Brief Genetics Report

Association Studies of Variants in the Genes Involved in Pancreatic β-Cell Function in Type 2 Diabetes in Japanese Subjects

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Because impaired insulin secretion is characteristic of type 2 diabetes in Asians, including Japanese, the genes involved in pancreatic β-cell function are candidate susceptibility genes for type 2 diabetes. We examined the association of variants in genes encoding several transcription factors (*TCF1*, *TCF2*, *HNF4A*, *ISL1*, *IPF1*, *NEUROG3*, *PAX6*, *NKX2–2*, *NKX6–1*, and *NEUROD1*) and genes encoding the ATP-sensitive K^+ channel subunits Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*) with type 2 diabetes in a Japanese cohort of 2,834 subjects. The exon 16 −3c/t variant rs1799854 in *ABCC8* showed a significant association ($P = 0.0073$), and variants in several genes showed nominally significant associations ($P < 0.05$) with type 2 diabetes. Although the E23K variant rs5219 in *KCNJ11* showed no association with diabetes in Japanese (for the K allele, odds ratio [OR] 1.08 [95% CI 0.97–1.21], $P = 0.15$), 95% CI around the OR overlaps in meta-analysis of European populations, suggesting that our results are not inconsistent with the previous studies. This is the largest association study so far conducted on these genes in Japanese and provides valuable information for comparison with other ethnic groups. *Diabetes* 55:2379–2386, 2006

Impaired insulin secretion and insulin resistance both contribute to the pathogenesis of type 2 diabetes. The former is a characteristic feature of type 2 diabetes, especially in Asians including Japanese (1), and genes encoding proteins critical in pancreatic β-cell function are therefore particularly good candidate susceptibility genes for type 2 diabetes for this population. Studies of maturity-onset diabetes of the young in humans (2) and knockout mice (3) have shown that mutations of transcription factors required for development, differentiation, and maintenance of the pancreatic β-cells can cause diabetes. Pancreatic β-cell ATP-sensitive K^+ channels (K_{ATP} channels) are crucial in the regulation of insulin secretion by coupling cell metabolism to membrane electrical activity. The pancreatic β-cell K_{ATP} channel comprises two subunits, the inwardly rectifying potassium channel Kir6.2 (*KCNJ11*) and the sulfonylurea receptor SUR1 (*ABCC8*) (4). Mutations in the genes (*ABCC8* and *KCNJ11*) can cause familial persistent hyperinsulinemic hypoglycemia of infancy (5) and permanent neonatal diabetes (6). Several polymorphisms in these genes also have been reported to be associated with type 2 diabetes in populations with distinct ethnic backgrounds (7–20). However, a large-scale association study of these genes has not been performed in type 2 diabetes in the Japanese population. Here, we report on the association of variants in genes encoding various transcription factors and pancreatic β-cell K_{ATP} channel subunits with type 2 diabetes in a large Japanese cohort.

A case-control association study using 1,590 Japanese diabetic subjects and 1,244 nondiabetic control subjects was performed. All subjects were genotyped for 33 variants of 12 genes including transcription factors (*TCF1*, *TCF2*, *HNF4A*, *ISL1*, *IPF1*, *NEUROG3*, *PAX6*, *NKX2–2*, *NKX6–1*, and *NEUROD1*) and β-cell K_{ATP} channel subunits (*KCNJ11* and *ABCC8*) (Table 1).

Results of Hardy-Weinberg equilibrium (HWE) tests are shown in Table 1 of the online appendix (available at http://diabetes.diabetesjournals.org). All genotypes were in HWE, except for departures in cases at *TCF2*_SNP (single nucleotide polymorphism) 5 rs2689, *TCF2*_SNP6

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Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org.

HWE, Hardy-Weinberg equilibrium; K_{ATP} channel, ATP-sensitive K^+ channel; LD, linkage disequilibrium; SNP, single nucleotide polymorphism.

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TABLE 1
Summary of association studies of 33 variants for 12 genes with type 2 diabetes

Number	Locus	HapMap data	Subject	Allele data (frequency) 1	Allele data (frequency) 3	Genotype data (frequency) 1	Genotype data (frequency) 2	Genotype data (frequency) 3	*P* value Allele 2*2	*P* value Genotype 2*3	OR (95% CI)
1	TCF1_SNP1			A	C	A/A	A/C	C/C			
	rs1169288	JPT	Case	1,590 (0.50)	1,590 (0.50)	385 (0.24)	820 (0.52)	385 (0.24)	0.4508	0.2388	1.04 (0.94–1.16)
	I27L		Control	1,270 (0.51)	1,218 (0.49)	332 (0.27)	606 (0.49)	306 (0.25)			
2	TCF1_SNP2			G	A	G/G	G/A	A/A			
	rs1169294	none	Case	1,702 (0.54)	1,478 (0.46)	443 (0.28)	816 (0.51)	331 (0.21)	0.3247	0.1566	1.06 (0.95–1.17)
	IVS1 −42		Control	1,298 (0.52)	1,190 (0.48)	348 (0.28)	602 (0.48)	294 (0.24)			
3	TCF1_SNP3			A	T	A/A	A/T	T/T			
	rs2071190	JPT	Case	482 (0.15)	2,698 (0.85)	38 (0.02)	406 (0.26)	1,146 (0.72)	0.8925	0.8617	1.01 (0.87–1.17)
	IVS2 −51		Control	373 (0.15)	2,115 (0.85)	26 (0.02)	321 (0.26)	897 (0.72)			
4	TCF2_SNP1			G	A	G/G	G/A	A/A			
	rs757210	JPT	Case	2,079 (0.65)	1,101 (0.35)	697 (0.44)	685 (0.43)	208 (0.13)	0.4565	0.2695	1.04 (0.93–1.17)
	IVS2 + 2916		Control	1,651 (0.66)	837 (0.34)	546 (0.44)	559 (0.45)	139 (0.11)			
5	TCF2_SNP2			A	G	A/A	A/G	G/G			
	rs757211	none	Case	1,460 (0.46)	1,720 (0.54)	342 (0.22)	776 (0.49)	472 (0.30)	0.6994	0.5473	1.02 (0.92–1.14)
	IVS2 + 2953		Control	1,156 (0.46)	1,332 (0.54)	262 (0.21)	632 (0.51)	350 (0.28)			
6	TCF2_SNP3			G	A	G/G	G/A	A/A			
	rs718960	JPT	Case	2,288 (0.72)	892 (0.28)	824 (0.52)	640 (0.40)	126 (0.08)	0.6121	0.8597	1.03 (0.92–1.16)
	IVS4 + 14307		Control	1,774 (0.71)	714 (0.29)	632 (0.51)	510 (0.41)	102 (0.08)			
7	TCF2_SNP4			T	A	T/T	T/A	A/A			
	rs1016991	JPT	Case	2,823 (0.89)	357 (0.11)	1,260 (0.79)	303 (0.19)	27 (0.02)	0.0105*	0.0399*	1.23 (1.05–1.45)
	IVS8 + 929		Control	2,152 (0.87)	336 (0.14)	938 (0.75)	276 (0.22)	30 (0.02)			
8	TCF2_SNP5			A	T	A/A	A/T	T/T			
	rs2689	JPT	Case	1,722 (0.54)	1,458 (0.46)	488 (0.31)	746 (0.47)	356 (0.22)	0.5195	0.3582	1.04 (0.93–1.15)
	+274 TGA		Control	1,325 (0.53)	1,163 (0.47)	355 (0.29)	615 (0.49)	274 (0.22)			
9	TCF2_SNP6			A	C	A/A	A/C	C/C			
	rs2688	JPT	Case	1,840 (0.58)	1,340 (0.42)	552 (0.35)	736 (0.46)	302 (0.19)	0.0563	0.0291*	1.11 (1.00–1.24)

	+444 TGA		Control	1,503 (0.60)	985 (0.40)	448 (0.36)	607 (0.49)	189 (0.15)			
10	HNF4A_SNP1			T	C	T/T	T/C	C/C			
	rs717247	JPT	Case	2,277 (0.72)	903 (0.28)	811 (0.51)	655 (0.41)	124 (0.08)	0.2071	0.4223	1.08 (0.96–1.22)
	−4229		Control	1,820 (0.73)	668 (0.27)	661 (0.53)	498 (0.40)	85 (0.07)			
11	HNF4A_SNP2			A	G	A/A	A/G	G/G			
	rs736820	none	Case	1,207 (0.38)	1,973 (0.62)	230 (0.14)	747 (0.47)	613 (0.39)	0.4482	0.6472	1.04 (0.94–1.16)
	IVS1 + 3889		Control	919 (0.37)	1,569 (0.63)	176 (0.14)	567 (0.46)	501 (0.40)			
12	HNF4A_SNP3			T	C	T/T	T/C	C/C			
	rs745975	JPT	Case	643 (0.20)	2,537 (0.80)	53 (0.03)	537 (0.34)	1,000 (0.63)	0.0470*	0.0769	1.15 (1.00–1.31)
	IVS1 −5		Control	450 (0.18)	2,038 (0.82)	39 (0.03)	372 (0.30)	833 (0.67)			
13	ISL1_SNP10			G	A	G/G	G/A	A/A			
	rs2303750	none	Case	2,842 (0.89)	338 (0.11)	1,271 (0.80)	300 (0.19)	19 (0.01)	0.5101	0.4453	1.06 (0.90–1.26)
	IVS3 −4		Control	2,209 (0.89)	279 (0.11)	987 (0.79)	235 (0.19)	22 (0.02)			
14	ISL1_SNP11			A	G	A/A	A/G	G/G			
	rs2303751	none	Case	2,466 (0.78)	714 (0.22)	958 (0.60)	550 (0.35)	82 (0.05)	0.0539	0.1224	1.14 (1.00–1.29)
	P165P		Control	1,983 (0.80)	505 (0.20)	787 (0.63)	409 (0.33)	48 (0.04)			
15	IPF1_SNP3			G	T	G/G	G/T	T/T			
	rs4430606	none	Case	1,688 (0.53)	1,492 (0.47)	451 (0.28)	786 (0.49)	353 (0.22)	0.1807	0.3405	1.06 (0.90–1.26)
	IVS1 + 512		Control	1,366 (0.55)	1,122 (0.45)	371 (0.30)	624 (0.50)	249 (0.20)			
16	IPF1_SNP4			A	C	A/A	A/C	C/C			
	rs1124607	JPT	Case	2,527 (0.79)	653 (0.21)	1,007 (0.63)	513 (0.32)	70 (0.04)	0.7609	0.6259	1.02 (0.90–1.16)
	IVS1 + 539		Control	1,968 (0.79)	520 (0.21)	788 (0.63)	392 (0.32)	64 (0.05)			
17	IPF1_SNP7			G	A	G/G	G/A	A/A			
	none	none	Case	2,836 (0.89)	344 (0.11)	1,260 (0.79)	316 (0.20)	14 (0.01)	0.1309	0.2318	1.14 (0.97–1.34)
	IVS1 + 1787		Control	2,186 (0.88)	302 (0.12)	953 (0.77)	280 (0.23)	11 (0.01)			

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TABLE 1
Continued

Number	Locus	HapMap data	Subject	Allele data (frequency) 1	Allele data (frequency) 3	Genotype data (frequency) 1	Genotype data (frequency) 2	Genotype data (frequency) 3	*P* value Allele 2*2	*P* value Genotype 2*3	OR (95% CI)
18	NEUROG3_SNP1			A	G	A/A	A/G	G/G			
	rs3812704	JPT	Case	1,472 (0.46)	1,708 (0.54)	337 (0.21)	798 (0.50)	455 (0.29)	0.2674	0.4687	1.06 (0.96–1.18)
	−1822		Control	1,114 (0.45)	1,374 (0.55)	252 (0.20)	610 (0.49)	382 (0.31)			
19	NEUROG3_SNP2			T	C	T/T	T/C	C/C			1.06 (0.95–1.19)
	rs4536103	JPT	Case	2,268 (0.71)	912 (0.29)	798 (0.50)	672 (0.42)	120 (0.08)	0.3129	0.2040	
	F199S		Control	1,743 (0.70)	745 (0.30)	616 (0.50)	511 (0.41)	117 (0.09)			
20	PAX6_SNP1			A	T	A/A	A/T	T/T			1.08 (0.97–1.20)
	rs2239789	none	Case	1,725 (0.54)	1,455 (0.46)	483 (0.30)	759 (0.48)	348 (0.22)	0.1697	0.2391	
	IVS6 + 282		Control	1,396 (0.56)	1,092 (0.44)	392 (0.32)	612 (0.49)	240 (0.19)			
21	PAX6_SNP2			C	T	C/C	C/T	T/T			1.01 (0.86–1.18)
	rs667773	none	Case	2,791 (0.88)	389 (0.12)	1,228 (0.77)	335 (0.21)	27 (0.02)	0.9358	0.8923	
	IVS7 + 218		Control	2,181 (0.88)	307 (0.12)	961 (0.77)	259 (0.21)	24 (0.02)			
22	NKX2-2_SNP1			T	C	T/T	T/C	C/C			1.08 (0.74–1.56)
	none	none	Case	3,117 (0.98)	63 (0.02)	1,530 (0.96)	57 (0.04)	3 (0.002)	0.7650	0.8727	
	+856 TGA		Control	2,435 (0.98)	53 (0.02)	1,193 (0.96)	49 (0.04)	2 (0.002)			
23	NKX2-2_SNP2			C	T	C/C	C/T	T/T			1.13 (1.02–1.25)
	rs3746741	none	Case	1,666 (0.52)	1,514 (0.48)	452 (0.28)	762 (0.48)	376 (0.24)	0.0251*	0.0563	
	+1163 TGA		Control	1,228 (0.49)	1,260 (0.51)	305 (0.25)	618 (0.50)	321 (0.26)			
24	NKX6-1_SNP1			A	C	A/A	A/C	C/C			1.03 (0.92–1.16)
	rs1017560	JPT	Case	2,182 (0.69)	998 (0.31)	747 (0.47)	688 (0.43)	155 (0.10)	0.6052	0.0144*	
	−15606		Control	1,724 (0.69)	764 (0.31)	625 (0.50)	474 (0.38)	145 (0.12)			
25	NKX6-1_SNP2			T	G	T/T	T/G	G/G			1.01 (0.83–1.23)
	none	none	Case	2,939 (0.92)	241 (0.08)	1,359 (0.85)	221 (0.14)	10 (0.01)	0.9750	0.9966	
	−8823		Control	2,298 (0.92)	190 (0.08)	1,062 (0.85)	174 (0.14)	8 (0.01)			

26	NKX6-1_SNP3			G	A	G/G	G/A	A/A			1.05 (0.95–1.17)
	rs1545330	JPT	Case	1,719 (0.54)	1,461 (0.46)	452 (0.28)	815 (0.51)	323 (0.20)	0.3348	0.5938	
	−8797		Control	1,312 (0.53)	1,176 (0.47)	338 (0.27)	636 (0.51)	270 (0.22)			
27	NKX6-1_SNP4			A	C	A/A	A/C	C/C			1.01 (0.90–1.13)
	rs2278671	JPT	Case	2,094 (0.66)	1,086 (0.34)	681 (0.43)	732 (0.46)	177 (0.11)	0.8884	0.5354	
	IVS2 + 28		Control	1,633 (0.66)	855 (0.34)	541 (0.43)	551 (0.44)	152 (0.12)			
28	NEUROD1_SNP1			C	G	C/C	C/G	G/G			1.03 (0.91–1.16)
	rs3916026	JPT	Case	893 (0.28)	2,287 (0.72)	132 (0.08)	629 (0.40)	829 (0.52)	0.6685	0.3769	
	−5425		Control	685 (0.28)	1,803 (0.72)	87 (0.07)	511 (0.41)	646 (0.52)			
29	NEUROD1_SNP2			G	T	G/G	G/T	T/T			1.03 (0.90–1.18)
	rs7420169	JPT	Case	2,538 (0.80)	642 (0.20)	1,022 (0.64)	494 (0.31)	74 (0.05)	0.6684	0.6459	
	−5084		Control	1,998 (0.80)	490 (0.20)	803 (0.65)	392 (0.32)	49 (0.04)			
30	ABCC8_SNP1			C	T	C/C	C/T	T/T			1.08 (0.97–1.20)
	rs1799854	JPT	Case	1,507 (0.47)	1,673 (0.53)	371 (0.23)	765 (0.48)	454 (0.29)	0.1664	0.0073†	
	IVS15 −3		Control	1,226 (0.49)	1,262 (0.51)	280 (0.23)	666 (0.54)	298 (0.24)			
31	ABCC8_SNP2			G	A	G/G	G/A	A/A			1.07 (0.91–1.25)
	rs4148643	JPT	Case	2,795 (0.88)	385 (0.12)	1,232 (0.77)	331 (0.21)	27 (0.02)	0.4419	0.7059	
	R1273R		Control	2,169 (0.87)	319 (0.13)	950 (0.76)	269 (0.22)	25 (0.02)			
32	ABCC8_SNP3			T	G	T/T	T/G	G/G			1.07 (0.96–1.19)
	rs757110	JPT	Case	1,884 (0.59)	1,296 (0.41)	570 (0.36)	744 (0.47)	276 (0.17)	0.2432	0.4293	
	S1369A		Control	1,513 (0.61)	975 (0.39)	463 (0.37)	587 (0.47)	194 (0.16)			
33	KCNJ11_SNP1			G	A	G/G	G/A	A/A			1.08 (0.97–1.21)
	rs5219	none	Case	1,954 (0.61)	1,226 (0.39)	610 (0.38)	734 (0.46)	246 (0.15)	0.1513	0.3343	
	E23K		Control	1,576 (0.63)	912 (0.37)	503 (0.40)	570 (0.46)	171 (0.14)			

Locus: experimental name of the SNP, followed by rs number and position. HapMap data: JPT, genotyped on Japanese in Tokyo. Nominal P values are listed for allele or genotype frequencies (*P <0.05; †P<0.01). IVS, intron variant sequence.

TABLE 2
Magnitude of LD (D′ and r^2) between *ABCC8* and *KCNJ11* variants

D'/r^2	ABCC8_SNP1	ABCC8_SNP2	ABCC8_SNP3	KCNJ11_SNP1
ABCC8_SNP1 rs1799854	—	0.0012	0.0177	0.0151
ABCC8_SNP2 rs4148643	0.0867	—	0.0919	0.0808
ABCC8_SNP3 rs757110	0.1708	0.9867	—	0.8703
KCNJ11_SNP1 rs5219	0.1653	0.9711	0.9794	—

rs2688, and *NKX2-2*_SNP1 (+856 TGA) and in controls at *NKX6-1*_SNP1 rs1017560 and *ABCC8*_SNP1 rs1799854 (online appendix Table 1). Although none of these are significant with correction for multiple comparisons, we reanalyzed several of the variants, including *NKX2-2*_SNP1 (+856 TGA) and *NKX6-1*_SNP1 rs1017560, and confirmed that there was no typing error for these variants. We also tested whether the observed departures were consistent with the genotype frequencies expected for a genetic disease model (21). The genotype distributions for *TCF2*_SNP5 rs2689, *TCF2*_SNP6 rs2688, *NKX2-2*_SNP1 (+856 TGA), and *ABCC8*_SNP1 rs1799854 are consistent with genetic models that best fit these data. In contrast, the departure from HWE observed in the control samples for *NKX6-1*_SNP1 rs1017560 is not consistent with any genetic model for disease. Thus, the observed departure from HWE in controls at *NKX6-1*_SNP1 rs1017560 is likely to be a chance observation. The remaining departures are unlikely to be attributable to genotyping errors and are consistent with the possibility that the selection of case and control samples from a population in HWE at a susceptibility locus (at the test marker or a polymorphism in strong linkage disequilibrium [LD]) has generated genotype distributions with the observed departures from HWE.

Among the 33 variants of 12 genes, 6 variants (*TCF2*_SNP4 rs1016991, *TCF2*_SNP6 rs2688, *HNF4A*_SNP3 rs745975, *NKX2-2*_SNP2 rs3746741, *NKX6-1*_SNP1 rs1017560, and *ABCC8*_SNP1 rs1799854) showed at least nominally significant associations ($P < 0.05$) with type 2 diabetes (Table 1 and online appendix Table 2). *ABCC8*_SNP1 rs1799854 showed the strongest association ($P = 0.0073$) with diabetes among the SNPs examined in this study. By further analysis of the variant, the T/T genotype was found in 454 (28.6%) and 298 (24.0%) subjects in the diabetic and control groups, respectively, a significant difference in the frequency of individuals with the T/T genotype between the two groups (C/C + C/T vs. T/T, $P = 0.0068$) (online appendix Table 2). The odds ratio (OR) for the T/T genotype was 1.27 (95% CI 1.07–1.50; C/C + C/T vs. T/T), indicating that the T/T genotype in *ABCC8*_SNP1 rs1799854 is associated with type 2 diabetes in Japanese subjects.

There was no association of other variants in *ABCC8* and *KCNJ11* with diabetes (Table 1 and online appendix Table 2). These include the E23K variant in *KCNJ11* (*KCNJ11*_SNP1 rs5219: for the K allele, OR 1.08 [95% CI 0.97–1.21], $P = 0.15$). To determine the extent of LD between the four variants in *ABCC8* and *KCNJ11*, we calculated D′ and r^2 (Table 2). There was modest LD between *ABCC8*_SNP2 rs4148643 and *ABCC8*_SNP3 rs757110. Strong LD was found between *ABCC8*_SNP3 rs757110 and *KCNJ11*_SNP1 rs5219. In the latter, we tested two-locus haplotypes having a frequency of >5% for association with diabetes and found no association of any of the haplotypes with diabetes (data not shown).

We examined the genes involved in pancreatic β-cell function (transcription factors and K_{ATP} channel subunits) in relation to type 2 diabetes in a large cohort of Japanese subjects. The study included 2,834 subjects, the largest case-control study so far conducted on these variants in a Japanese population. For disease susceptibility allele frequencies in the range of 0.3–0.5, our sample had >99% power to detect a susceptibility gene with a genotype relative risk in the range of 1.5–1.85 (for any genetic model of inheritance). For allele frequencies in this range, we had >80% power to detect susceptibility genes with genotype relative risk in the range of 1.25–1.55. Power was similarly good for dominant models with lower susceptibility allele frequencies (0.1–0.3) or recessive models with higher susceptibility allele frequencies (0.5–0.9). The sample was reasonably powered (>90%) to detect recessive susceptibility alleles at low frequencies (0.1–0.3) for higher genotype relative risks (2.1–4.0) but was not sufficiently powered to detect very common (>0.7) dominant susceptibility genes (genotype relative risk >100).

*ABCC8*_SNP1 rs1799854 (exon 16 −3c/t variant) was significantly associated with type 2 diabetes, primarily due to increased frequency of T/T homozygotes among patients. Since this variant is located in the 3′ splice site, it might impair normal splicing. Alternatively, the variant could be in strong LD with an unidentified functional variant in the unscreened region harboring the *ABCC8* gene. There have been two case-control association studies (22,23) conducted for the variant in Japanese populations, both of which found no association of this variant with type 2 diabetes. However, because these studies were based on a relatively small number of subjects (167 subjects in 22; 456 subjects in 23), their power to detect associations is limited. In Caucasians, several studies (7,8,11–13,17) have reported association of the variant with type 2 diabetes, although other studies found no association of the variant with type 2 diabetes (14–16). On the other hand, several studies have reported an association of the E23K variant in *KCNJ11* (*KCNJ11*_SNP1 rs5219 in this study) with type 2 diabetes in Caucasians (9,10,16,18). Recent meta-analyses (19,20) of the variant support this association. Although our present study finds no association of the E23K variant with diabetes in Japanese subjects (for the K allele, OR 1.08 [95% CI 0.97–1.21], $P = 0.15$), 95% CI around the OR overlaps in meta-analysis of European populations, suggesting that our results are not inconsistent with the previous studies on the E23K variant in *KCNJ11*.

The International HapMap Project aims to determine the common patterns of DNA sequence variation in the human genome (24). In the initial phase of the project, genetic data are being gathered from four populations with African, Chinese, Japanese, and European ancestry. Twenty of 33 SNPs used in this study were genotyped on Japanese subjects in the HapMap project, providing important information for determining whether the genes of interest are associated with type 2 diabetes in a Japanese cohort. To

clarify the relationships between our SNPs and those of the HapMap, the patterns of LD between SNPs for each gene are shown in online appendix Fig. 1. Among nine genes (*TCF1*, *TCF2*, *HNF4A*, *ISL1*, *PAX6*, *NKX6-1*, *NEUROD1*, *ABCC8*, and *KCNJ11*) that have a relatively large number of genotyped SNPs in the HapMap, four genes (*ISL1*, *NKX6-1*, *NEUROD1*, and *KCNJ11*) show a relatively strong LD, while five genes (*TCF1*, *TCF2*, *HNF4A*, *PAX6*, and *ABCC8*) show a weak LD across each gene. For the former genes, our data provide considerable information on the association of genes of interest with type 2 diabetes. However, for the latter genes, we could provide only partial information on their association with type 2 diabetes. In contrast, there are none or few genotyped SNPs in the HapMap for three genes (*IPF1*, *NEUROG3*, and *NKX2-2*). For these genes, our data provide valuable information on both the SNPs and their association with type 2 diabetes.

Associations of *HNF4A* variants in the upstream promoter region with type 2 diabetes have recently been reported in several populations (25–27). Using Japanese samples in the HapMap data, we calculated LD between our SNPs (rs717247 and rs745975) and those (rs1884614, rs2144908, and rs4810424) showing association with type 2 diabetes. However, LD was not found (online appendix Fig. 1), indicating that the association of our SNPs with type 2 diabetes is different from that of other studies. In this study, modest associations ($P < 0.05$) with type 2 diabetes were also detected for several of the candidate genes examined (*TCF2*_SNP4 rs1016991, *TCF2*_SNP6 rs2688, *NKX2-2*_SNP2 rs3746741, and *NKX6-1*_SNP1 rs1017560). As there has been no large association study for these variants, these associations need to be confirmed by further replication studies. Nevertheless, this is the largest association study so far conducted on these genes in Japanese subjects, providing valuable information not only for this population, but also for comparison with other ethnic groups.

RESEARCH DESIGN AND METHODS

We examined 1,590 unrelated Japanese type 2 diabetic subjects recruited from nine university hospitals and affiliated hospitals located in seven prefectures in Japan. Type 2 diabetes was diagnosed using World Health Organization criteria. The clinical data on these type 2 diabetic subjects are as follows (continuous data are given as median [interquartile range]): male 54.2%, age at diagnosis 49 years (40–57), and BMI 22.9 kg/m^2 (21.1–25.2). We also examined 1,244 nondiabetic control subjects matched for geographic region under the following criteria: aged ≥60 years, no past history of diagnosis of diabetes, HbA_{1c} <5.6%, and no diabetes within third-degree relatives. Analyses were performed on the whole population of subjects. Genetic analysis of human subjects was approved by the ethics committee at each university. Appropriate informed consent was obtained from all of the subjects examined.

Selection of SNPs and genotyping. We resequenced several target genes using DNA samples of Japanese subjects and selected SNPs for an association study mainly based on a minor allele frequency >0.10 and the possibilities of haplotype construction with the SNPs used in this study. We also selected several SNPs from previous publications (9,10,22).

Genomic DNA was extracted from peripheral blood samples by standard procedures. Genotyping of SNPs was performed by MassARRAY system (Sequenom, San Diego, CA), chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of primer extension products following the PCR amplification. Extension primers, extended across the SNP site, were designed using SpectroDESIGNER software (Sequenom, San Diego, CA). The extension reaction is controlled by a mixture of dideoxy-terminated nucleotides, such that one single-base extension product is created and one double-base extension product is created corresponding to an SNP allele. This scheme creates two peaks in the mass spectrometer that are separated by ~300 Da. The primer extension reaction products were loaded onto SpectroCHIPs preloaded with matrix. SpectroCHIPs were analyzed in fully automated mode by MassARRAY mass spectrometer (Bruker-Sequenom). Quality values are attached to each genotyping result, and samples with low quality value were reanalyzed.

Statistical analyses. Differences in distribution of allele or genotype frequencies between type 2 diabetic and control subjects were assessed using χ^2 tests. The extent of LD and haplotype frequencies were estimated using the Hitagene software (Hitachi Europe, Dublin, Ireland) and PowerMarker software (Kejun Liu and Spencer Muse, PowerMarker: new genetic data analysis software, version 3.0; free program distributed over the internet available from http://www.powermarker.net). Power calculations were completed using the Genetics Power Calculator (28). The pairwise r^2 values for SNPs in the HapMap were calculated by the Haploview (29).

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