ancestry from the Centre d'Etude du Polymorphisme Humain collection
FPG	Fasting plasma glucose
GWA studies	Genome-wide association studies
JPT	Japanese in Tokyo
LD	Linkage disequilibrium
RAF	Risk allele frequency
SNP	Single nucleotide polymorphism

S. Yamaguchi
Internal Medicine III, Shimane University School of Medicine,
Izumo, Japan

S. Kono
Department of Preventive Medicine,
Graduate School of Medical Sciences, Kyushu University,
Fukuoka, Japan

R. Takayanagi
Department of Medicine and Bioregulatory Science,
Graduate School of Medical Sciences, Kyushu University,
Fukuoka, Japan

Y. Yamori
Mukogawa Women's University,
Institute for World Health Development,
Nishinomiya, Japan

S. Kobayashi
Shimane University Hospital,
Izumo, Japan

T. Ogihara
Osaka General Medical Center,
Osaka, Japan

Introduction

Fasting plasma glucose (FPG) levels are associated with the future risk of type 2 diabetes and cardiovascular diseases [1, 2] and are tightly regulated despite considerable variation in food intake [3]. It has been reported that genetic effects explain 54.8% of the variance of glucose levels in a European population [4]. Recent progress in complex-trait genetics has allowed the identification of loci regulating FPG levels [5–20].

Several loci influencing FPG levels have been identified or verified by genome-wide association (GWA) studies of Europeans; these include glucokinase (*GCK*) [5–7], glucokinase regulatory protein (*GCKR*) [8–10, 13], glucose-6-phosphatase catalytic subunit 2 (*G6PC2*), the ATP-binding cassette, subfamily B (*MDR/TAP*), member 11 (*ABCB11*) [14–17], and melatonin receptor 1B (*MTNR1B*) [16–18].

All the associations were originally identified in populations of European ancestry. While some studies have shown reproducible associations [9, 11, 12, 19, 20], it remains to be further defined to what degree loci discovered in Europeans will show an association in populations of different ancestries. In addition, to localise the variant(s) responsible for an association signal, we need to generate a comprehensive list of potential causal variants in the regions of interest, i.e. to conduct fine-mapping after GWA studies. As discussed elsewhere [21], this fine-mapping will be challenging and genetic information from populations of different ancestries is expected to be useful [21–24].

Apart from assessing the previously identified variants in two Asian populations, Japanese of East Asian ancestry and Sri Lankan of South Asian ancestry, we also explored index single nucleotide polymorphism (SNP) markers, which either tag the SNPs attaining a locus-wise significance level in the GWA scan of Japanese or were previously reported in Europeans. This was done to advance the fine-mapping of the associated loci [5–20].

Methods

Study populations

A replication study of the previously identified variants was performed in the general Japanese and Sri Lankan

populations (Electronic supplementary material [ESM] Table 1, ESM Study samples for continuous traits), using 5,456 Japanese samples (including 4,813 non-diabetic participants) consecutively enrolled in a population-based setting as described elsewhere [25] and 3,012 Sri Lankan samples (including 2,319 non-diabetic participants) who had participated in the baseline survey of the Ragama Health Study [26] in Sri Lanka. Complementary to this replication study, we organised genetic studies of FPG levels as part of an ongoing GWA scan for cardiometabolic disorders among the Japanese population (ESM Study samples for continuous traits). We used 776 population-based, non-diabetic Japanese samples for preliminary screening of association with FPG levels. Then, the association signals were examined in the general populations mentioned above. In addition to quantitative trait analysis, type 2 diabetes associations were tested for index SNPs at *G6PC2–ABCB11* in a Japanese case–control study panel comprising 5,629 cases and 6,406 controls as previously reported [27], and in a Sri Lankan case–control study panel (ESM Study samples for type 2 diabetes case–control studies). All participants from these different studies provided written informed consent and the local Ethics Committees approved the protocols.

Type 2 diabetes was diagnosed according to the WHO criteria as described in ESM Study samples for type 2 diabetes case–control studies.

SNP genotyping and quality control

In the replication study, samples were genotyped using the TaqMan assay (Life Technologies Japan, Tokyo, Japan) for five SNPs from four gene loci previously identified in European-descent populations [5–10, 13–18]. These included *GCK* (rs1799884), *GCKR* (rs780094), *G6PC2–ABCB11* (rs560887) and *MTNR1B* (rs1387153 and rs10830963).

In the GWA scans, genotyping was performed with a bead array (Infinium HumanHap550; Illumina, San Diego, CA, USA) as described elsewhere [27] (ESM Fig. 1, ESM SNP genotyping, ESM Quality control of the GWA scan data). After the GWA scan, three additional SNPs in the *G6PC2–ABCB11* region, rs483234, rs3755157 and rs853778, were genotyped with the TaqMan assay for follow-up.

Statistical analysis

SNP association analysis SNPs were tested for association with FPG levels by using linear regression analysis in the additive genotype model (ESM SNP-based association analysis). A p value of <0.05 was considered statistically significant. For an association to be considered significant, it had to involve the same risk allele as that reported in Europeans and was accordingly assessed with a one-tailed test. To assess the proportion of variance for FPG that could be explained by a SNP, we calculated the coefficient of determination R^2. The per-allele gradients, which correspond to the increase in FPG levels by additional 'high FPG' alleles of associated SNPs, were calculated in the linear regression model (including age, sex and BMI as covariates) as previously reported [14, 18] (ESM Evaluation of cumulative effect of multiple loci on FPG). We used PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/), the R software (version 2.8.1; www.r-project.org) and the rmeta package (http://cran.r-project.org) for association test and meta-analysis (websites accessed 15 October 2009).

Haplotype analysis In the *G6PC2–ABCB11* region, we selected SNPs attaining a locus-wise significance level (p<0.002 by Bonferroni's correction for 23 SNPs genotyped in the relevant region) or reported in European studies, inferring the haplotypes using PLINK [28] and PHASE [29] software (http://depts.washington.edu/ventures/UW_Technology/Express_Licenses/PHASEv2.php). We then tested which haplotypes were strongly associated with the trait. In parallel, haplotypes were inferred from the genotype data of the SNPs in HapMap (www.hapmap.org) Utah residents with northern and western European ancestry from the Centre d'Etude du Polymorphisme Humain collection (CEU) and Japanese in Tokyo (JPT) categories using HaploView software (www.broad.mit.edu/mpg/haploview/) [30] and in South Asians from the Human Genome Diversity Panel (http://hagsc.org/hgdp/files.html) [31]. Haplotype-tagging SNPs were selected and characterised in the large study panels.

Stepwise regression analysis for testing of independent associations To test the most likely explanation for the signal of association among the index SNPs and their genotyped correlates, we performed stepwise linear regression analysis for FPG levels by forward selection (ESM Index SNPs showing an independent association). If two SNPs simultaneously included in the model each attained significance (p<0.05), they could have independent associations. Further, when two haplotype classes that are distant in the phylogeny have an opposite effect and are tagged by two SNPs showing independent associations, the haplotype classes are presumably linked to different causative variants, thus implying allelic heterogeneity (ESM Haplotype explaining index association).

Cross-population filtering of causal variants To appreciably narrow the location of potential causal variants, we closely inspected subsets of SNPs and haplotypes shared by multiple ethnicities. We partitioned all the HapMap SNPs

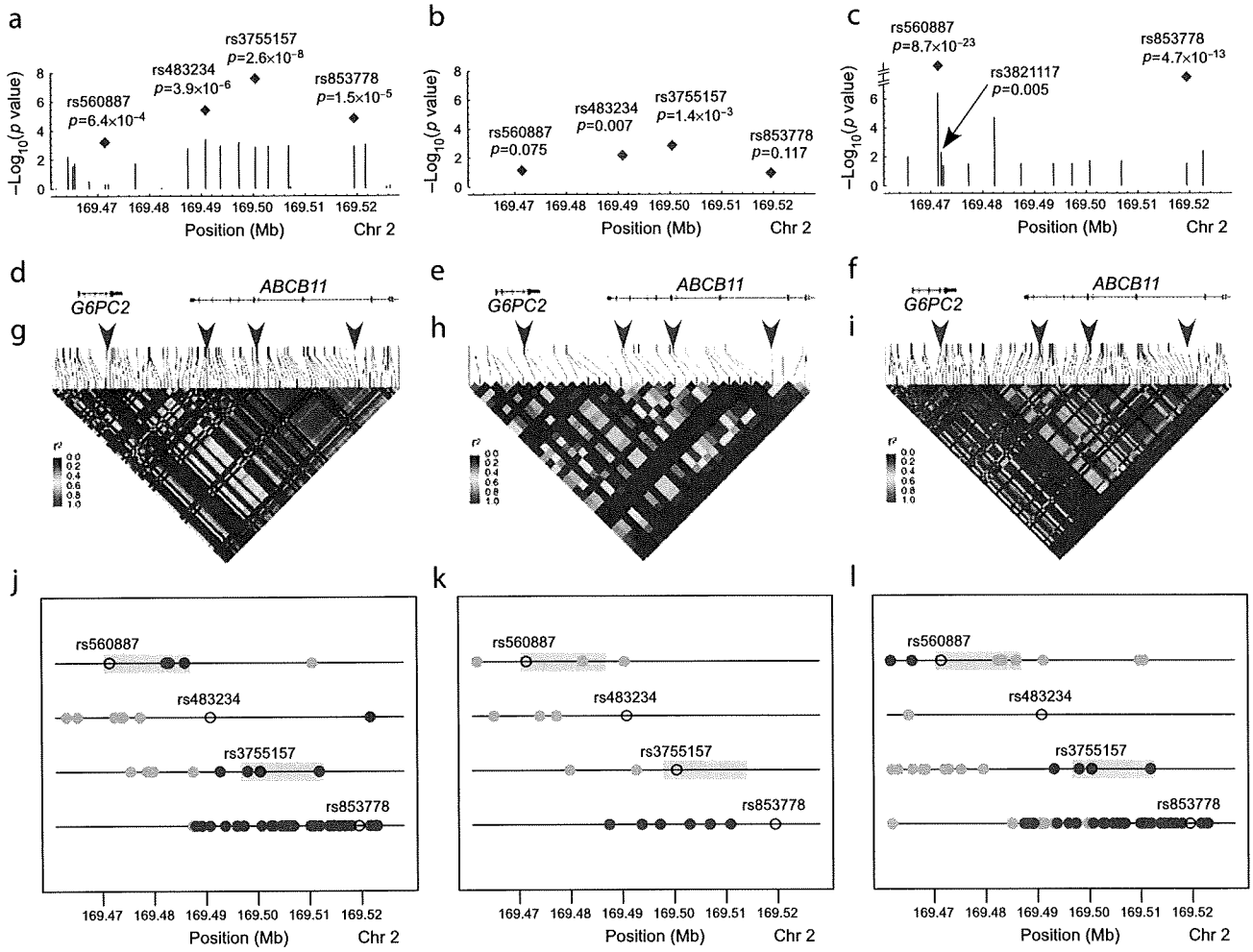

Fig. 1 Plots of FPG association, and LD and SNP partitioning for the *G6PC2–ABCB11* region in Japanese (**a**, **d**, **g**, **j**), Sri Lankan (**b**, **e**, **h**, **k**) and Europeans (**c**, **f**, **i**, **l**). Association results for Europeans are drawn from the published studies [14, 17]. **a–c** Bar graphs of all genotyped SNPs that passed the quality control (ESM Table 6) in the Japanese GWA scan (**a**) and those in the published European GWA scan [14] (**c**) with $-\log_{10}$ (p values) for FPG plotted against chromosome position in Mb. Red diamonds, p values for the genotypes of general populations (n=4,813 in Japanese, n=2,319 in Sri Lankan) and meta-analysis data (>12,000 in the ENGAGE consortium [17]). **d–f** Genomic location of *G6PC2* and *ABCB11* genes with intron and exon structure (NCBI Build 36) in the relevant populations. **g–l** WGAViewer (http://people.genome.duke.edu/~dg48/WGAViewer/whatis.php) [36] plot of LD (r^2) for all SNPs across the regions for the HapMap populations JPT (**g**) and CEU (**i**), and for the Human Genome Diversity Panel, ethnic groups belonging to South Asia (**h**). **j–l** SNPs in the LD block were partitioned into four subsets using the extent of LD with lead SNPs and/or haplotype-tagging SNPs, i.e. rs560887, rs483234, rs3755157 and rs853778 (see Methods). Red circles, SNP with $0.8 \leq r^2 \leq 1.0$ to the index SNP; orange circles, SNP with $0.6 \leq r^2 < 0.8$ to the index SNP; green bars, intervals where causative variants are most likely to be located

located in the *G6PC2–ABCB11* region (Fig. 1) so that SNPs in the strongest linkage disequilibrium (LD) with one index SNP (e.g. rs3755157) rather than with other index SNPs (e.g. rs560887, rs483234 and rs853778) were grouped into a bin of rs3755157 correlates. We then narrowed target intervals by investigating a subset (or subsets) of variants that could show a consistent pattern of trait association across different ethnic groups (ESM Narrowing target intervals in fine-mapping).

Results

Association with FPG and metabolic traits at four loci

Significant (p<0.05 by one-tailed test) association was replicated for all five SNPs from four tested loci in the Sri Lankan and Japanese populations (Table 1). Together with the previous reports in Europeans and Chinese [8, 11, 12, 17, 18, 20], we performed meta-analysis of FPG associa-

Table 1 Association of SNPs with fasting plasma glucose level

SNP	Neighbouring gene(s)	Alleles		Japanese panel (n=4,813)			Sri Lankan panel (n=2,319)			Europeans	
		FPG+[a]	FPG−[a]	Allele frequency[b]	Per-allele effect[c]	p value	Allele frequency[b]	Per-allele effect[c]	p value	Allele frequency[d]	Per-allele effect[c,e]
Reported in European studies											
rs780094	*GCKR*	G	A	0.44	0.032 (0.012, 0.052)	0.002	0.80	0.074 (0.035, 0.113)	2.1×10^{-4}	0.62	0.067 (0.045, 0.090)
rs560887	*G6PC2–ABCB11*	G	A	0.97	0.103 (0.044, 0.162)	6.4×10^{-4}	0.91	0.050 (−0.005, 0.105)	0.075	0.67	0.064 (0.056, 0.072)
rs1799884	*GCK*	A	G	0.18	0.075 (0.049, 0.101)	1.1×10^{-8}	0.12	0.076 (0.028, 0.123)	0.002	0.20	0.062 (0.048, 0.076)
rs1387153	*MTNR1B*	T	C	0.41	0.058 (0.038, 0.078)	9.7×10^{-9}	0.38	0.036 (0.005, 0.068)	0.024	0.28	0.07 (0.05, 0.08)
rs10830963	*MTNR1B*	G	C	0.42	0.056 (0.036, 0.075)	2.9×10^{-8}	0.45	0.064 (0.033, 0.094)	3.6×10^{-5}	0.30	0.072 (0.062, 0.082)
Tested in addition[f]											
rs483234	*G6PC2–ABCB11*	A	G	0.51	0.046 (0.026, 0.065)	3.9×10^{-6}	0.42	0.043 (0.012, 0.074)	0.007	0.70	–
rs3755157	*G6PC2–ABCB11*	T	C	0.38	0.057 (0.037, 0.078)	2.6×10^{-8}	0.16	0.069 (0.027, 0.111)	0.001	0.07	–
rs853778	*G6PC2–ABCB11*	A	G	0.40	0.044 (0.024, 0.064)	1.5×10^{-5}	0.35	0.026 (−0.006, 0.058)	0.117	0.46	–

Type 2 diabetes participants were excluded from the analysis; FPG association of each SNP was tested using linear regression models with adjustment for BMI, age and sex

[a] FPG-increasing (+) and decreasing (−)

[b] Of FPG-increasing allele

[c] Effect (95% CI) (mmol/l)

[d] Of FPG-increasing allele in HapMap CEU panel

[e] Association results were drawn from the previous studies: rs560887, rs4607517 (in substitution for rs1799884, $r^2=1$ in CEU) and rs10830963 [17]; rs1260326 (in substitution for rs780094, $r^2=0.93$ in CEU) [8]; rs1387153 [18]

[f] In the *G6PC2–ABCB11* region

tions to compare the effect sizes among different ethnic groups (ESM Fig. 2). Among the four loci, significant cross-population heterogeneity was detected for rs1387153 (*MTNR1B*; $p=0.03$). The variance for FPG that was explained by the associated SNPs totalled 2% in both the Japanese and Sri Lankan populations (ESM Table 2). Per-allele gradients in the two Asian populations ($\beta=0.055$ and 0.069 mmol/l in Japanese and Sri Lankans, respectively) were almost equivalent to those reported in populations of European descent ($\beta=0.07$ mmol/l) [18] (ESM Fig. 3, ESM Evaluation of cumulative effect of multiple loci on FPG).

Besides FPG levels, we analysed the relationship of SNPs with lipid traits (ESM Tables 3 and 4). Notably, rs780094 (*GCKR*) significantly and consistently modulated triacylglycerol levels in both ethnic groups ($p=2.2\times10^{-10}$ in Japanese, $p=1.4\times10^{-4}$ in Sri Lankan populations), where glucose-increasing alleles were associated with lower triacylglycerol levels as previously reported [8, 9, 11, 13]. Furthermore, glucose-increasing alleles at rs1799884 (*GCK*) and rs10830963 (*MTNR1B*) were significantly associated with reduced beta cell function (HOMA-B; $p=0.037$ for rs1799884, $p=2.6\times10^{-4}$ for rs10830963 in the Sri Lankan population), with no appreciable effect on fasting insulin or insulin sensitivity (ESM Table 5).

Refinement of genetic association in the *G6PC2–ABCB11* region

In fine-mapping with Japanese GWA scan data, we identified in the *G6PC2–ABCB11* region a novel associated SNP rs3755157, which was proven to be independent of the SNPs previously reported by GWA studies in Europeans [14–17].

In our GWA scan, multiple and significant SNPs were found in the *G6PC2–ABCB11* region ($p=0.0004$ to 0.002; Fig. 1, ESM Table 6) but not in the other candidate regions (ESM Tables 7–9). We therefore performed a detailed investigation of the *G6PC2–ABCB11* region. With reference to the LD and haplotype data (ESM Tables 10–12), we chose four haplotype-tagging SNPs (rs3755157, rs483234, rs853778 and rs560887) for genotyping the general Japanese population ($n=4{,}813$), which resulted in concordant evidence of associations between the tests of individual SNPs and those of haplotypes (Tables 1 and 2, ESM Haplotype explaining index association). The most significantly associated haplotype, class 5 (frequency = 0.35, $p=2.8\times10^{-7}$) (Table 2), was almost unequivocally tagged by rs3755157, which showed the strongest association by SNP-based test in the general Japanese population ($p=2.6\times10^{-8}$; Table 1).

We then performed a stepwise linear regression (for FPG levels) to test whether one of the four haplotype-tagging SNPs was necessary and sufficient to explain the association signal (ESM Tables 13–17, ESM Fig. 4, ESM Index SNP showing an independent association). The FPG association remained significant ($p<0.05$) when two haplotype-tagging SNPs, rs3755157 and rs560887, were included in the regression model (ESM Table 13). This independent association had gone unnoticed among more significant associations of SNPs that were in strong LD with a leading SNP, rs560887, among Europeans [14, 15]. Thus the presence of a novel SNP, rs3755157, and of allelic heterogeneity (ESM Fig. 5)

Table 2 Fasting glucose association according to haplotypes in the *G6PC2–ABCB11* region

Haplotype class	Tested SNPs[a]				Japanese panel[b]			Sri Lankan panel[c]			Europeans
	rs560887	rs483234	rs3755157	rs853778	Frequency	Effect (mmol/l)	*p* value	Frequency	Effect (mmol/l)	*p* value	Frequency[d]
1	G[a]	G	C	G	0.48	−0.047	2.3×10^{-6}	0.56	−0.041	9.6×10^{-3}	0.26
2	G[a]	G	C	A[a]	0	–	–	0.02	−0.029	0.65	0
3	G[a]	A[a]	C	G	0.09	0.004	0.81	0.08	0.035	0.23	0.28
4	G[a]	A[a]	C	A[a]	0.01	0.048	0.31	0.09	0.030	0.28	0.07
5	G[a]	A[a]	T[a]	A[a]	0.35	0.054	2.8×10^{-7}	0.16	0.072	9.8×10^{-4}	0.08
6	G[a]	A[a]	T[a]	G	0.02	0.053	0.12	0	–	–	0
7	A	A[a]	C	A[a]	0.02	−0.098	2.5×10^{-3}	0.08	−0.078	8.1×10^{-3}	0.28

FPG association was tested with adjustment for BMI, age and sex

[a] Allele increasing fasting glucose

[b] 4,792 complete observations

[c] 2,306 complete observations

[d] Haplotype frequency estimated in the HapMap CEU panel

Table 3 Association of FPG-altering SNPs with type 2 diabetes in the *G6PC2–ABCB11* region

SNP	rs560887 (FPG-increasing allele: G)	rs483234 (FPG-increasing allele: A)	rs3755157 (FPG-increasing allele: T)
Japanese (JPN)[a]			
Frequency, cases (n=5,629)	0.974	0.508	0.380
Frequency, controls (n=6,406)	0.969	0.490	0.360
OR (95% CI)	1.20 (1.03–1.41)	1.07 (1.02–1.13)	1.09 (1.03–1.15)
p value for trend	0.019	0.0056	0.0017
Sri Lankan (SL)[b]			
Frequency, cases (n=599)	0.917	0.422	0.169
Frequency, controls (n=515)	0.896	0.408	0.159
OR (95% CI)	1.28 (0.95–1.73)	1.06 (0.89–1.26)	1.08 (0.86–1.36)
p value for trend	0.08	0.51	0.52
JPN and SL combined			
OR (95% CI)	1.22 (1.06–1.40)	1.07 (1.02–1.13)	1.09 (1.03–1.15)
European-descent			
OR (95% CI)	0.93 (0.89–0.97)[c]	1.05 (0.99–1.13)[d]	1.00 (0.91–1.09)[d]
p value	0.0017	0.12	0.93

Type 2 diabetes association was tested with the Cochran–Armitage trend test in the case–control analysis

[a] SNPs were genotyped in a Japanese case–control study panel independently of the general Japanese population [27]

[b] In the Sri Lankan population, 515 controls are part of those used for FPG association analysis, whereas 599 cases were independent participants

[c] Results for 18,236 cases and 64,453 controls from a previous study [17]

[d] Results for 4,549 cases and 5,579 controls from the DIAGRAM consortium [37]

has become evident in the *G6PC2–ABCB11* region for the first time, as a result of comparing the GWA scan data between European and Japanese populations.

In the *G6PC2–ABCB11* region, *G6PC2* and *ABCB11* are both biologically plausible candidate genes [15, 32, 33]. During fine-mapping, we attempted to partition the LD block into intervals, each containing SNPs strongly correlated with an index SNP, in the hope that correlation coefficients r^2 would reflect phylogenic closeness once the index SNPs were selected from a reasonably dense set of SNP markers. This partitioning approach helped to prioritise the target interval for fine-mapping, thereby reducing the potential candidate variants to manageable proportions. For the *G6PC2–ABCB11* region, the target intervals were estimated to be 14 kb (in the *ABCB11* gene) for rs3755157 and 14 kb (in the *G6PC2* gene and between the genes) for rs560887 when the LD threshold was set at $r^2 \geq 0.6$ in the HapMap data (Fig. 1, ESM Table 18, ESM Narrowing target intervals in fine-mapping).

Concordance of association for FPG levels and type 2 diabetes

In addition to the quantitative trait analysis of FPG, we performed case–control analysis of type 2 diabetes for three of four haplotype-tagging SNPs, rs3755157, rs483234 and rs560887, in the *G6PC2–ABCB11* region and confirmed a significant ($p<0.05$) association in a relatively large study panel comprising 5,629 cases and 6,406 controls. The strongest association was found for rs3755157 (OR 1.09, 95% CI 1.03–1.15, $p=1.7\times10^{-3}$; Table 3), which was in good agreement with the FPG association mentioned above. To confirm the consistency of associations with increases in FPG levels and the risk of type 2 diabetes, we examined the changes in risk allele frequency (RAF) between the diabetes subgroup and non-diabetic participants in quartiles of FPG levels stratified in the general populations for unbiased estimates (ESM Fig. 6, ESM Table 19). In the Japanese and Sri Lankan populations, the RAF in the diabetes subgroup reached the second highest quartile, supporting the concordant association for FPG levels and the risk of type 2 diabetes in the *G6PC2–ABCB11* region.

Discussion

The present study has proven that common variant loci influencing FPG levels are reproducible in two populations of Asian descent, Japanese (East Asians) and Sri Lankan

(South Asians). To our knowledge, this is the first study investigating the genetic associations with FPG and related metabolic traits at four candidate loci, *GCK*, *GCKR*, *G6PC2–ABCB11* and *MTNR1B*, in South Asians, who are known to have high prevalence of type 2 diabetes [34]. The combined impact of associated SNPs is almost equivalent across the ethnic groups despite some cross-population diversity in the effect size of individual loci (ESM Figs 2 and 3, ESM Table 2). Other novel aspects of the present study include a fine-mapping approach using Japanese GWA scan data and consistent associations of FPG and type 2 diabetes in the *G6PC2–ABCB11* region.

According to genome-wide patterns of SNPs examined in the Human Genomic Diversity Panel [31], much of sub-Saharan Africa, Europe, South and Central Asia (including Sri Lanka), and East Asia appear to be homogeneous and individuals from these populations can be distinguished from each other. Although limited in the number of examples, our study has provided evidence supporting the importance of human genetic diversity in complex disease studies. For instance, beside replicating FPG association at four candidate loci in two Asian populations, our data also clarified the genetic architecture of the *G6PC2–ABCB11* region with regard to ethnic diversity. Using the GWA scan data, we found a novel SNP, rs3755157, to be a leading SNP among Japanese and independent of a leading SNP, rs560887, in Europeans (ESM Table 13–15).

As a fine-mapping approach, we listed HapMap SNPs having the strongest r^2 (in the range of $r^2 \geq 0.6$) with each of the index SNPs in the *G6PC2–ABCB11* region (Fig. 1, ESM Table 18). We performed cross-population filtering, which appreciably decreased the number of potential causal variants from 79 to 8 in the *G6PC2–ABCB11* region (ESM Narrowing target intervals in fine-mapping). The novel SNP rs3755157 and its correlated SNPs are located in the 3′-side (introns) of the *ABCB11* gene. While four different mRNAs, two alternatively spliced variants and two unspliced forms, are known to be transcribed from the *ABCB11*, it is possible that the potential causal variant(s) will influence the selective production of any of the 3′-side mRNA variants or the alteration of mRNA expression. Thus, closer inspection of subsets of SNP haplotypes shared by multiple ethnicities may allow us to appreciably narrow the field of potential causal variants before starting in-depth resequencing and functional follow-up studies, as demonstrated for the *G6PC2–ABCB11* region. During the preparation of our manuscript, replication of the *G6PC2* association was also reported in a Chinese population [19], where four SNPs were selected from the HapMap database so as to tag common variations near the *G6PC2* gene. Although the index SNP (rs560887) originally detected in Europeans was not tested, three (of four) SNPs appeared to show significant association in the Chinese population, in agreement with our findings in Japanese.

Our data also verified concordance of association for FPG levels and type 2 diabetes risk by using a systematic study design; i.e. unbiased estimates with stratification of general populations plus large-scale case–control studies involving 12,035 Japanese and 1,114 Sri Lankan samples (Table 3, ESM Fig. 6). It has been debated whether the genetic determinants regulating FPG levels in physiological states differ from those increasing type 2 diabetes risk. Some studies report that carriers of glucose-increasing alleles at three loci (*MTNR1B*, *GCK* and *GCKR*) show a higher risk of type 2 diabetes [8, 17, 18], although there is no significant association between *G6PC2–ABCB11* variants and type 2 diabetes in populations of European descent [14, 17, 35]. In this context, our data not only supported the concordant association of *G6PC2–ABCB11* variants for FPG and type 2 diabetes in two Asian populations, but also indicated that genetic determinants regulating FPG levels could, at least in part, differ from those increasing type 2 diabetes risk (ESM Fig. 6, ESM Consistent association of fasting glucose and type 2 diabetes in the *G6PC2–ABCB11* region). It is likely that genetic susceptibility for FPG levels increases type 2 diabetes risk in the population at large, but that some diabetic patients will develop the disease independently of a predisposition to elevated FPG levels.

In summary, despite the overall reproducibility of FPG association across the populations, ethnic diversity in allele frequencies led to the discovery of allelic heterogeneity in the *G6PC2–ABCB11* region. The diversity in the LD pattern also helped to reduce the probable causative variants in the corresponding region. The prevalence of the phenomena described here in human complex trait genetics is another research area warranting investigation. For applicable cases, the use of ethnic diversity in genetic studies can constitute an efficient approach subsequent to GWA scan.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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