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A Common P2 Promoter Polymorphism of the Hepatocyte Nuclear Factor-4α Gene Is Associated with Insulin Secretion in Non-Obese Japanese with Type 2 Diabetes

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Abstract. Aims. Heterozygous mutations of the hepatocyte nuclear factor (HNF)-4α gene cause a particular form of maturity-onset diabetes of the young (MODY1). Recent genetic studies have shown that single nucleotide polymorphisms (SNPs) of the β-cell type P2 promoter of the HNF-4α gene are associated with type 2 diabetes in some populations. In the Japanese population, a haplotype consisting of two SNPs (rs1884614 and rs2144908) in the P2 promoter region is reported to show a significant association with type 2 diabetes. Methods. Both rs1884614 and rs2144908 were genotyped in 349 type 2 diabetic patients and 203 non-diabetic controls. The relation of these SNPs to clinical characteristics was also examined in the diabetic subjects. Results. There were no differences in the genotype distribution of the two SNPs between the control and diabetic subjects, and the haplotype distribution was also similar in the two groups. However, the rs1884614 T/T genotype was significantly associated with a smaller area under the plasma insulin curve (AUC) during the OGTT in non-obese (BMI <25 kg/m^2) patients ($p = 0.0272$; adjusted for age and sex). Conclusions. SNP rs1884614 in the P2 promoter region of the HNF-4α gene may influence insulin secretion in non-obese Japanese subjects with type 2 diabetes.

Key words: HNF-4α, Single nucleotide polymorphism, Type 2 diabetes, Insulin

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HEPATOCYTE nuclear factor (HNF)-4α, a transcription factor belonging to the nuclear hormone receptor superfamily (NR2A1), is expressed in the liver, kidneys, intestine, and pancreas [1, 2]. Maturity-onset diabetes of the young (MODY) is a genetically heterogenous monogenic disorder that accounts for 2 to 5% of type 2 diabetes. It is characterized by autosomal dominant inheritance and an early age of onset (usually <25 years old). We have previously shown that heterozygous mutations of the HNF-4α gene cause a particular form of MODY (MODY1) [3]. Clinical studies have indicated that the primary cause of MODY1 is impairment of acute insulin secretion by pancreatic β-cells in response to a glucose load [4, 5], indicating that loss of HNF-4α leads to abnormal insulin secretion by these cells. Targeted disruption of HNF-4α in mouse pancreatic β-cells has revealed that HNF-4α controls insulin secretion, at least partly, by regulating K_{ATP} channel function [6, 7].

Interestingly, recent genetic studies have shown that single nucleotide polymorphisms (SNPs) of the β-cell type P2 promoter (located about 46 kb upstream of the original liver type P1 promoter) of the HNF-4α gene are associated with type 2 diabetes in some populations [8–13]. These findings indicate that HNF-4α mutations do not only cause MODY, and that variations of the HNF-4α gene may also be associated with

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Table 1. Clinical characteristics of the study subjects

	Type 2 diabetic subjects	nondiabetic subjects	p
n	349	203	
Age (years)	62.3 ± 0.6	67.7 ± 0.7	<0.001
Sex (M/F)	190/159	94/109	N.S.
BMI (kg/m^2)	24.2 ± 0.3	23.0 ± 0.3 (201)	0.006
FPG (mg/dl)	162.9 ± 3.1	92.8 ± 0.5	<0.001
HbA_{1c} (%)	8.2 ± 0.1	4.9 ± 0.0 (171)	<0.001
F-IRI (μU/ml)	10.5 ± 1.0 (152)	6.2 ± 0.2 (167)	<0.001
HOMA-IR	2.4 ± 0.1 (141)	1.4 ± 0.0 (167)	<0.001

Data are n or mean ± SEM. HOMA-IR = FPG (mg/dl) × F-IRI (μU/ml)/405.
Numbers of available data are shown in each parenthesis. The clinical characteristics of diabetic patients are those recorded at the time of hospitalization.

a predisposition to common type 2 diabetes. In the Japanese population, it has been reported that a haplotype consisting of two SNPs (rs1884614 and rs2144908) in the P2 promoter region is significantly associated with type 2 diabetes [14]. In contrast, another study failed to detect any risk associated with this haplotype in the same population [15]. In the present study, we examined the contribution of these two SNPs to the susceptibility of Japanese to type 2 diabetes and their influence on quantitative parameters of insulin secretion.

Subjects and Methods

Subjects

We enrolled 349 unrelated Japanese subjects with type 2 diabetes who had been admitted to Osaka University Hospital and 203 unrelated Japanese nondiabetic control subjects. The clinical characteristics of the subjects are shown in Table 1. Type 2 diabetes was diagnosed in accordance with the World Health Organization criteria. In all patients, type 2 diabetes was first detected after the age of 40 years. Patients with type 1 diabetes or other types of diabetes (such as maturity onset diabetes of the young) were excluded from the study. This study was approved by the ethics committee of Osaka University and written informed consent was obtained from each participant.

Biological measurements

Chronic hyperglycemia is one of the factors that has a detrimental effect on early-phase insulin secretion (glucotoxicity) [16–18], and this impaired insulin response due to glucotoxicity is partly reversible after treatment of hyperglycemia. After hospitalization, the diabetic patients were treated by diet alone, or regular/ultrarapid insulin before each meal, for at least 2 weeks. At the time when FPG was below 126 mg/dl as a result of treatment, a 75-g OGTT was performed in 109 subjects after an overnight fast, as described previously [19]. Blood samples were collected at 0, 30, 60, and 120 minutes. The insulinogenic index was defined as the ratio of the increment of insulin to that of plasma glucose at 30 minutes after the glucose load (Δinsulin 0–30 minutes/ΔPG 0–30 minutes), and was calculated to assess early-phase insulin secretion [20]. Homeostasis model assessment of insulin resistance (HOMA-IR) was used to estimate insulin sensitivity, which was calculated as follows: [fasting plasma glucose (mg/dl)] × [fasting IRI (μU/ml)]/405. In addition, a glucagon stimulation test was performed by infusing 1 mg of glucagon (Novo Nordisk Pharma. Ltd., Tokyo, Japan) intravenously after an overnight fast. Blood samples were collected at 0 and 5 minutes.

Genotyping of HNF-4α polymorphisms

Genotyping of two single nucleotide polymorphisms (rs1884614 and rs2144908) of HNF-4α was performed by using the TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA). The PCR primers and oligonucleotide probes were purchased from Applied Biosystems. The PCR parameters were 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C and 60 s at 60°C. Then the alleles of the PCR products were determined with an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems).

Table 2. Comparison of genotypic and allelic distribution of the SNPs between type 2 diabetic and nondiabetic subjects

Polymorphisms	Allele frequency (%)		p	Genotype distribution (%)			p
rs1884614	C	T		C/C	C/T	T/T	
T2DM (n = 349)	55.0	45.0		29.5	51.0	19.5	
NDM (n = 203)	53.4	46.6	0.614	30.5	45.8	23.6	0.405
rs2144908	G	A		G/G	G/A	A/A	
T2DM (n = 349)	54.6	45.4		28.9	51.3	19.8	
NDM (n = 203)	52.2	47.8	0.447	29.1	46.3	24.6	0.359

T2DM: type 2 diabetic subjects. NDM: nondiabetic subjects.

When these two SNPs were sequenced in 20 randomly selected subjects, the results were compatible with the genotyping data obtained by using the TaqMan assay.

Statistical analysis

Results are shown as the mean ± SE. Data on the insulinogenic index were logarithmically transformed to approximate a normal distribution. The Hardy-Weinberg equilibrium was assessed by χ^2 analysis. Linkage disequilibrium (LD) and haplotype analyses were performed with SNPAlyze version 5.1 software (Dynacom, Japan). The frequencies of alleles or genotypes were compared by the χ^2 test. Differences of continuous variables among genotypes were evaluated by one-way ANOVA. All statistical analyses were performed with StatView Ver. 5.0 software (SAS Institute Inc., Cary, NC), and statistical significance was defined as $P<0.05$.

Results

Lack of association between HNF-4α SNPs and type 2 diabetes

We genotyped two SNPs (rs1884614 and rs2144908) of HNF-4α in Japanese subjects (Table 2). The frequency of each genotype of these two SNPs was in accord with the Hardy-Weinberg equilibrium ($P = 0.53$ in controls and $P = 0.90$ in diabetic patients for rs1884614, $P = 0.59$ in controls and $P = 0.87$ in diabetic patients for rs2144908). There were no differences in the genotype distribution of rs1884614 between control and diabetic subjects, and the genotype distribution of rs2144908 was also similar in the two groups. The two SNPs showed strong linkage disequilibrium ($r^2 = 0.98$ in diabetic subjects and $r^2 = 0.95$ in control subjects). A minor haplotype (TG for rs1884614 and rs2144908) has been reported to show an association with type 2 diabetes in the Japanese population [14]. However, the existence of this haplotype was not estimated in either our control or diabetic subjects in the present study (Table 3), in accordance with the report of Takeuchi *et al.* [15]. The frequencies of the haplotypes detected were similar in the control and diabetic groups.

Table 3. Haplotype frequencies of rs1884614 and rs2144908 between type 2 diabetic and nondiabetic subjects

Haplotype		Frequency (this study)		Frequency (Takeuchi *et al.*)	
rs1884614	rs2144908	T2DM	NDM	T2DM	NDM
C	G	0.544	0.522	0.548	0.572
T	A	0.451	0.466	0.450	0.426
C	A	0.004	0.012	0.002	0.002
T	G	—	—	—	—

T2DM: type 2 diabetic subjects. NDM: nondiabetic subjects.

Association between rs1884614 and AUC-insulin

To examine the effect of the two SNPs on clinical parameters, 75 g-OGTT was performed in 109 diabetic subjects after minimization of any potential influence of glucotoxity. Since rs1884614 and rs2144908 showed strong linkage disequilibrium, we only examined the influence of rs1884614 in these assessments. No differences were observed with respect to the fasting plasma glucose, HbA_{1c}, BMI, diabetic duration, treatment of diabetes, fasting insulin, homeostasis model assessment of insulin resistance (HOMA-IR),

Table 4. Clinical characteristics of obese/non-obese Type 2 diabetic subjects in whom 75 g-OGTT was performed

	total	obese subjects	non-obese subjects	p (obese vs. non-obese)
n	109	54	55	
Age (years)	61.5 ± 0.9	58.9 ± 1.2	64.0 ± 1.2	0.005
Sex (M/F)	51/58	22/32	29/26	N.S.
BMI (kg/m^2)	25.1 ± 0.5	28.7 ± 0.7	21.6 ± 0.3	<0.001
Duration (years)	12.2 ± 0.8	10.6 ± 1.0	13.7 ± 1.3	N.S.
Treatment (Diet/OHA/Insulin)	17/68/24	9/33/12	8/35/12	N.S.
Family History (+/–)	57/52	31/24	26/28	N.S.
FPG (mg/dl)	115.8 ± 1.7	113.4 ± 2.2	118.1 ± 2.5	N.S.
HbA_{1c} (%)	9.1 ± 0.2	9.2 ± 0.2	9.0 ± 0.3	N.S.
F-IRI (μU/ml)	7.4 ± 0.5	9.3 ± 0.8	5.6 ± 0.5	<0.001
HOMA-IR	2.1 ± 0.2	2.6 ± 0.2	1.6 ± 0.2	<0.001
I.I.	0.18 ± 0.02	0.22 ± 0.02	0.13 ± 0.02	0.005
u-CPR (μg/day)	65.9 ± 4.1 (100)	68.8 ± 6.2 (50)	63.0 ± 5.5 (50)	N.S.
ΔCPR	2.3 ± 0.1 (105)	2.8 ± 0.2 (53)	1.9 ± 0.2 (52)	<0.001
AUC-insulin	63.8 ± 5.1	81.6 ± 8.6	46.3 ± 4.8	<0.001

Data are n or mean ± SEM. HOMA-IR = FPG (mg/dl) × F-IRI (μU/ml)/405.
Obese: BMI ≥25 kg/m^2, non-obese: BMI <25 kg/m^2, I.I.: insulinogenic index. AUC: area under the curve. Family History: presence of type 2 diabetes among their third-degree relatives. Data of FPG, F-IRI, and HOMA-IR at the time of 75 g-OGTT are shown. Numbers of available data are shown in each parenthesis.

Table 5. Genotypes of rs1884614 and clinical data of non-obese (BMI <25 kg/m^2) diabetic subjects

	C/C	C/T	T/T	C/C + C/T	p (CC + CT vs. TT)
n	14	28	13	42	
BMI (kg/m^2)	21.9 ± 0.6	21.1 ± 0.5	22.3 ± 0.6	21.3 ± 0.4	0.4864
Duration (years)	13.2 ± 3.3	13.3 ± 1.7	15.2 ± 1.9	13.3 ± 1.6	0.5817
Treatment (Diet/OHA/Insulin)	1/7/6	6/19/3	1/9/3	7/26/9	0.7241
Family History (+/–)	6/8	19/9	6/7	25/17	0.3956
FPG (mg/dl)	115.4 ± 6.0	118.1 ± 3.2	121.2 ± 4.8	117.2 ± 2.9	0.3848
HbA_{1c} (%)	8.9 ± 0.4	9.1 ± 0.5	8.9 ± 0.4	9.0 ± 0.4	0.5497
F-IRI (μU/ml)	5.4 ± 1.1	5.6 ± 0.8	5.6 ± 0.8	5.5 ± 0.6	0.0886
HOMA-IR	1.6 ± 0.4	1.7 ± 0.3	1.6 ± 0.2	1.6 ± 0.2	0.9990
I.I.	0.18 ± 0.03	0.14 ± 0.02	0.07 ± 0.02	0.15 ± 0.02	0.0680
u-CPR (μg/day)	79.5 ± 11.3	60.4 ± 8.1 (24)	48.9 ± 8.0	67.5 ± 6.7	0.3781
ΔCPR	2.1 ± 0.3	2.0 ± 0.2 (26)	1.6 ± 0.3	2.0 ± 0.2	0.5755
AUC-insulin	56.2 ± 6.6	46.6 ± 8.2	35.2 ± 5.6	49.8 ± 6.0	0.0272

Data are n or mean ± SEM. HOMA-IR = FPG (mg/dl) × F-IRI (μU/ml)/405.
Obese: BMI ≥25 kg/m^2, non-obese: BMI <25 kg/m^2, I.I.: insulinogenic index. AUC: area under the curve. Family History: presence of type 2 diabetes among their third-degree relatives. Data of FPG, F-IRI, and HOMA-IR at the time of 75 g-OGTT are shown. Numbers of available data are shown in each parenthesis.

insulinogenic index (I.I.), urinary C-peptide level, ΔCPR in the glucagon test, and area under the plasma insulin curve (AUC-insulin) during the OGTT in relation to the genotypes of rs1884614 (data not shown). However, among non-obese subjects (BMI <25 kg/m^2), we found a significant association between rs1884614 and AUC-insulin (Table 5). The T/T genotype of rs1884614 was significantly associated with a smaller AUC-insulin (35.2 ± 5.6 for T/T vs. 49.8 ± 6.0 for C/C + C/T, p = 0.0272) after adjustment for age and sex. In contrast, the association was not found in obese patients (BMI ≥25 kg/m^2). Although not significant, there was also a tendency of association between the T/T genotype of rs1884614 and the lower I.I. in non-obese diabetic patients (p = 0.068; adjusted for age and sex).

Discussion

Recent genetic studies have shown that SNPs such as rs1884614 in the P2 promoter region of the HNF-4α gene display a significant association with type 2 diabetes in several populations [8–13]. However, the influence of variations in the P2 region of the HNF-4α gene remains controversial with respect to the Japanese population. One study has shown that a haplotype consisting of rs1884614 (T) and rs2144908 (G) is significantly associated with type 2 diabetes in Japanese [14]. In contrast, this haplotype was not detected among either diabetic or non-diabetic Japanese subjects in another study [15]. In the present study, we also did not find this haplotype in our cohort and failed to demonstrate any significant association of these SNPs with type 2 diabetes. Further studies in larger cohorts will be required to fully define the influence of these SNPs of the HNF-4α gene on the development of type 2 diabetes in the Japanese population.

Impaired acute secretion of insulin by pancreatic β-cells is a characteristic of HNF-4α deficiency in both humans and mice [4–7]. One study from Finland reported that non-diabetic subjects with the risk of A allele for rs2144908 (in nearly perfect linkage disequilibrium with the T allele for rs1884614) had a weaker plasma insulin response to glucose [8]. In addition, the T/T genotype of rs1884614 is significantly associated with a lower insulin secretion index in Thais [21]. Therefore, we examined the relation of rs1884614 to various clinical characteristics of Japanese diabetic subjects. Consistent with previous observations, we also detected a significant association between the T/T genotype of rs1884614 and a smaller AUC-insulin during the OGTT in non-obese diabetic patients. These associations across several studies strongly suggest that the risk allele of rs1884614 (or rs2144908) contributes to insulin response. When we adjusted AUC-insulin data for duration of diabetes, in addition to age and sex, the difference between T/T and C/C + C/T genotypes disappeared ($p = 0.836$). Since it is difficult to determine the precise date of onset of type 2 diabetes, the lack of association after adjustment for duration of diabetes may not rule out the association between rs1884614 and insulin secretion. The region between –27 kb upstream and 17 kb downstream of the P2 promoter is a single large block that shows strong linkage disequilibrium in Japanese individuals, and rs1884614 is located in this block [14]. One possibility is that rs1884614 may directly influence HNF-4α gene expression and insulin secretion, but the observed association may also reflect linkage disequilibrium with an unknown causative variation. The effects of these SNPs on P2 promoter activity of the HNF-4α gene remain unclear at this stage. Further investigation is required to elucidate the functional importance of these SNPs.

Interestingly, an effect of the T/T genotype on AUC-insulin was not detected in obese (BMI ≥25 kg/m^2) diabetic patients. We have no good explanation as to why the association was only observed in non-obese patients. One cannot exclude an interaction between genes or environmental factors involved in both obesity and rs1884614 to cause susceptibility to defective insulin secretion. Obesity is generally associated with hyperinsulinemia. The detrimental effect of this SNP might have been obscure in obese patients with hyperinsulinemia.

In conclusion, there was no association between two SNPs of HNF-4α (rs1884614 and rs2144908) and the occurrence of type 2 diabetes in Japanese subjects. However, SNP rs1884614 in the P2 promoter region of the HNF-4α gene may influence insulin secretion in non-obese diabetic patients.

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