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GPR40 gene Arg211His polymorphism may contribute to the variation of insulin secretory capacity in Japanese men

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Abstract

GPR40 is a member of the G-protein–coupled receptors. Recent studies suggest that GPR40 is highly expressed in pancreatic β cells and insulin-secreting cell lines, and that fatty acids increase intracellular calcium concentration and amplify glucose-stimulated insulin secretion by activating GPR40. Despite identification of the Arg211His polymorphism in the GPR40 gene, there have been no clinical studies concerning this polymorphism. The present study was performed to investigate the effects of the GPR40 gene Arg211His polymorphism on clinical and metabolic parameters, including serum insulin level, in 327 healthy Japanese men, using the TaqMan polymerase chain reaction method. Serum insulin level, homeostasis model of insulin resistance (HOMA-IR), and beta-cell function (HOMA-β) were significantly different (P = .0075, .0152, and .0039, respectively) and were lowest in Arg/Arg homozygotes and highest in His/His homozygotes, although plasma glucose and serum lipids were not significantly different. Multiple regression analyses showed that serum insulin level, HOMA-IR, and HOMA-β were significantly correlated with this polymorphism after adjusting for age and body mass index. After Bonferroni's correction for multiple comparisons was made, only HOMA-β was significantly different among the 3 genotypes. These results suggest that the Arg211His polymorphism in the GPR40 gene may contribute to the variation of insulin secretory capacity in Japanese men.

1. Introduction

Type 2 diabetes mellitus is characterized by impaired insulin secretion and/or insulin resistance [1]. There is evidence of impaired insulin action and pancreatic β-cell dysfunction as early metabolic features in nondiabetic first-degree relatives of type 2 diabetic patients [2,3]. Although considerable effort has been devoted to identifying genes that contribute to diabetes susceptibility, the genetic basis for β-cell dysfunction in common forms of type 2 diabetes has not yet been elucidated [4].

Long-chain fatty acids exert pleiotropic effects on pancreatic β cells. Although fatty acids augment insulin secretion in response to glucose in the short term [5], chronically elevated fatty acid levels adversely affect β-cell function in the long term [6-8]. The effects of fatty acids have been thought to be mediated by their intracellular metabolism. However, the recent observation [9-11] that fatty acids activate GPR40, a G-protein–coupled cell-surface receptor, shows a new perspective on the mode of action of fatty acids. GPR40 has been cloned, along with GPR41-43, downstream of CD22 on the human chromosomal locus [12].

Recently, Haga et al [13] identified the GPR40 gene Arg211His (cGc/cAc) single nucleotide polymorphism. They reported that the allele frequency of A was 0.784 and that of G was 0.216 in the Japanese population. To our knowledge, however, there have been no clinical studies concerning this polymorphism. In the present study, we investigated the effects of the GPR40 Arg211His polymorphism on clinical and metabolic parameters, including serum insulin level, homeostasis model of insulin resistance (HOMA-IR), and beta-cell function (HOMA-β), in healthy Japanese men.

2. Subjects and methods

2.1. Subjects

This study included 327 healthy Japanese men aged 30 to 65 years (49.5 ± 8.8 [mean ± SD] years; body mass index

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[BMI], 23.2 ± 2.4 kg/m^2) who underwent an annual health checkup, with normal fasting plasma glucose (FPG <110 mg/dL). Subjects with endocrine disease, significant renal or hepatic disease, and those receiving medication for diabetes mellitus or hyperlipidemia were excluded. A part of this group of subjects has previously been evaluated for leptin receptor gene polymorphisms [14]. The present study was conducted according to the principles expressed in the Declaration of Helsinki. Informed consent was obtained from each subject before the study, and the protocol was approved by the ethical review committees of the Health Center and the Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan.

2.2. Measurements

Height, weight, FPG, serum insulin, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and free fatty acid levels were measured in the morning after an overnight fast. Plasma glucose and lipids were assayed by routine automated laboratory methods, as described previously [14-18]. Serum insulin level was measured by an enzyme immunoassay, using a commercially available kit (Tosoh, Tokyo, Japan) with intra- and interassay coefficients of 2.9% to 4.6% and 4.5% to 7.0%, respectively. The indices of basal insulin secretion and sensitivity were evaluated by homeostasis model assessment and calculated as follows: HOMA-IR = F-IRI × FPG/22.5, HOMA-β = (20 × F-IRI)/(FPG − 3.5), where F-IRI is fasting serum insulin level (μU/mL) and FPG is fasting plasma glucose level (mmol/L) [19].

2.3. Determination of polymorphism

Genomic DNA was isolated from peripheral blood according to standard procedures. The Arg211His polymorphism was determined by the TaqMan (Applied Biosystems, Tokyo, Japan) polymerase chain reaction method, as described previously [20,21]. The following primers and probes were included in the reaction: forward primer, 5′-GCC ATC ACA GCC TTC TGC TAC-3′; reverse primer, 5′-CCA CGT TGG AGG CGT TGT A-3′; Arg-allele–specific probe, 5′-VIC-CAC TGG CCC *G*CT C-MGB-3′; and His-allele–specific probe, 5′-FAM-ACT GGC CC*A* CTC C-MGB-3′. Polymerase chain reaction was performed with an ABI Prism 7700 (Applied Biosystems) under the following conditions: initial denaturation at 95°C for 10 minutes, followed by 35 cycles of 92°C for 15 seconds and 60°C for 60 seconds.

2.4. Statistical analysis

All statistical analyses were performed using the StatView program for Windows (version 5.0-J; SAS Institute Inc, Cary, NC). The population samples were tested for Hardy-Weinberg equilibrium using the χ^2 test vs the expected distribution pattern. Relations between the GPR40 Arg211His polymorphism and metabolic parameters were examined by nonparametric Kruskal-Wallis test. Bonferroni's correction for multiple comparisons was performed where appropriate. Differences in serum insulin level, HOMA-IR, and HOMA-β due to the genotypes were analyzed by multiple regression analyses incorporating age and BMI as independent variables. Because serum insulin level, HOMA-IR, and HOMA-β were normally distributed after logarithmic transformation, the logarithms of these parameters were used for multiple regression analyses. All data are expressed as mean ± SD, and P values less than 0.05 were considered statistically significant.

3. Results

The population samples were in Hardy-Weinberg equilibrium. In this study, only 15 (4.6%) were Arg/Arg homozygous, 107 (32.7%) were heterozygous, and 205 (62.7%) were His/His homozygous for the GPR40 gene. So the frequency of the Arg211 allele was 0.21 and the frequency of the His211 allele was 0.79.

As shown in Table 1, we compared clinical and metabolic parameters among these genotypes in 327 healthy Japanese men. Among the 3 genotypes, serum insulin level (P = .0075), HOMA-IR (P = .0152), and

Table 1
Relations between GPR40 Arg211His genotype and metabolic parameters in 327 healthy men

Parameter	Arg/Arg	Arg/His	His/His	P[a] for 3 groups
No. of subjects	15 (4.6%)	107 (32.7%)	205 (62.7%)	
Age (y)	54 ± 9	48 ± 8	50 ± 9	.0416
BMI (kg/m^2)	22.1 ± 1.9	23.3 ± 2.3	23.3 ± 2.5	NS
Glucose (mg/dL)	96 ± 7	94 ± 6	94 ± 6	NS
Insulin (μU/mL)	4.1 ± 2.9	5.1 ± 2.6	5.5 ± 2.4	.0075
HOMA-IR	1.0 ± 0.7	1.2 ± 0.6	1.3 ± 0.6	.0152
HOMA-β	46 ± 29	60 ± 31	65 ± 29	.0039
Total cholesterol (mg/dL)	212 ± 21	206 ± 30	211 ± 30	NS
Triglycerides (mg/dL)	90 ± 69	112 ± 74	125 ± 94	NS
HDL cholesterol (mg/dL)	59 ± 11	60 ± 13	58 ± 13	NS
LDL-cholesterol (mg/dL)	130 ± 22	122 ± 29	126 ± 27	NS
Free fatty acids (mEq/L)	0.63 ± 0.24	0.70 ± 0.24	0.74 ± 0.37	NS

Values are mean ± SD. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; NS, not significant (P > .05).
[a] Kruskal-Wallis test.

Table 2
Multiple regression analyses with serum insulin level (A), HOMA-IR (B), and HOMA-β (C) as dependent variables

(A) Parameter	R	P
Age	−0.132	.0076
BMI	0.412	<.0001
Genotype	0.151	.0023

(B) Parameter	R	P
Age	−0.082	NS
BMI	0.422	<.0001
Genotype	0.138	.0055

(C) Parameter	R	P
Age	−0.271	<.0001
BMI	0.315	<.0001
Genotype	0.173	.0005

R denotes standardized regression coefficient.
Genotypes were determined as follows: Arg/Arg = 0, Arg/His = 1, and His/His = 2.

HOMA-β (P = .0039) were significantly different, and were lowest in Arg/Arg homozygotes, intermediate in heterozygotes, and highest in His/His homozygotes. There was a marginally significant age difference among the 3 genotypes (P = .0416). There were no significant differences among the 3 genotypes in any other parameters measured. After Bonferroni's correction for multiple comparisons was made, only HOMA-β was significantly different among the 3 genotypes.

As shown in Table 2, we next performed multiple regression analyses to confirm the relations between the genotypes and serum insulin level, HOMA-IR, and HOMA-β. Significant associations between the GPR40 Arg211His polymorphism and serum insulin level (R = 0.151, P = .0023), HOMA-IR (R = 0.138, P = .0055), and HOMA-β (R = 0.173, P = .0005) were observed in multiple regression analyses with age and BMI as independent variables.

4. Discussion

GPR40 is a member of G-protein–coupled receptors, and was originally isolated from a human genomic DNA fragment. The GPR40 gene is located downstream of CD22, which was previously mapped to 19q13.1, and GPR40 encodes a 300–amino acid protein containing the characteristic 7 transmembrane domains. The entire coding sequence is in one exon, and there are no introns in the GPR40 gene. The Arg211 residue is located between transmembrane 5 domain and transmembrane 6 domain, that is, in the intracellular region [12].

Long-chain fatty acids augment glucose-stimulated insulin secretion from pancreatic β cells via intracellular metabolism and generation of lipid-derived molecules such as fatty acyl-CoA. The recent observation that fatty acids activate GPR40 indicates that they can also act as extracellular receptor ligands. Itoh et al [9] showed that GPR40, which was abundantly expressed in the pancreas, functioned as a receptor for long-chain fatty acids. They also showed that long-chain fatty acids amplified glucose-stimulated insulin secretion from pancreatic β cells by activating GPR40. Furthermore, GPR40 was significantly expressed in pancreatic β-cell lines. Briscoe et al [10] reported that medium- to long-chain fatty acids were able to induce an elevation of intracellular calcium concentration in human GPR40-expressing cells. Quantitative reverse transcriptase polymerase chain reaction showed that GPR40 was specifically expressed in the human brain and pancreas, with localized expression in insulin-producing β cells of the rodent pancreas. Kotarsky et al [11] also reported that GPR40 was expressed in pancreatic β cells and that GPR40 was specifically activated by medium- to long-chain fatty acids and thiazolidinedione drugs. These novel findings add a new insight into the complex cellular effects of fatty acids, particularly relating to the control of insulin release and its dysfunction in type 2 diabetes mellitus.

In the present study, the allele frequencies of the GPR40 Arg211His variant were not different compared with previous reports [13]. We also determined the Arg211His polymorphism in 203 type 2 diabetic men. However, the genotype and allele frequencies of this variant did not differ between type 2 diabetic patients and healthy subjects (data not shown). Among healthy subjects, we found that serum insulin level, HOMA-IR, and HOMA-β were significantly lower in Arg/Arg homozygotes than in His/His homozygotes of the GPR40 gene, although plasma glucose and serum lipids were not different among the genotypes. The exact mechanisms of these differences are not clear, because there have been no functional studies concerning this polymorphism either in vitro or in vivo. Briscoe et al [10] reported a significant increase in expression of GPR40 messenger RNA (mRNA) in parallel with insulin mRNA in the pancreas of *ob*/*ob* mice compared to control lean mice. Therefore, we speculate that human GPR40 with the Arg211 allele reduces transducing activity and insulin secretory capacity under equivalent glucose and fatty acid conditions, or that human GPR40 with the His211 allele may potentiate insulin secretion and predispose to insulin resistance. Although we do not know whether the GPR40 gene polymorphism predominantly affects insulin secretion or insulin resistance, our finding that only HOMA-β was significantly different among the genotypes after Bonferroni's correction for multiple comparisons may suggest that insulin secretion is affected more. Furthermore, our finding that the lipid profile was not significantly different among the genotypes may suggest that insulin resistance is affected less.

We also found that there were significant associations between the GPR40 Arg211His polymorphism and serum

insulin level, HOMA-IR, and HOMA-β in multiple regression analyses with age and BMI as independent variables. Because it was not clear whether the effect of this polymorphism on insulin secretory capacity might be codominant, we performed analyses of the difference between Arg/His groups and His/His groups. Although we found higher tendencies for serum insulin level, HOMA-IR, and HOMA-β in His/His homozygotes, these differences between the 2 groups were not statistically significant (P = .0765, .0891, and .0710 by Mann-Whitney U test). Therefore, there is a possibility that the effect of this polymorphism may be His dominant instead of codominant.

Although the so-called gold standard tests, glucose clamp methods, are useful for intensive physiological studies on small numbers of subjects, a simpler tool such as HOMA is more appropriate for large epidemiological or genetic studies. HOMA is a measure of basal insulin sensitivity and β-cell function and is not intended to give information about the stimulated state. In addition, HOMA has several other limitations: relatively poor reproducibility and impossible or difficult assessment in those taking exogenous insulin [22]. It is unfortunate that we were not able to perform oral glucose or meal tolerance test in this study. A greater difference in insulin level might be observed after a glucose load because GPR40 was activated more at high glucose concentrations [9].

In conclusion, the present study showed relations between the GPR40 Arg211His polymorphism and serum insulin level, HOMA-IR, and especially HOMA-β in healthy Japanese men. It is suggested that genetic variation in the GPR40 gene, together with other genes, may contribute to the variation of insulin secretory capacity, which may possibly link β-cell dysfunction and type 2 diabetes mellitus. Future studies in vitro and in vivo may further clarify the role of the GPR40 gene in human β-cell function.

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