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Variants in *KCNQ1* are associated with susceptibility to type 2 diabetes mellitus

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We carried out a multistage genome-wide association study of type 2 diabetes mellitus in Japanese individuals, with a total of 1,612 cases and 1,424 controls and 100,000 SNPs. The most significant association was obtained with SNPs in *KCNQ1*, and dense mapping within the gene revealed that rs2237892 in intron 15 showed the lowest *P* value (6.7×10^{-13}, odds ratio (OR) = 1.49). The association of *KCNQ1* with type 2 diabetes was replicated in populations of Korean, Chinese and European ancestry as well as in two independent Japanese populations, and meta-analysis with a total of 19,930 individuals (9,569 cases and 10,361 controls) yielded a *P* value of 1.7×10^{-42} (OR = 1.40; 95% CI = 1.34–1.47) for rs2237892. Among control subjects, the risk allele of this polymorphism was associated with impairment of insulin secretion according to the homeostasis model assessment of β-cell function or the corrected insulin response. Our data thus implicate *KCNQ1* as a diabetes susceptibility gene in groups of different ancestries.

In Japan, the prevalence of type 2 diabetes mellitus is increasing rapidly, and more than 10% of individuals over 40 years of age are affected. Relatively few diabetic individuals in Japan are obese, and impairment of insulin secretion often develops before the onset of diabetes[1]. As part of a national project designated the Millennium Genome Project in Japan, in 2002 we began a multistage genome-wide association study (GWAS) to identify disease-associated SNPs for type 2 diabetes mellitus using 100,000 SNPs from a collection of

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Table 1 Positive SNPs identified in the third screening

				Panel 1 (187 cases)				Panel 2 (752 cases, 752 controls)				Panel 3 (672 cases, 672 controls)			
dbSNP ID	Risk allele	Chr.	Gene	RAF(DM)	RAF(NC)	OR (95% CI)	*P* value	Control	RAF(DM)	RAF(NC)	OR (95% CI)	*P* value	RAF(DM)	RAF(NC)	OR (95% CI)	*P* value
rs151290	C	11	*KCNQ1*	0.63	0.57	1.30 (1.03–1.65)	0.027	ODG	0.62	0.55	1.34 (1.16–1.55)	7.4×10^{-5}	0.61	0.54	1.36 (1.16–1.58)	1.1×10^{-4}
rs163184	G	11	*KCNQ1*	0.51	0.43	1.33 (1.06–1.67)	0.015	JDC	0.49	0.44	1.22 (1.06–1.41)	0.0064	0.48	0.42	1.27 (1.09–1.48)	0.0021
rs2237895	C	11	*KCNQ1*	0.45	0.35	1.53 (1.22–1.93)	2.8×10^{-4}	JDC	0.42	0.33	1.49 (1.28–1.73)	1.4×10^{-7}	0.42	0.33	1.45 (1.24–1.70)	3.4×10^{-6}
rs2250402	C	15	*EIF2AK4*	0.20	0.27	1.45 (1.09–1.93)	0.011	JDC	0.24	0.21	1.20 (1.01–1.43)	0.035	0.26	0.21	1.34 (1.11–1.60)	0.0018
rs2307027	C	12	*KRT4*	0.14	0.22	1.68 (1.20–2.36)	0.0024	ODG	0.20	0.17	1.23 (1.02–1.47)	0.031	0.21	0.16	1.37 (1.12–1.67)	0.0017
rs3741872	C	12	*FAM60A*	0.29	0.23	1.37 (1.06–1.76)	0.015	ODG	0.29	0.24	1.29 (1.09–1.52)	0.0024	0.28	0.23	1.28 (1.07–1.52)	0.0060
rs574628	G	20	*ANGPT4*	0.56	0.64	1.38 (1.09–1.74)	0.0066	ODG	0.65	0.61	1.17 (1.01–1.36)	0.037	0.64	0.59	1.28 (1.10–1.50)	0.0018
rs2233647	G	6	*SPDEF*	0.92	0.86	1.87 (1.07–3.27)	0.026	ODG	0.88	0.86	1.24 (1.00–1.54)	0.047	0.89	0.86	1.29 (1.02–1.62)	0.033
rs3785233[a]	C	16	*A2BP1*	0.20	0.17	1.20 (0.90–1.61)	0.22	ODG	0.19	0.16	1.25 (1.03–1.51)	0.023	0.19	0.16	1.23 (1.01–1.50)	0.039
rs2075931	A	1		0.71	0.64	1.37 (1.07–1.75)	0.013	ODG	0.68	0.65	1.17 (1.01–1.37)	0.038	0.68	0.64	1.18 (1.00–1.38)	0.048

P values were calculated for allele data. For panel 1, two control groups (ODG, other disease group; JDC, Japanese database control) were used for association studies and the lower *P* values are listed. RAF(DM) and RAF(NC), risk allele frequencies in cases and controls, respectively. OR, odds ratio for risk allele.
[a]This SNP was selected for the second stage on the basis of the recessive model (OR = 2.59, CI = 1.20–5.58, $P = 0.012$).

standard Japanese SNPs[2] (which we refer to as the JSNP Genome Scan (JGS)), as part of the multi-disease collaborative genome scan (**Supplementary Fig. 1** online).

Among 100,000 SNPs genotyped by multiplex PCR–based Invader analysis in the first stage of the study, 82,343 autosomal polymorphisms passed our typing quality control in 187 individuals with diabetes (**Supplementary Table 1** online). We then carried out two separate association analyses to compare the 187 individuals with diabetes with two different control groups, which we considered as population controls: one to compare allele frequencies with reference data for 752 individuals representing the general Japanese population deposited in the JSNP database (referred to as the 'JSNP database control' (JDC)), and one to compare allele or genotype frequencies with those of the 752 individuals in the initial panels for the other four disease groups (Alzheimer's disease, gastric cancer, hypertension and asthma) of the national project (referred to as the 'other disease group' (ODG)). The combination of two types of association analysis resulted in the selection of 2,880 SNPs for the second stage of the study. An independent case-control panel (panel 2) was analyzed, and 201 positive SNPs ($P < 0.05$) were selected for the third stage (see **Supplementary Table 2a** online). Ten SNPs yielded a *P* value of <0.05 at the third stage using another case-control panel (panel 3; **Table 1** and **Supplementary Table 2b**). These SNPs showed variable *P* values in the first stage, suggestive of a limited power of the study design. The most significant association ($P = 3.4 \times 10^{-6}$) was obtained with rs2237895, which is located in intron 15 of *KCNQ1*. Another two SNPs (rs151290 and rs163184) were also located in the same intron, yielding *P* values of 1.1×10^{-4} and 0.0021, respectively. Panels 2 and 3 combined (panel 2+3) were analyzed for these 10 SNPs, yielding even lower *P* values for all the SNPs (**Supplementary Table 2b**). The genotype-based Cochran-Armitage trend test gave *P* values similar to those based on the allele data (**Supplementary Table 2b**).

We further analyzed *KCNQ1*, which was the only gene that yielded positive results according to the standard criterion (*P* value of $<5 \times 10^{-7}$) recently proposed for GWAS[3]. The three SNPs of *KCNQ1* that passed the third scan (rs151290, rs163184 and rs2237895) were in moderate linkage disequilibrium (LD) with each other (**Fig. 1**). The SNP with the lowest *P* value, rs2237895, yielded D' and r^2 values of 0.54 and 0.12 with rs151290 and 0.83 and 0.46 with rs163184, respectively. We isolated 49 additional SNPs of *KCNQ1* from dbSNP of NCBI and typed them together with the three originally positive SNPs in panel 2+3 (**Fig. 1**). Among these 52 SNPs, rs2237892, which is also located in intron 15, showed the strongest association with diabetes ($P = 6.7 \times 10^{-13}$), with OR =1.49 and 95% CI = 1.34–1.66; the *P* value for the trend test was 1.7×10^{-12} (**Table 2**). The D' and r^2 values for rs2237895 and rs2237892 were 0.95 and 0.30, respectively.

We also sequenced all the exons and the 47-kb genomic region corresponding to intron 15 of *KCNQ1* in 24 Japanese individuals and identified 212 variations, including three synonymous and two nonsynonymous (P448R and G643S) polymorphisms (**Supplementary Table 3a** online). We then genotyped ten of the newly identified SNPs of intron 15 and the two nonsynonymous polymorphisms in panel 2+3. None of these SNPs showed a stronger association with diabetes than did rs2237892 (**Fig. 1** and **Supplementary Table 3b**).

We next examined the possible association of *KCNQ1* with diabetes in several additional subject panels, including those of other ancestral groups, by genotyping rs2237892, rs2237895 and rs2074196, the three SNPs that showed the strongest association in the original study. Two independent Japanese panels revealed a strong association of these

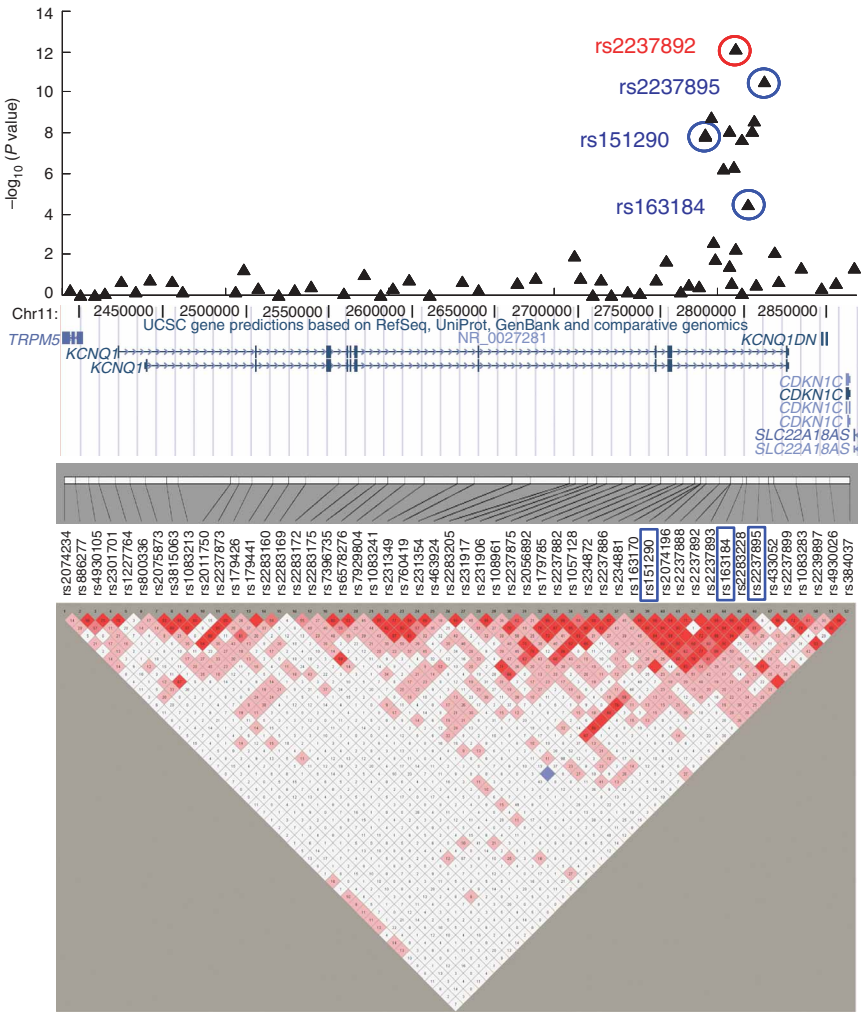

Figure 1 Dense mapping analysis of *KCNQ1*. The top panel shows the association $-\log_{10}$ (*P* value) in panel 2+3 for 64 SNPs of *KCNQ1*. The three blue circles represent the positive SNPs in the third screening. The red circle (rs2237892) indicates the SNP showing the most significant association with type 2 diabetes. The upper middle panel shows the physical position of *KCNQ1* and neighboring genes on chromosome 11 (UCSC Genome Browser). The lower middle panel shows the positions and rs numbers of the 52 previously identified SNPs. Blue rectangles indicate the positive SNPs in the third screening. The bottom panel shows a Haploview representation of LD (*D′*) based on genotyping data from control subjects in panel 2+3 ($n = 1{,}424$).

polymorphisms with diabetes (**Table 2** and **Supplementary Table 4** online); rs2237892, for example, showed allelic *P* values of 9.6×10^{-10} and 6.9×10^{-10} in the replication 1 and 2 panels, respectively. The three Japanese panels (panel 2+3 and replication 1 and 2), which included a total of 4,378 cases and 4,412 controls, yielded an allelic *P* value of 2.8×10^{-29} and OR of 1.43 (95% CI = 1.34–1.52) for rs2237892. The association was also reproduced in the replication 3 (Chinese) and replication 4 (Korean) panels; the allelic *P* values for rs2237892 in these two panels were 1.3×10^{-8} and 1.7×10^{-5}, respectively (**Table 2** and **Supplementary Table 4**). Meta-analysis of the Asian populations yielded a *P* value of 2.5×10^{-40} and OR of 1.42 (95% CI = 1.34–1.49) for rs2237892. We also examined rs2237892 and rs2074196 in the replication 5 panel (recruited from Sweden), with both SNPs showing a positive association ($P = 7.8 \times 10^{-4}$ and 0.017, respectively). With the inclusion of the replication 5 panel, meta-analysis with a total of 19,930 individuals (9,569 cases and 10,361 controls) yielded a *P* value of 1.7×10^{-42} and OR of 1.40 (95% CI = 1.34–1.47) for rs2237892 (**Table 2** and **Supplementary Fig. 2** online).

We next investigated the relation of rs2237892 to clinical phenotype. Among 1,424 individuals with diabetes in panel 2+3, no association was found between this SNP and clinical parameters such as body mass index (BMI) and the level of insulin resistance. Among the 948 control subjects in panel 2+3 whose fasting plasma glucose and insulin levels were available, homozygotes for the risk allele of rs2237892 (CC) showed a significantly lower homeostasis model assessment of β-cell function (HOMA-β)[4] than did those with the other genotypes (**Supplementary Table 5** online). Among nondiabetic subjects of the Botnia prospective cohort (**Supplementary Methods** online), the corrected insulin response (CIR) at the follow-up visit was significantly lower for individuals with the CC genotype of rs2237892 than for those with the other two genotypes in both an additive and recessive model for this SNP ($P = 0.024$ and 0.010, respectively; **Supplementary Table 5**). These results suggested that the risk allele of *KCNQ1* might contribute to diabetes susceptibility by impairing insulin secretion.

The multistage strategy for GWASs has an advantage in the effective elimination of a large number of false-positive results and has proved to be successful[5]. Indeed, we detected the association of several SNPs of *KCNQ1* with diabetes in the JGS, and this association was reproduced in two independent Japanese panels. *KCNQ1*, which encompasses 404 kb, is located at chromosome 11p15.5, not far from a candidate region at 11p13–p12 with suggestive evidence of linkage to type 2 diabetes in two independent studies of affected Japanese sibpairs[6,7]. We also reproduced the association of *KCNQ1* with diabetes in Chinese and Korean panels, establishing *KCNQ1* as a diabetes susceptibility gene for populations of East Asian descent. We further showed the association to be significant in individuals of European descent. Given that *KCNQ1* was not implicated as a diabetes susceptibility gene in two recent GWASs with individuals of European descent[8,9], we examined SNPs of *KCNQ1* in the available datasets (**Supplementary Fig. 3** and **Supplementary Table 6a,b** online). Within the LD block of *KCNQ1* that includes the SNPs associated with diabetes in Japanese, 11 SNPs in the WTCCC dataset[8] and 9 SNPs in the DGI dataset[9] had been typed, and none of them had been selected for further analysis. This apparent discrepancy may be due mainly to the allele frequencies of the causative SNPs (the minor allele frequency of rs2237892 was 0.28–0.41 and 0.05–0.07 in populations of East Asian and European descent, respectively). Indeed, in a recent meta-analysis of three GWASs (DGI, WTCCC and FUSION; see URLs section in Methods)[10], the risk alleles of both rs2237892 and rs2074196 identified in the present study were associated with an increased risk of type 2 diabetes ($P = 0.01$ and 0.02,

Table 2 Association study results for SNPs in *KCNQ1* and type 2 diabetes

SNP ID	Risk allele	Panel	RAF(DM)	RAF(NC)	P_{allele}	OR	95% CI		P_{trend}	Meta-analysis OR (95% CI) *P* value
rs2074196	G	2+3 (dense mapping)	0.63	0.55	1.7×10^{-9}	1.39	1.25	1.54	1.8×10^{-9}	
		Replication 1 (Japanese)	0.61	0.54	1.4×10^{-7}	1.32	1.19	1.46	2.1×10^{-7}	
		Replication 2 (Japanese)	0.62	0.55	4.7×10^{-7}	1.31	1.18	1.46	6.2×10^{-7}	
		All Japanese (4,378 cases, 4,412 controls)	**0.62**	**0.55**	**4.6×10^{-21}**	**1.34**	**1.26**	**1.42**	**9.8×10^{-21}**	**1.34 (1.26–1.42), $P = 4.8 \times 10^{-21}$**
		Replication 3 (Chinese)	0.71	0.63	1.2×10^{-9}	1.40	1.26	1.56	9.8×10^{-10}	
		Replication 4 (Korean)	0.66	0.58	3.0×10^{-5}	1.39	1.19	1.62	2.1×10^{-5}	
		All Asian (6,552 cases, 6,621 controls)	**0.64**	**0.57**	**9.9×10^{-32}**	**1.35**	**1.28**	**1.42**	**2.1×10^{-31}**	**1.36 (1.29–1.42), $P = 7.9 \times 10^{-33}$**
		Replication 5 (European)	0.96	0.95	0.017	1.23	1.04	1.46	0.017	
		All	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	**1.35 (1.28–1.41), $P = 8.6 \times 10^{-34}$**
rs2237892	C	2+3 (dense mapping)	0.69	0.60	6.7×10^{-13}	1.49	1.34	1.66	1.7×10^{-12}	
		Replication 1 (Japanese)	0.66	0.59	9.6×10^{-10}	1.39	1.25	1.54	1.6×10^{-9}	
		Replication 2 (Japanese)	0.68	0.60	6.9×10^{-10}	1.41	1.26	1.57	1.1×10^{-9}	
		All Japanese (4,378 cases, 4,412 controls)	**0.68**	**0.59**	**2.8×10^{-29}**	**1.43**	**1.34**	**1.52**	**1.7×10^{-28}**	**1.43 (1.34–1.52), $P = 3.0 \times 10^{-29}$**
		Replication 3 (Chinese)	0.72	0.65	1.3×10^{-8}	1.38	1.24	1.55	4.2×10^{-9}	
		Replication 4 (Korean)	0.69	0.61	1.7×10^{-5}	1.41	1.21	1.65	1.0×10^{-5}	
		All Asian (6,552 cases, 6,621 controls)	**0.69**	**0.61**	**2.0×10^{-39}**	**1.41**	**1.34**	**1.48**	**2.5×10^{-39}**	**1.42 (1.34–1.49), $P = 2.5 \times 10^{-40}$**
		Replication 5 (European)	0.95	0.93	7.8×10^{-4}	1.29	1.11	1.50	7.2×10^{-4}	
		All	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	**1.40 (1.34–1.47), $P = 1.7 \times 10^{-42}$**
rs2237895	C	2+3 (dense mapping)	0.41	0.33	3.1×10^{-11}	1.44	1.30	1.61	4.0×10^{-11}	
		Replication 1 (Japanese)	0.38	0.33	4.5×10^{-5}	1.25	1.12	1.38	4.7×10^{-5}	
		Replication 2 (Japanese)	0.41	0.34	5.8×10^{-8}	1.35	1.21	1.50	5.5×10^{-8}	
		All Japanese (4,378 cases, 4,412 controls)	**0.40**	**0.33**	**1.3×10^{-20}**	**1.34**	**1.26**	**1.43**	**1.7×10^{-20}**	**1.34 (1.26–1.43), $P = 1.4 \times 10^{-20}$**
		Replication 3 (Chinese)	0.40	0.34	3.5×10^{-5}	1.25	1.12	1.39	3.4×10^{-5}	
		Replication 4 (Korean)	0.35	0.30	3.2×10^{-3}	1.27	1.08	1.49	2.7×10^{-3}	
		All Asian (6,552 cases, 6,621 controls)	**0.39**	**0.33**	**2.7×10^{-25}**	**1.31**	**1.24**	**1.38**	**2.7×10^{-25}**	**1.31 (1.25–1.38), $P = 6.1 \times 10^{-26}$**
		Replication 5 (European)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	

RAF(DM) and RAF(NC), risk allele frequencies in cases and controls, respectively. P_{allele} values were calculated for allele data. OR, odds ratio for risk allele. P_{trend} values were calculated by the Cochran-Armitage trend test. Meta-analysis was performed by the Mantel-Haenszel method (fixed-effects models). n.a., not applicable.

respectively). These results provide further support for *KCNQ1* as a general susceptibility gene for diabetes, and they also highlight the need to extend GWAS to different populations.

Alternative splicing has been found to generate several variants of *KCNQ1* mRNA (see Accession codes section in Methods), but we do not know whether the identified candidate SNPs in intron 15 affect the splicing pattern of the primary transcript. Although neighboring genes seem to be located outside the LD block containing rs2237892, we are not able to exclude completely the possibility that the SNPs identified in the present study affect the expression of other causative genes. We did not find any microRNA harboring rs2237892 in the miRBase database.

KCNQ1 encodes the pore-forming subunit of a voltage-gated K^+ channel (KvLQT1) that is essential for the repolarization phase of the action potential in cardiac muscle[11]. Mutations in this gene are associated with cardiac diseases such as hereditary long QT syndrome (Romano-Ward syndrome[12] and Jervell and Lange-Nielsen syndrome[13]) and familial atrial fibrillation[14]. This K^+ channel is also expressed in other tissues, including brain, adipose tissues and pancreas[15,16]. The lower HOMA-β or CIR apparent for CC homozygotes of rs2237892 among Japanese and Europeans in the present study may reflect a functional role for this channel in insulin-producing cells. We examined the abundance of *Kcnq1* mRNA by reverse transcription and real-time PCR analysis in the islets of 12-week-old diabetic KK-Ay mice, which manifested both hyperglycemia and hyperinsulinemia. The amount of the mRNA was significantly increased ($P = 0.0004$) by a factor of 1.6 compared with that in the islets of C57BL6 control mice (data not shown). The KCNQ1 protein was previously shown to be expressed in insulin-secreting INS-1 cells, and the KCNQ1 blocker 293B was found to stimulate insulin secretion in the presence of tolbutamide[17]. It is also possible that fine-tuning of the membrane potential by this channel might modulate the survival of pancreatic β cells in the long term. Further studies are necessary to elucidate the precise mechanism by which the risk allele of *KCNQ1* confers susceptibility to diabetes.

We may have missed a substantial number of susceptibility genes in our screening, given that the strategy we adopted seven years ago lacks sufficient analytical power[18] relative to that now achievable as a result of recent progress in genomic studies. The genomic coverage of the SNP set was not robust, in part because the IMS-JST Japanese SNP (JSNP) database was designed to focus on 'gene-centric' SNPs[2]. Several comprehensive studies based on new platforms for GWASs have recently been described, with about ten genes being found to be

reproducibly associated with type 2 diabetes in individuals of European ancestry[8,9,19–23]. None of these genes showed a positive association in our JGS typing data. Given that some of these genes were recently shown to confer susceptibility to diabetes in Japanese[24–26], the lack of association in our study might be due to the limited sample size of the first scan or to weak LD between the SNPs we used and the causative variants; actually, some genes were totally missed in our JGS (**Supplementary Table 6c**).

In summary, with a comprehensive multistage SNP association study in Japanese, we have identified *KCNQ1* as a previously unreported susceptibility gene as well as several other candidate genes for type 2 diabetes mellitus. Replication studies further confirmed the association of *KCNQ1* with diabetes in individuals of East Asian and European descent. Our findings may provide new insight into the pathophysiology of diabetes as well as a basis for the development of new therapeutic agents.

METHODS

Study participants. We assembled three independent subject panels for multistage genome-wide screening. Panel 1 consisted of 188 cases only, panel 2 of 752 cases and 752 controls and panel 3 of 672 cases and 672 controls. The inclusion criteria for diabetic patients were as follows: (i) age of disease onset of 40 to 55 years, (ii) maximum BMI of <30 kg/m^2, (iii) insulin treatment not initiated until at least three years after diagnosis and (iv) absence of antibodies to glutamic acid decarboxylase. Most Japanese diabetic individuals have a BMI of <30 kg/m^2, and we aimed to focus on the most common subtype of type 2 diabetes in Japan. The criteria for controls in panels 2 and 3 were as follows: (i) age of >60 years, (ii) no past history of diagnosis of diabetes and (iii) hemoglobin A_{1c} content of $<5.6\%$. The cases in the three panels and the controls in panels 2 and 3 were recruited at 11 core facilities located in various regions of Japan. Panels 2 and 3 were assembled simultaneously. Genomic DNA was extracted from peripheral blood by standard methods. We also obtained clinical information such as BMI, blood biochemistry (including plasma glucose and insulin levels) and family history of diabetes. The replication panels are described in **Supplementary Methods**. The clinical characteristics of subjects in each panel are summarized in **Supplementary Table 1**. The study protocol was approved by the local ethics committee of each institution, and written informed consent was obtained from all participants.

Study design. The general design and power for the multistage screening in the Millennium Genome Project (**Supplementary Fig. 1**), referred to as the JSNP Genome Scan (JGS), have been described previously[18]. In the first stage, 188 individuals with each disease (panel 1 for diabetes) were genotyped for 100,000 SNPs in the IMS-JST JSNP database (see URLs section below)[2]. The coverage of the nucleotide sequences of the RefSeq NM exonic regions (as defined by 5′ UTR + CDS (coding sequences) + 3′ UTR) achieved by the JSNP 'gene-centric' genome-wide LD mapping is estimated to be $\sim$35%, if we assume an average extent of LD of 10 kb for each SNP with a minor allele frequency (MAF) of $>15\%$. We also previously evaluated the power of the first two stages of the JGS by a simulation experiment[18]. For example, this analysis would yield a sensitivity of $\sim$13% for SNPs with an odds ratio of 1.5 and a disease-associated genotype frequency of 30%.

One subject did not yield a genotype call for any SNP in the first stage. We then carried out two separate association analyses to compare the 187 diabetic individuals with two different control groups, which we referred to as JDC and ODG, respectively. We did not detect significant population stratification among individuals of the initial panels of the five disease groups by standard methods such as genomic control[27] (inflation factor = 1.06 with 1,025 SNPs selected for genomic control analysis). The genotype-based analysis was done with dominant and recessive models. First, SNPs whose MAF was $>10\%$ in the database and which showed either a genotype OR of >1.5 or an allele OR of >1.3 in either association analysis were selected. If multiple SNPs in the same gene with positive association were in strong LD ($r^2 > 0.9$), only one SNP was chosen for the next step to avoid redundancy. A total of 2,880 SNPs for each disease was then selected for the second screening in order of P value; for diabetes, 2,343 and 1,111 SNPs were selected by the association analyses with ODG and JDC, respectively, with 574 SNPs being selected by both analyses.

In the second stage, an independent case-control panel (panel 2) was analyzed, generating valid data for 2,827 SNPs after a quality check. Thirty-eight SNPs gave no results for all the samples in panel 2, whereas five and three SNPs yielded no data for all case or control samples, respectively, by multiplex PCR-based Invader analysis, and seven probes were not annotated on the updated human genome. The call rate for the 2,827 SNPs was 0.993. A total of 201 positive SNPs ($P < 0.05$) was selected for the third stage of the study on the basis of allelic data (**Supplementary Table 2a**). In the third stage, another case-control panel (panel 3) was typed; one SNP could not be typed by SSP-PCR-FCS analysis (see below) for any of the subjects in panel 3, with the call rate for the other 200 SNPs being 0.990. The ten positive SNPs ($P < 0.05$; **Table 1**) were also then analyzed in the combined panels 2 and 3 (panel 2+3, 1,424 cases and 1,424 controls). Panel 2 was genotyped again for these ten SNPs by SSP-PCR-FCS analysis, and the concordance rate with the Invader method used in the second screening was 0.992. The possibility of stratification in panels 2 and 3 was assessed by typing of 28 diabetes-unrelated SNPs followed by (i) comparison of allele and genotype frequencies by the χ^2 test, (ii) principal component analysis or (iii) STRUCTURE analysis (see URLs section below). None of these analyses showed evidence of stratification among cases and controls of panels 2 and 3 (data not shown).

The list of SNPs used for the initial screening and the allele and genotype frequency data for the first and the second stages of the JGS for the five diseases studied in the Millennium Genome Project of Japan, including diabetes, have been deposited in the Genome Medicine Database of Japan (GeMDBJ, see URLs section below).

Dense SNP mapping for *KCNQ1*. We first selected 49 additional SNPs of *KCNQ1* from the dbSNP database of NCBI, with an average interval of $\sim$10 kbp, and typed these polymorphisms in panel 2+3 together with the three positive SNPs originally included in the JGS. We sequenced 24 control Japanese subjects for the gene, including all the exons and the putative promoter region (4 kbp upstream from the transcription start site), in order to comprehensively identify genetic variants in Japanese. We also sequenced the regions surrounding the positive SNPs of *KCNQ1*, spanning 47 kbp (intron 15). Ten of the SNPs identified in the 47-kbp region were selected on the basis of LD and MAF ($>10\%$). These 10 SNPs and the two identified nonsynonymous variants were genotyped in panel 2+3. A total of 64 SNPs was thus genotyped for *KCNQ1*, including 18 SNPs in the 35.6-kbp region between rs151290 and rs2237895, with an average interval of 2 kbp (see **Supplementary Table 3b**).

Typing methods. In the first and second stages of the study, genotyping was done by the multiplex PCR-based Invader assay (Third Wave Technologies) as previously described[28]. In the third stage and for dense mapping, genome-wide amplified DOP degenerate oligonucleotide–primed (DOP)-PCR templates were genotyped by sequence-specific primer (SSP)-PCR analysis followed by fluorescence correlation spectroscopy (FCS)[29]. Some SNPs included in dense mapping were therefore re-genotyped in panel 2 by the SSP-PCR-FCS method. Some SNPs were genotyped by real-time PCR analysis with TaqMan probes (Applied Biosystems). For replication panels, we applied either SSP-PCR-FCS or the TaqMan method.

Statistical analysis. In the first screening, we performed two case-control evaluations as described above. We examined allele or genotype (dominant or recessive models) data in 2×2 contingency tables for comparison with ODG, as well as allele data in 2×2 contingency tables for comparison with JDC (for which genotype data were not available). In the second and third screening and dense mapping, we analyzed allele data in 2×2 contingency tables by the χ^2 test. LD and haplotype analyses were done with Haploview 3.31 software[30]. A P value of <0.05 was considered statistically significant. For ten positive SNPs in the JGS, rs2237892 and rs2074196, genotype-based analyses were also performed by the Cochran-Armitage trend test. Meta-analysis was done by the Mantel-Haenszel method (fixed-effects models) with the "meta" package of the R Project; the P values for heterogeneity among panels joined in the Mantel-Haenszel tests were all >0.05.

URLs. Genome Medicine Database of Japan, https://gemdbj.nibio.go.jp/dgdb/; DGI, WTCCC and FUSION, http://www.well.ox.ac.uk/DIAGRAM/; miRBase database, http://microrna.sanger.ac.uk/sequences/; IMS-JST JSNP database, http://snp.ims.u-tokyo.ac.jp/; STRUCTURE analysis, http://pritch.bsd.uchicago.edu/software.html.

Accession codes. GenBank: *KCNQ1* mRNA, NM_000218.2 and NM_181798.1.

Note: Supplementary information is available on the Nature Genetics website.

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