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Absence of an association between the polymorphisms in the genes encoding adiponectin receptors and type 2 diabetes

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Abstract *Aims/hypothesis:* Secreted by adipocytes, adiponectin is a hormone that acts as an antidiabetic and anti-atherogenic adipokine. We recently cloned the genes encoding two adiponectin receptors (*ADIPOR1* and *ADIPOR2*). The aim of this study was to examine whether *ADIPOR1* and/or *ADIPOR2* play a major role in genetic susceptibility to insulin resistance or type 2 diabetes in the Japanese population. *Methods:* By direct sequencing and a search of public databases, we identified single nucleotide polymorphisms (SNPs) in *ADIPOR1* and *ADIPOR2*, and investigated whether these SNPs are associated with insulin resistance and type 2 diabetes in the Japanese population. *Results:* The linkage disequilibrium (LD) in the chromosomal region of *ADIPOR1* was almost completely preserved, whereas the LD in *ADIPOR2* was less well preserved. None of the SNPs in *ADIPOR1* or *ADIPOR2* were significantly associated with insulin resistance or type 2 diabetes. No differences in *ADIPOR1* or *ADIPOR2* haplotype frequencies were observed between type 2 diabetic and non-diabetic subjects. *Conclusions/interpretation:* Genetic variations in *ADIPOR1* or *ADIPOR2* are unlikely to lead to a common genetic predisposition to insulin resistance or type 2 diabetes in the Japanese population.

Keywords Association · Polymorphism · Susceptibility gene

Abbreviations LD: linkage disequilibrium · ESM: electronic supplementary material · HOMA: homeostasis model assessment · SNP: single nucleotide polymorphism

Introduction

Adiponectin (also known as the 30-kDa adipocyte complement-related protein, or Acrp30) [1–4] is a hormone secret-

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ed by adipocytes that acts as an antidiabetic adipokine [5–9]. Levels of adiponectin in the blood are decreased in subjects with obesity, insulin resistance or type 2 diabetes [10, 11]. In animal models, decreased plasma adiponectin is causally involved in insulin resistance and glucose intolerance [6–9]. In humans, polymorphisms in the gene encoding adiponectin have been shown to be associated with insulin resistance and type 2 diabetes [12–14].

We recently cloned cDNAs encoding adiponectin receptors 1 and 2 (*ADIPOR1* and *ADIPOR2*) [15]. These receptors mediate increases in the AMP kinase [16] and peroxisome proliferator-activated receptor-α ligand activities [17] of adiponectin [15], and are likely to mediate the insulin-sensitising actions of adiponectin. Therefore, *ADIPOR1* and *ADIPOR2* may be viewed as plausible candidate genes for susceptibility to insulin resistance and type 2 diabetes.

The aim of this study was to investigate whether single nucleotide polymorphisms (SNPs) in *ADIPOR1* and *ADIPOR2* influence insulin resistance and susceptibility to type 2 diabetes in the Japanese population.

Subjects and methods

Subjects The inclusion criteria for the diabetic and non-diabetic subjects enrolled in this study have been described previously [13]. Diabetes was diagnosed according to the criteria of the World Health Organization [18]. All subjects enrolled in this study were of full Japanese ancestry. SNPs in *ADIPOR1* and *ADIPOR2* were genotyped in 192 diabetic and 192 non-diabetic subjects. The clinical characteristics of the subjects are described in Table 1 of the electronic supplementary material (ESM). Written informed consent was obtained from the subjects, and the study was approved by the Ethics Committee of the University of Tokyo.

Biological measurements Insulin resistance and beta cell function were assessed using homeostasis model assessment (HOMA). The HOMA of insulin resistance (HOMA-IR) was calculated as fasting insulin (μU/ml)×glucose (mmol/l)/22.5, as described elsewhere [19]. Data are expressed as means±SEM. Since the use of insulin therapy or oral hypoglycaemic agents in subjects with type 2 diabetes is likely to interfere with insulin levels, the correlations between SNPs and insulin resistance were only assessed in non-diabetic subjects.

Screening and selection of SNPs in ADIPOR1 and ADIPOR2 To establish an SNP map encompassing *ADIPOR1* and *ADIPOR2*, SNPs were identified by direct sequencing and a search of public databases. All eight exons in *ADIPOR1* and all nine exons in *ADIPOR2*, plus 50–100 bases of the 5′ and 3′ intronic regions flanking the exons, were amplified and directly sequenced in 30 type 2 diabetic subjects. The conditions and the sequences of the primers used

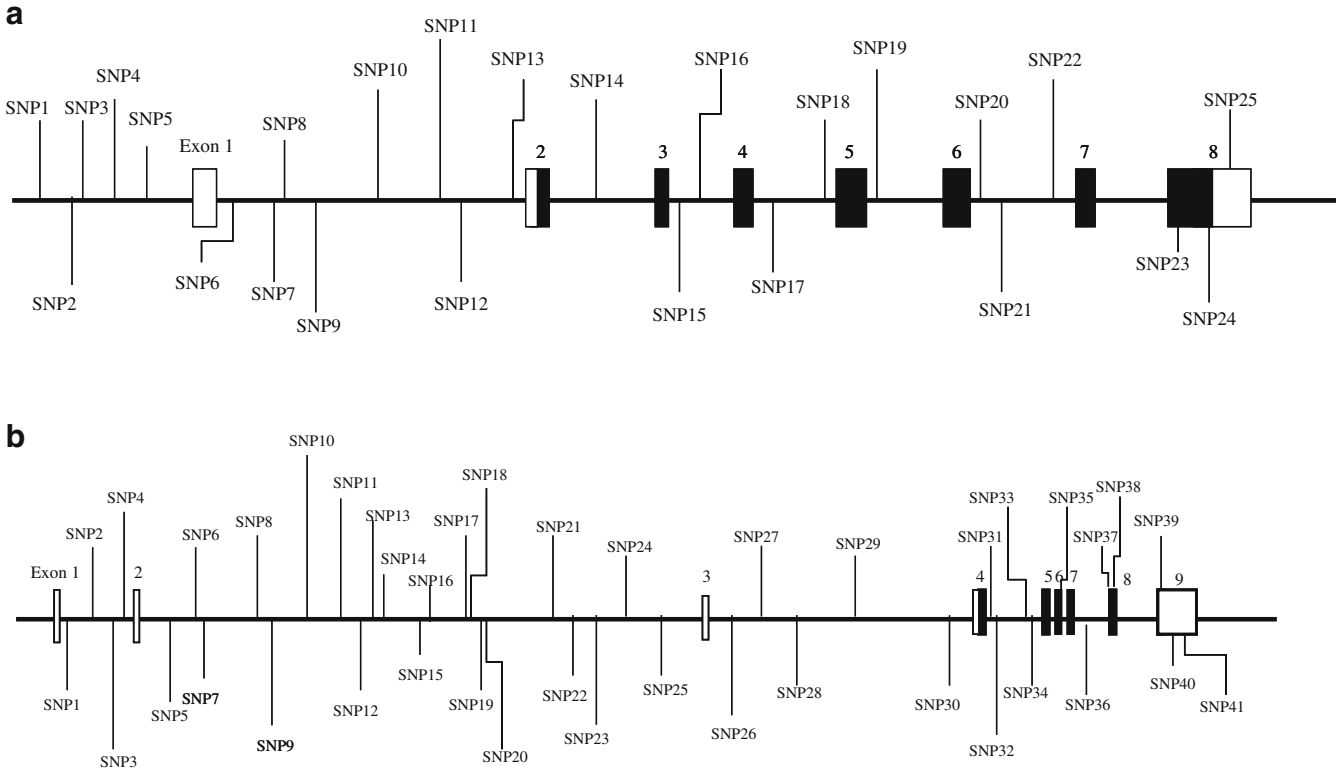

Fig. 1 Genomic structure of *ADIPOR1* (**a**) and *ADIPOR2* (**b**) and the locations of the SNPs genotyped in the present study. Exons are shown as *boxes*, and introns and flanking sequences as *lines connecting the boxes*. Coding sequences are represented as *closed boxes*, and untranslated regions as *open boxes*. The SNPs are numbered in order of appearance from the 5′ to 3′ ends of the genes

in the PCR are described in Table 2 of the ESM. The SNPs were identified based on the sequences reported in the GenBank database (http://www.ncbi.nih.gov/index.html), which contains *ADIPOR1* (accession number NT_004671) and *ADIPOR2* (accession number NT_009759). From the public database, 14 SNPs in *ADIPOR1* (rs6666089, rs10920534, rs12039275, rs12733285, rs1539355, rs2275738, rs2275737, rs2275735, rs1342387, rs3737884, rs2275736, rs11581, rs1043268, rs1043280) and 29 SNPs in *ADIPOR2* (rs2058033, rs6489322, rs12579507, rs11061935, rs7975600, rs10773982, rs11829703, rs12810020, rs11061947, rs11612383, rs11612726, rs9888418, rs7976827, rs12582624, rs10848566, rs7297509, rs12818963, rs10848569, rs11061974, rs2068485, rs7974924, rs12831353, rs12828908, rs10848571, rs7974422, rs2286385, rs730032, rs12342, rs1044471) were selected and validated in 30 type 2 diabetic subjects. From the database, we chose SNPs with a minor allele frequency higher than 10%; we excluded those SNPs for which information on allele frequency was not presented. In total, 25 SNPs in *ADIPOR1* and 41 SNPs in *ADIPOR2* were identified. Minor allele frequency was determined and Hardy–Weinberg equilibrium was assessed. We eliminated SNPs that deviated from Hardy–Weinberg equilibrium or that had a minor allele frequency lower than 10% from further study. In total, 14 SNPs in *ADIPOR1* and 24 SNPs in *ADIPOR2* were analysed, resulting in an average SNP density of one SNP per 1.2 kb in *ADIPOR1* and one SNP per 3.9 kb in *ADIPOR2*.

Genotyping of SNPs used in the association study We genotyped the SNPs in *ADIPOR1* and *ADIPOR2* in type 2 diabetic subjects and non-diabetic subjects using direct sequencing. PCR was performed under standard conditions. Sequencing reactions were performed using the Big Dye terminator kit (Applied Biosystems, Foster City, CA, USA), and the products were resolved using an ABI 3700 automated DNA sequencer (Applied Biosystems). The results were integrated using a Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA), and individual SNPs were manually genotyped. Ambiguous base assignments were eliminated from further analysis.

Statistical analysis The proportions of genotypes or alleles between subjects with or without type 2 diabetes were compared using a chi square (χ^2) test. The differences between subjects with different SNP genotypes were statistically tested using an ANOVA. A Bonferroni adjustment was used to avoid type 1 errors caused by multiple testing. The level of significance for SNPs in *ADIPOR1* and *ADIPOR2* was 0.001 (0.05 divided by 38, the total number

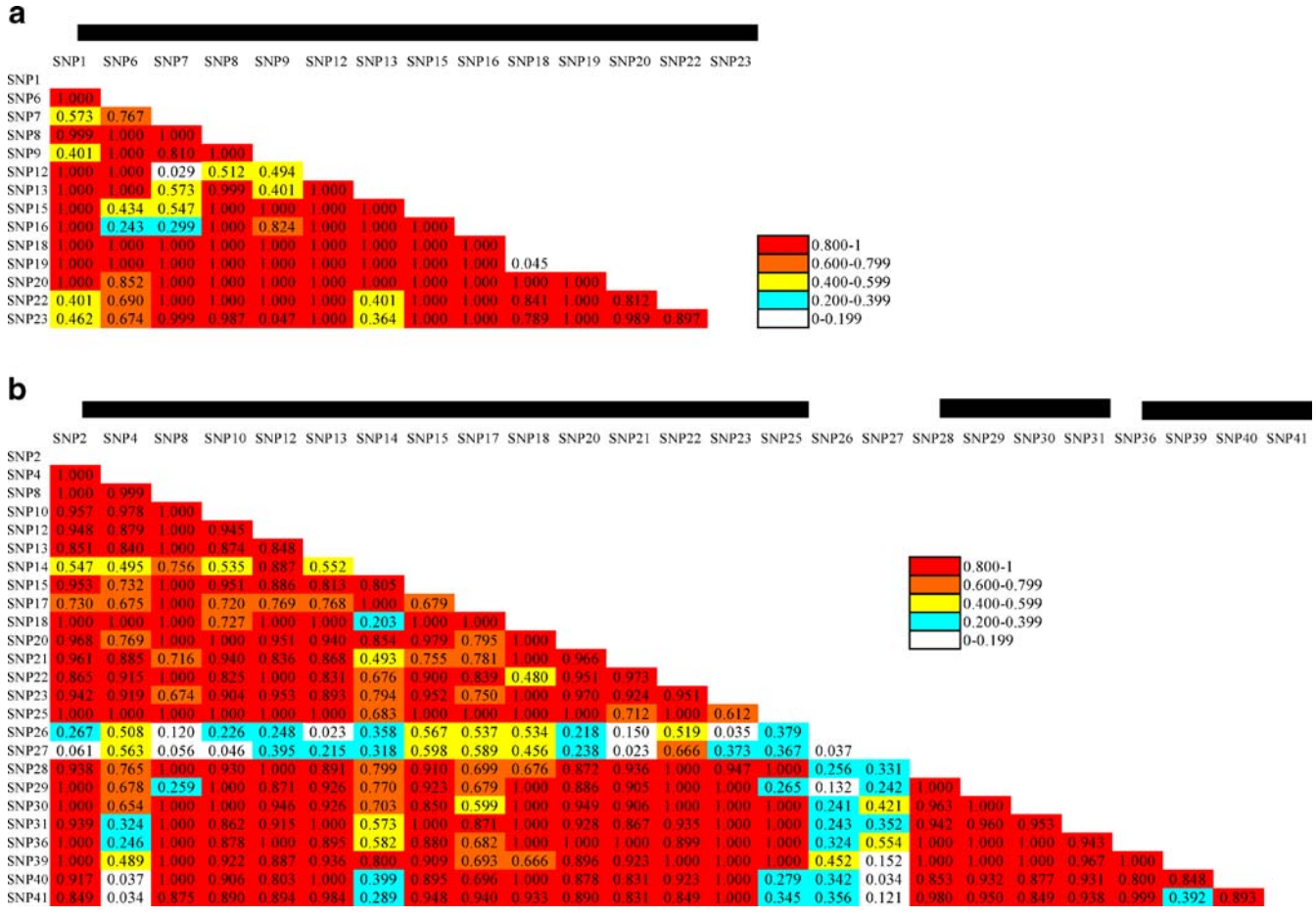

Fig. 2 The pairwise marker LD between SNPs in *ADIPOR1* (**a**) and *ADIPOR2* (**b**). The LD between a pair of markers is indicated by the colour of the block (*blue to red*). Block structure is indicated at the top of each figure as a *closed box*

of SNPs for which the association between SNPs and type 2 diabetes was investigated in the present study). We further genotyped SNP15 in *ADIPOR2*, which showed a tendency towards an association with HOMA (p=0.04), in a second panel to test reproducibility. The statistical analyses, except for those for haplotype estimation, were performed using JUMP for Windows, version 4.00 (SAS Institute, Cary, NC, USA).

Haplotype analysis Tagged SNPs were selected for haplotype analysis using HaploBlockFinder software (http://www.cgi.uc.edu/cgi-bin/kzhang/haploBlockFinder.cgi, last accessed in April 2005). The tagged SNPs consisted of a minimal set of SNPs that were uniquely distinguishable from at least 90% of the common haplotypes. After selecting the tagged SNPs, the frequency of each haplotype was estimated and differences in haplotype frequencies between non-diabetic and diabetic subjects were assessed

Table 1 Comparison of genotypic and allelic distribution of SNPs in *ADIPOR1* between type 2 diabetic subjects and non-diabetic subjects

SNP	Rs number	Position (kb)	Genotype, *n* (%)			*p* value	Allele, *n* (%)		*p* value
SNP1	6666089	−8.505	11	12	22		1	2	
NDM			45 (23.4%)	101 (52.6%)	46 (24.0%)		191 (49.7%)	193 (50.3%)	
T2DM			49 (25.5%)	100 (52.1%)	43 (22.4%)	0.871	198 (51.6%)	186 (48.4%)	0.613
SNP6	12039275	−5.692	11	12	22		1	2	
NDM			78 (40.6%)	90 (46.9%)	24 (12.5%)		246 (64.1%)	138 (35.9%)	
T2DM			79 (41.1%)	87 (45.3%)	26 (13.5%)	0.934	245 (63.8%)	139 (36.2%)	0.94
SNP7		−4.612	11	12	22		1	2	
NDM			146 (76.0%)	42 (21.9%)	4 (2.1%)		334 (87.0%)	50 (13.0%)	
T2DM			147 (76.6%)	42 (21.9%)	3 (1.6%)	0.929	336 (87.5%)	48 (12.5%)	0.829
SNP8		−4.519	11	12	22		1	2	
NDM			155 (81.2%)	34 (17.8%)	2 (1.0%)		344 (90.1%)	38 (9.9%)	
T2DM			151 (78.6%)	38 (19.8%)	3 (1.6%)	0.790	340 (88.5%)	44 (11.5%)	0.499
SNP9		−2.635	11	12	22		1	2	
NDM			165 (85.9%)	27 (14.1%)	0 (0.0%)		357 (93.0%)	27 (7.0%)	
T2DM			157 (81.8%)	34 (17.7%)	1 (0.5%)	0.368	348 (90.6%)	36 (9.4%)	0.237
SNP12	2275738	−0.106	11	12	22		1	2	
NDM			132 (68.8%)	55 (28.6%)	5 (2.6%)		319 (83.1%)	65 (16.9%)	
T2DM			135 (70.3%)	52 (27.1%)	5 (2.6%)	0.943	322 (83.9%)	62 (16.1%)	0.771
SNP13	2275737	−0.102	11	12	22		1	2	
NDM			134 (69.8%)	54 (28.1%)	4 (2.1%)		322 (83.9%)	62 (16.1%)	
T2DM			144 (75.0%)	44 (22.9%)	4 (2.1%)	0.502	332 (86.5%)	52 (13.5%)	0.31
SNP15		2.850	11	12	22		1	2	
NDM			126 (65.6%)	60 (31.3%)	6 (3.1%)		312 (81.3%)	72 (18.8%)	
T2DM			138 (71.9%)	50 (26.0%)	4 (2.1%)	0.396	326 (84.9%)	58 (15.1%)	0.178
SNP16		3.000	11	12	22		1	2	
NDM			115 (59.9%)	59 (30.7%)	18 (9.4%)		289 (75.3%)	95 (24.7%)	
T2DM			120 (62.5%)	56 (29.2%)	16 (8.3%)	0.860	296 (77.1%)	88 (22.9%)	0.553
SNP18	1342387	5.841	11	12	22		1	2	
NDM			45 (23.4%)	103 (53.6%)	44 (22.9%)		193 (50.3%)	191 (49.7%)	
T2DM			53 (27.6%)	97 (50.5%)	42 (21.9%)	0.644	203 (52.9%)	181 (47.1%)	0.47
SNP19	3737884	6.993	11	12	22		1	2	
NDM			151 (79.1%)	38 (19.9%)	2 (1.0%)		340 (89.0%)	42 (11.0%)	
T2DM			151 (78.6%)	39 (20.3%)	2 (1.0%)	0.995	341 (88.8%)	43 (11.2%)	0.929
SNP20		7.477	11	12	22		1	2	
NDM			75 (39.1%)	93 (48.4%)	24 (12.5%)		243 (63.3%)	141 (36.7%)	
T2DM			67 (34.9%)	98 (51.0%)	27 (14.1%)	0.685	232 (60.4%)	152 (39.6%)	0.414
SNP22	2275736	8.712	11	12	22		1	2	
NDM			136 (70.8%)	53 (27.6%)	3 (1.6%)		325 (84.6%)	59 (15.4%)	
T2DM			139 (72.4%)	49 (25.5%)	4 (2.1%)	0.847	327 (85.2%)	57 (14.8%)	0.840
SNP23	10581	9.879	11	12	22		1	2	
NDM			150 (78.1%)	39 (20.3%)	3 (1.6%)		339 (88.3%)	45 (11.7%)	
T2DM			140 (72.9%)	49 (25.5%)	3 (1.6%)	0.477	329 (85.7%)	55 (14.3%)	0.284

NDM Non-diabetic subjects; *T2DM* type 2 diabetic subjects; *11* major/major; *12* major/minor; *22* minor/minor

Table 2 Comparison of the genotypic and allelic distribution of SNPs in *ADIPOR2* between type 2 diabetic subjects and non-diabetic subjects

SNP	Rs number	Position, kb	Genotype, *n* (%)			*p* value	Allele, *n* (%)		*p* value
SNP2	2058033	−77.219	11	12	22		1	2	
NDM			99 (51.6%)	78 (40.6%)	15 (7.8%)		276 (71.9%)	108 (28.1%)	
T2DM			91 (47.4%)	84 (43.8%)	17 (8.9%)	0.710	266 (69.3%)	118 (30.7%)	0428
SNP4	12579507	−71.725	11	12	22		1	2	
NDM			84 (43.8%)	88 (45.8%)	20 (10.4%)		256 (66.7%)	128 (33.3%)	
T2DM			79 (41.1%)	89 (46.4%)	24 (12.5%)	0.770	247 (64.3%)	137 (35.7%)	0.495
SNP8		−60.706	11	12	22		1	2	
NDM			93 (48.4%)	82 (42.7%)	17 (8.9%)		268 (69.8%)	116 (30.2%)	
T2DM			89 (46.4%)	84 (43.8%)	19 (9.9%)	0.894	262 (68.2%)	122 (31.8%)	0.640
SNP10	10773982	−53.950	11	12	22		1	2	
NDM			52 (27.1%)	98 (51.0%)	42 (21.9%)		202 (52.6%)	182 (47.4%)	
T2DM			62 (32.3%)	96 (50.0%)	34 (17.7%)	0.419	220 (57.3%)	164 (42.7%)	0.192
SNP12	12810020	−52.196	11	12	22		1	2	
NDM			77 (40.1%)	92 (47.9%)	23 (12.0%)		246 (64.1%)	138 (35.9%)	
T2DM			85 (44.3%)	84 (43.8%)	23 (12.0%)	0.684	254 (66.1%)	130 (33.9%)	0.545
SNP13	11061947	−50.799	11	12	22		1	2	
NDM			123 (64.1%)	62 (32.3%)	7 (3.6%)		308 (80.2%)	76 (19.8%)	
T2DM			120 (62.5%)	63 (32.8%)	9 (4.7%)	0.863	303 (78.9%)	81 (21.1%)	0.655
SNP14	11612383	−50.744	11	12	22		1	2	
NDM			56 (29.2%)	96 (50.0%)	40 (20.8%)		208 (54.2%)	176 (45.8%)	
T2DM			51 (26.6%)	101 (52.6%)	40 (20.8%)	0.835	203 (52.9%)	181 (47.1%)	0.718
SNP15	11612726	−47.171	11	12	22		1	2	
NDM			129 (67.2%)	57 (29.7%)	6 (3.1%)		315 (82.0%)	69 (18.0%)	
T2DM			121 (63.0%)	64 (33.3%)	7 (3.6%)	0.691	306 (79.7%)	78 (20.3%)	0.409
SNP17	7976827	−40.160	11	12	22		1	2	
NDM			69 (36.1%)	103 (53.9%)	19 (9.9%)		241 (63.1%)	141 (36.9%)	
T2DM			64 (33.3%)	97 (50.5%)	31 (16.1%)	0.197	225 (58.6%)	159 (41.4%)	0.203
SNP18	12582624	−39.956	11	12	22		1	2	
NDM			71 (37.0%)	94 (49.0%)	27 (14.1%)		236 (61.5%)	148 (38.5%)	
T2DM			78 (40.6%)	90 (46.9%)	24 (12.5%)	0.744	246 (64.1%)	138 (35.9%)	0.455
SNP20		−39.862	11	12	22		1	2	
NDM			63 (32.8%)	99 (51.6%)	30 (15.6%)		225 (58.6%)	159 (41.4%)	
T2DM			57 (29.7%)	99 (51.6%)	36 (18.8%)	0.655	213 (55.5%)	171 (44.5%)	0.382
SNP21	7297509	−33.122	11	12	22		1	2	
NDM			75 (39.1%)	92 (47.9%)	25 (13.0%)		242 (63.0%)	142 (37.0%)	
T2DM			89 (46.4%)	81 (42.2%)	22 (11.5%)	0.352	259 (67.4%)	125 (32.6%)	0.198
SNP22	12818963	−30.884	11	12	22		1	2	
NDM			67 (34.9%)	96 (50.0%)	29 (15.1%)		230 (59.9%)	154 (40.1%)	
T2DM			79 (41.1%)	87 (45.3%)	26 (13.5%)	0.451	245 (63.8%)	139 (36.2%)	0.265
SNP23		−29.409	11	12	22		1	2	
NDM			74 (38.5%)	92 (47.9%)	26 (13.5%)		240 (62.5%)	144 (37.5%)	
T2DM			81 (42.2%)	90 (46.9%)	21 (10.9%)	0.647	252 (65.6%)	132 (34.4%)	0.367
SNP25	10848569	−21.557	11	12	22		1	2	
NDM			48 (25.0%)	101 (52.6%)	43 (22.4%)		197 (51.3%)	187 (48.7%)	
T2DM			52 (27.1%)	95 (49.5%)	45 (23.4%)	0.823	199 (51.8%)	185 (48.2%)	0.885
SNP26	11061974	−15.081	11	12	22		1	2	
NDM			45 (23.4%)	99 (51.6%)	48 (25.0%)		189 (49.2%)	195 (50.8%)	
T2DM			47 (24.5%)	98 (51.0%)	47 (24.5%)	0.971	192 (50.0%)	192 (50.0%)	0.829
SNP27	2068485	−13.571	11	12	22		1	2	
NDM			63 (32.8%)	95 (49.5%)	34 (17.7%)		221 (57.6%)	163 (42.4%)	
T2DM			60 (31.3%)	97 (50.5%)	35 (18.2%)	0.947	21 (56.5%)	167 (43.5%)	0.771
SNP28	7974924	−9.821	11	12	22		1	2	
NDM			56 (29.2%)	96 (50.0%)	40 (20.8%)		208 (54.2%)	176 (45.8%)	

Table 2 (continued)

SNP	Rs number	Position, kb	Genotype, *n* (%)			*p* value	Allele, *n* (%)		*p* value
T2DM			51 (26.6%)	96 (50.0%)	45 (23.4%)	0.768	198 (51.6%)	186 (48.4%)	0.470
SNP29	12831353	−6.333	11	12	22		1	2	
NDM			95 (49.5%)	74 (38.5%)	23 (12.0%)		264 (68.8%)	120 (31.3%)	
T2DM			78 (40.6%)	89 (46.4%)	25 (13.0%)	0.208	245 (63.8%)	139 (36.2%)	0.147
SNP30	12828908	−2.713	11	12	22		1	2	
NDM			50 (26.0%)	94 (49.0%)	48 (25.0%)		194 (50.5%)	190 (49.5%)	
T2DM			36 (18.8%)	96 (50.0%)	60 (31.3%)	0.163	168 (43.8%)	216 (56.3%)	0.060
SNP31	10848571	0.374	11	12	22		1	2	
NDM			104 (54.2%)	76 (39.6%)	12 (6.3%)		284 (74.0%)	100 (26.0%)	
T2DM			91 (47.4%)	83 (43.2%)	18 (9.4%)	0.305	265 (69.0%)	119 (31.0%)	0.129
SNP36	2286385	8.490	11	12	22		1	2	
NDM			56 (29.2%)	98 (51.0%)	38 (19.8%)		210 (54.7%)	174 (45.3%)	
T2DM			48 (25.0%)	100 (52.1%)	44 (22.9%)	0.584	196 (51.0%)	188 (49.0%)	0.312
SNP39		13.274	11	12	22		1	2	
NDM			52 (27.1%)	98 (51.0%)	42 (21.9%)		202 (52.6%)	182 (47.4%)	
T2DM			61 (31.8%)	96 (50.0%)	35 (18.2%)	0.503	218 (56.8%)	166 (43.2%)	0.246
SNP40	12342	14.800	11	12	22		1	2	
NDM			55 (28.6%)	97 (50.5%)	40 (20.8%)		207 (53.9%)	177 (46.1%)	
T2DM			46 (24.0%)	99 (51.6%)	47 (24.5%)	0.500	191 (49.7%)	193 (50.3%)	0.248
SNP41	1044471	14.858	11	12	22		1	2	
NDM			78 (40.6%)	90 (46.9%)	24 (12.5%)		246 (64.1%)	138 (35.9%)	
T2DM			70 (36.5%)	94 (49.0%)	28 (14.6%)	0.661	234 (60.9%)	150 (39.1%)	0.371

NDM Non-diabetic subjects; *T2DM* type 2 diabetic subjects; *11* major/major; *12* major/minor; *22* minor/minor

using a piece of software based on the Expectation Maximisation algorithm (SNPAlyze; Dynacom, Tokyo, Japan). The differences in the haplotype frequencies were then analysed using the chi square test and the permutation test.

Results

We identified a total of 25 SNPs in *ADIPOR1* (Fig. 1a) and 41 SNPs in *ADIPOR2* (Fig. 1b). All of the SNPs that were identified had genotype frequencies that were in Hardy–Weinberg equilibrium in non-diabetic and type 2 diabetic subjects ($p>0.05$). Among these, SNPs with a minor allele frequency higher than 10% were investigated for linkage disequilibrium (LD) in *ADIPOR1* and *ADIPOR2*, and then association with type 2 diabetes and insulin resistance was evaluated. We estimated the degree of LD between pairs of SNPs using an absolute value of D' ($|D'|$). For *ADIPOR1*, the LD extended over 20 kb of the chromosomal region and covered one haplotype block (Fig. 2a). In contrast, the LD in the chromosomal region was less preserved for *ADIPOR2* and was split into three haplotype blocks (Fig. 2b). No differences were observed between the diabetic and non-diabetic subjects in terms of the distribution of the genotypes or alleles of the SNPs in *ADIPOR1* (Table 1) and *ADIPOR2* (Table 2). Only one nominal association was found; this was between SNP15 in *ADIPOR2* and HOMA-IR (11/12/22: 1.27±0.05/1.35±0.08/1.81±0.21, $p=0.04$) (see Tables 3 and 4 of the ESM). When a Bonferroni adjustment was performed (adopted to avoid type 1 errors

Table 3 Distribution of the haplotypes composed of the tagged SNPs in *ADIPOPR1* and *ADIPOR2* in type 2 diabetic subjects and non-diabetic subjects

Haplotype	T2DM	NDM	*p* value	Permutation *p* value
ADIPOR1				
00000	0.4790	0.4521	0.4549	0.3863
10010	0.0500	0.0509	0.9545	0.2367
10001	0.0782	0.0876	0.6367	0.3687
11001	0.1858	0.1934	0.7882	0.6101
11011	0.0600	0.0746	0.4194	0.2117
11111	0.1093	0.0892	0.3521	0.2400
ADIPOR2				
00000	0.5289	0.5260	0.9358	0.6748
11010	0.0653	0.0436	0.1852	0.1443
11011	0.1530	0.1878	0.1992	0.1974
11111	0.1694	0.1626	0.8010	0.6435
ADIPOR2				
00	0.6235	0.6809	0.0949	0.1020
10	0.0521	0.0567	0.7787	0.6882
11	0.3075	0.2582	0.1290	0.0720
ADIPOR2				
00	0.5079	0.5470	0.2782	0.0926
10	0.1011	0.0761	0.2226	0.0585
11	0.3836	0.3614	0.5249	0.1326

The '0' and '1' used for haplotype notation stand for 'major allele' and 'minor allele', respectively
NDM Non-diabetic subjects; *T2DM* type 2 diabetic subjects

caused by multiple testing; threshold of significance, p=0.001), no association was found between HOMA-IR and SNP15 in *ADIPOR2*. Moreover, when SNP15 in *ADIPOR2* was further genotyped in 384 additional type 2 diabetic subjects and 384 additional non-diabetic subjects (second panel) to avoid type 2 errors, no significant differences were observed in the HOMA results when compared according to SNP15 genotype (11/12/22: 1.66±0.09/1.62±0.07/1.67±0.13, p=0.91). There were no differences in clinical parameters, such as sex, age, BMI, HbA_1c and fasting glucose, between the genotypes of any of the SNPs investigated in the present study (data not shown). We then performed a haplotype analysis, which may be a more sensitive method for detecting associations than the assessment of individual SNPs. First, the haplotype blocks in *ADIPOR1* and *ADIPOR2* were determined. The tagged SNPs that represented more than 90% of the haplotypes in each block were then selected, and the difference in the frequency of each haplotype between the type 2 diabetic subjects and the non-diabetic subjects was analysed. As shown in Table 3, none of the haplotypes in *ADIPOR1* or *ADIPOR2* were associated with type 2 diabetes.

Discussion

After constructing a dense map of SNPs in *ADIPOR1* and *ADIPOR2* and performing haplotype analysis, no evidence of a major role for *ADIPOR1* or *ADIPOR2* in susceptibility to type 2 diabetes or insulin resistance was found in a Japanese population. Our results may reflect a type 2 error (false-negative result), but this is unlikely. First, SNP densities of one SNP every 1.2 kb in *ADIPOR1* and one SNP every 3.9 kb in *ADIPOR2* were used for the association study. The distance between each SNP was short, and the LD between them was fully analysed. We estimated that more than 90% of the haplotypes in *ADIPOR1* and *ADIPOR2* were covered. Second, the sample size used in the present study had an 80% power to detect the effect of a polymorphism, conferring an odds ratio of 2.0 at a significance level of 5% (assuming an allele frequency of 40% in the control population). However, it cannot be excluded that SNPs in *ADIPOR1* and/or *ADIPOR2* had a minor effect on susceptibility to type 2 diabetes.

Consistent with our results for *ADPOR1*, an American study recently reported that SNPs in *ADIPOR1* were not associated with type 2 diabetes in Caucasians or African Americans [20]. However, they reported that the level of expression of *ADIPOR1* in lymphocytes from type 2 diabetic subjects was reduced compared with that in lymphocytes from non-diabetic subjects, implicating *ADIPOR1* in the pathogenesis of type 2 diabetes. Further analysis is needed to clarify the role played by *ADIPOR2* in susceptibility to type 2 diabetes in different ethnic groups.

In summary, the genetic variations in *ADIPOR1* or *ADIPOR2* investigated in the present study were not associated with insulin resistance or type 2 diabetes. However, further studies using denser SNPs and larger samples may be required to conclusively determine that genetic variations in *ADIPOR1* or *ADIPOR2* are not major genetic determinants of the development of type 2 diabetes or insulin resistance.

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