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Association of soluble epoxide hydrolase gene polymorphism with insulin resistance in type 2 diabetic patients

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Abstract

The insulin resistance found in diabetes is influenced by vascular tone and local blood flow. Endothelial-derived hyperpolarizing factor (EDHF) functions as a potent vasodilator to regulate vascular tone, and its production is regulated by soluble epoxide hydrolase (sEH). In this study, we examined the genotype distribution and allele frequency of sEH gene G860A (Arg287Gln) polymorphism in Japanese subjects ($n = 499$) (non-diabetic subjects, $n = 205$; type 2 diabetic patients, $n = 294$). Also, to accurately evaluate insulin resistance, we performed the euglycemic hyperinsulinemic clamp test for each type 2 diabetic patient ($n = 86$) from whom agreement was obtained, and then examined a possible association of sEH gene G860A polymorphism with insulin resistance status. There was no significant difference in genotype distribution and allele frequency between non-diabetic subjects and type 2 diabetic patients. Interestingly, however, there was close association of sEH gene G860A (Arg287Gln) polymorphism with insulin resistance in type 2 diabetic patients, which was not observed in non-diabetic subjects. These results suggest that sEH and EDHF play some important role in the pathogenesis of insulin resistance found in type 2 diabetes.

Keywords: Diabetes; Insulin resistance; Euglycemic hyperinsulinemic clamp; Soluble epoxide hydrolase; Polymorphism

Type 2 diabetes is the most prevalent and serious metabolic disease affecting people around the world. The hallmark of the disease is insulin resistance which is influenced by vascular tone and local blood flow. Glucose uptake into various tissues has been reported to be enhanced by insulin-mediated vasodilation and the decrease in local blood flow to lead to the development of insulin resistance [1,2]. An endothelial-derived hyperpolarizing factor (EDHF) that functions as a potent vasodilator is thought to be epoxyeicosatrienoic acids (EETs), lipid metabolites of arachidonic acid which are synthesized by the cytochrome P450 system [3–5]. EETs function as regulators of the vascular tone as well as an anti-inflammatory molecules [6–14]. Their production is regulated by soluble epoxide hydrolase (sEH), a ubiquitous enzyme that catalyzes the degradation of EETs. It has been reported that a common polymorphism in exon 8 of the sEH gene, which results in an amino acid substitution from arginine to glutamine at codon 287 (G860A), reduces its enzymatic activity and decreases its protein stability [15,16]. Very recently, the coronary artery risk development in young adults (CARDIA) study demonstrated that the sEH Arg287Gln polymorphism is associated with coronary artery calcification, suggesting a role of sEH in the pathogenesis of atherosclerosis [17]. In this study, we examined whether sEH Arg287Gln polymorphism is associated with insulin resistance in type 2 diabetes. This is the first report showing the association of sEH and insulin resistance.

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Methods

Study subjects. Japanese type 2 diabetic patients ($n = 294$, aged 30–76 years, duration of diabetes: 12.1 ± 9.2 years) undergoing periodic follow-up examinations at the Diabetes Clinic in Osaka University Hospital were enrolled in this study. The determination of type 2 diabetes was based on World Health Organization criteria. Patients were recruited for the study if they met the following inclusion criteria: (1) no episodes of ketoacidosis, (2) absence of overt diabetic nephropathy, and (3) absence of cardiovascular, cerebral vascular, and peripheral artery disease. Of the 294 diabetic patients, 72 patients were controlled with diet only, 114 with oral agents, and 108 with insulin injection. As control subjects, we enrolled healthy non-diabetic subjects ($n = 205$, aged 21–66 years) without cardiovascular, cerebral vascular or peripheral artery disease. Written informed consent was obtained from all subjects enrolled in this study.

Euglycemic hyperinsulinemic clamp test. For non-diabetic subjects ($n = 205$), insulin sensitivity was determined using fasting insulin and glucose concentrations by homeostasis model assessment of insulin resistance (HOMA). Plasma insulin concentrations were measured by radioimmunoassay (SRL, Tokyo, Japan). For type 2 diabetic patients, insulin sensitivity was determined by the euglycemic hyperinsulinemic clamp test. Of type 2 diabetic patients ($n = 294$), 86, from whom agreement was obtained, were subjected to determination of insulin-mediated glucose uptake by the euglycemic hyperinsulinemic clamp technique using an artificial pancreas (STG22; Nikkiso, Tokyo, Japan). Before the clamp study, the patients were hospitalized at Osaka University Hospital for at least 2 weeks and were confirmed to be free of glucose toxicity. Briefly, regular insulin (Humalin-R Eli Lilly, Indianapolis, IN, USA) was infused in a primed continuous manner at a rate of 8.7 pmol/kg/min for 2 h. Normoglycemia was maintained by adjusting the rate of a 10% D-glucose infusion based on plasma glucose measurements performed at 1-min intervals. Glucose infusion rate (GIR) was calculated by averaging the glucose infusion rates achieved over the last 30 min of the clamp as endogenous glucose production is completely suppressed at the elevated concentrations achieved.

Table 1
Clinical characteristics of non-diabetic and type 2 diabetic subjects

Variables	Non-diabetic subjects	Type 2 diabetic patients
n	205	294
Male/female	132/73	210/84
Age (year)	44.1 ± 0.8	57.4 ± 0.6
Duration (years)	—	12 ± 0.5
BMI (kg/m^2)	22.3 ± 0.2	23.7 ± 0.2
Systolic blood pressure (mmHg)	122 ± 0.9	133 ± 1.0
Diastolic blood pressure (mmHg)	75 ± 0.7	76 ± 0.5
HbA1c (%)	4.9 ± 0.02	7.7 ± 0.1
Total cholesterol (mg/dl)	193 ± 2.0	203 ± 2.1
Triglycerides (mg/dl)	95 ± 3.0	133 ± 4.2
HDL cholesterol (mg/dl)	61 ± 1.0	52 ± 0.9

Data are shown as means ± SE.

Genotype determination. Whole blood was first collected in a Vacutainer CPT tube (Becton–Dickinson, Franklin Lakes, NJ, USA). After the tube was centrifuged in a horizontal, swing-out rotor (20 min, 1500*g*, 24 °C), the leukocyte-enriched fraction was collected. This was then added to lysing buffer and DNA was extracted using the QIAamp Blood Kit (Qiagen, Chatsworth, CA, USA). Genotyping for the G860A (Arg287Gln) polymorphism was performed using the TaqMan assay kit (Applied Biosystems) as described previously [17]. A 64-bp product was amplified by polymerase chain reaction from 15-ng DNA using 0.9 μmol/L each of forward primer (AGA TCC CTG CTC TGG CCC) and reverse primer(TCT CCA TAG CCT TTC ATG TCC A). The sequence-specific probes (FAM-TAG GAC CcG GTA ACC and VIC-CTA GGA CCt GGT AAC C) were used in the allele discrimination assay, and allele detection and genotype calling were performed using the ABI7700 instrument and Sequence Detection System software.

Statistical analysis. The difference in quantitative variables was examined by one-way ANOVA and differences in allele frequency were examined by χ^2 analysis. Data are shown as means ± SEM.

Results

Genotype distribution and allele frequency of soluble epoxide hydrolase gene G860A (Arg287Gln) polymorphism in Japanese non-diabetic and diabetic subjects

We first examined the genotype distribution and allele frequency of sEH gene G860A (Arg287Gln) polymorphism in Japanese subjects ($n = 499$). Table 1 shows clinical characteristics of non-diabetic ($n = 205$) and type 2 diabetic subjects ($n = 294$), and Table 2 shows genotype distribution and allele frequency in non-diabetic subjects and type 2 diabetic patients. Genotype distribution and allele frequency of sEH G860A polymorphism in non-diabetic subjects and type 2 diabetic patients were in accordance with the Hardy–Weinberg equilibrium. Also, as shown in Table 2, there was no significant difference in genotype distribution (χ^2 analysis; genotype: $\chi^2 = 0.081$, $p = 0.97$) and allele frequency ($\chi^2 = 0.074$, $p = 0.85$) between Japanese non-diabetic subjects ($n = 205$) and type 2 diabetic patients ($n = 294$).

Association of soluble epoxide hydrolase gene polymorphism with insulin resistance in type 2 diabetic patients

We evaluated a possible association of sEH gene G860A polymorphism with insulin resistance status in non-diabetic subjects and type 2 diabetic patients. The

Table 2
Genotype distribution and allele frequency of sEH gene G860A polymorphism in non-diabetic subjects and type 2 diabetic patients

	n	Genotype			Allele frequency	
		GG	GA	AA	G	A
Non-diabetic subjects	205	126 (61.5%)	71 (34.6%)	8 (3.9%)	0.788	0.212
Type 2 diabetic patients	294	177 (60.2%)	105 (35.7%)	12 (4.1%)	0.781	0.219

number of homozygous mutants was so small that we combined the data of homozygous (AA) and heterozygous mutants (GA) in the following analysis. To evaluate insulin resistance in non-diabetic subjects, we calculated their HOMA values. We did not perform the euglycemic hyperinsulinemic clamp test in non-diabetic subjects from the ethical point of view. As shown in Table 3, there was no significant difference in age, BMI, HbA1c, blood pressure, and cholesterol levels between with (GA + AA) and without the 860A allele (GG) in both non-diabetic ($n = 205$) and diabetic subjects ($n = 294$). Also, there was no difference in HOMA values between with (GA + AA) and without the 860A allele (GG) in non-diabetic subjects. Next, to accurately evaluate insulin resistance in type 2 diabetic patients from whom agreement was obtained ($n = 86$), we performed the euglycemic hyperinsulinemic clamp test and calculated their glucose infusion rate which indicates insulin sensitivity in the whole body. As shown in Table 4, there was no significant difference in age, BMI, HbA1c levels between with (GA + AA) and without the 860A allele (GG). Interestingly, however, the glucose infusion rate in type 2 diabetic patients with the sEH gene 860A allele (GA + AA) was significantly lower than those without the 860A allele (GG) (5.06 ± 0.26 vs. 6.01 ± 0.28 mg/kg/min, $p = 0.038$). It is noted that glucose infusion rate indicates insulin sensitivity in the whole body. Thus, insulin sensitivity in type 2 diabetic patients with the sEH gene 860A allele (GA + AA) was significantly lower than those without the 860A allele (GG). These results indicate that the presence of the 860A allele in the sEH gene is associated with the insulin resistance status found in type 2 diabetic patients.

Table 4
Association of sEH gene G860A polymorphism with insulin resistance in type 2 diabetic patients

	Genotype		
	GG	GA or AA	
n	51	35	
Gender (male/female)	39/17	24/11	n.s.
Age (years)	50.1 ± 1.8	52.4 ± 2.2	n.s.
BMI (kg/m^2)	25.2 ± 0.8	25.9 ± 0.8	n.s.
HbA1c (%)	7.5 ± 0.3	7 ± 0.3	n.s.
Glucose infusion rate (mg/kg/min)	6.01 ± 0.28	5.05 ± 0.26	$p = 0.038$

Data are shown as means ± SE.

Discussion

In this study, we examined the genotype distribution and allele frequency of sEH gene G860A (Arg287Gln) polymorphism in Japanese subjects ($n = 499$) (non-diabetic subjects, $n = 205$; type 2 diabetic patients, $n = 294$) and a possible association of sEH gene G860A polymorphism with insulin resistance status which was accurately evaluated by the euglycemic hyperinsulinemic clamp technique using artificial pancreas. The main finding in this

Table 3
Clinical characteristics according to G860A polymorphism of sEH gene

	Non-diabetic subjects			Type 2 diabetic patients		
	GG	GA or AA	*p*	GG	GA or AA	*p*
Gender (male/female)	81/45	51/28		130/47	80/37	
Age (years)	44.1 ± 0.9	41.2 ± 1.2	n.s.	57.8 ± 0.8	57.2 ± 1.0	n.s.
BMI (kg/m^2)	22.3 ± 0.3	22.8 ± 0.4	n.s.	23.8 ± 0.3	23.6 ± 0.3	n.s.
Systolic BP (mmHg)	122 ± 1.2	122 ± 1.3	n.s.	132 ± 1.2	133 ± 1.6	n.s.
Diastolic BP (mmHg)	75 ± 0.1	76 ± 0.1	n.s.	76 ± 0.7	76 ± 0.9	n.s.
HbA1c (%)	4.9 ± 0.03	4.8 ± 0.03	n.s.	7.9 ± 0.1	7.6 ± 0.1	n.s.
Total cholesterol (mmol/L)	194 ± 2.5	190 ± 3.5	n.s.	203 ± 2.6	203 ± 3.7	n.s.
Triglycerides (mmol/L)	92 ± 3.7	100 ± 5.2	n.s.	130 ± 5.0	137 ± 7.1	n.s.
HDL cholesterol (mmol/L)	61 ± 1.3	62 ± 1.5	n.s.	51 ± 1.1	52 ± 1.4	n.s.
Insulin (pmol/L)	7.2 ± 0.3	7.1 ± 0.3	n.s.	—	—	
HOMA-R	1.63 ± 0.06	1.64 ± 0.08	n.s.	—	—	
HT-Risk[a]	17	11	n.s.	41	25	n.s.
HL-Risk[a]	31	8	n.s.	48	30	n.s.
SM-Risk[a]	29	5	n.s.	50	28	n.s.
Nephropathy[a]	—	—		155/22	99/18	n.s.
Retinopathy[a]	—	—		149/28	96/21	n.s.
Treatment of diabetes[a]	—	—		45/68/64	23/49/45	n.s.

Data are shown as means ± SE. HT-Risk, number of subjects with either systolic >160, diastolic pressure >95 mmHg, or taking anti-hypertensive drugs; HL-Risk, number of subjects with either total cholesterol >240 mg/dl, triglyceride >150 mg/dl, HDL-cholesterol <40 mg/dl or taking hypolipidemic drugs; SM-Risk, number of subjects who smoke more than one pack of cigarettes per day for 20 years; nephropathy, normoalbuminuria + albuminuria/proteinuria with normal serum creatinine; retinopathy, no diabetic retinopathy + background diabetic retinopathy/preproliferative diabetic retinopathy + proliferative diabetic retinopathy; treatment of diabetes, diet therapy/oral agents/insulin injection.

[a] χ^2 test.

study is that sEH gene G860A (Arg287Gln) polymorphism is closely associated with insulin resistance in type 2 diabetic patients. These results suggest that sEH and EDHF play some important role in the pathogenesis of insulin resistance found in type 2 diabetes.

In contrast to type 2 diabetic patients, there was no association between sEH gene G860A polymorphism and insulin sensitivity in non-diabetic subjects. Since endothelial cell dysfunction is often observed in type 2 diabetic patients, but not in non-diabetic subjects, we assume that the presence of the 860A allele in the sEH gene, together with endothelial cell dysfunction, leads to the substantial progression of insulin resistance. It is noted that although the development of obesity and/or poor glycemic control are well known to induce insulin resistance, there was no difference in BMI and HbA1c levels between the two groups. Thus, we assume that the sEH gene G860A polymorphism directly affects the insulin resistance status in type 2 diabetic patients by altering vascular tone. Although we could not measure the plasma EET levels in the patients due to the very short half-life of EETs, we assume that plasma EET levels are lower in type 2 diabetic patients with sEH G860A, which leads to a disturbed vascular tone and to an increase of insulin resistance.

In conclusion, although no significant difference was observed in the genotype distribution and allele frequency of sEH gene G860A (Arg287Gln) polymorphism between Japanese non-diabetic subjects and type 2 diabetic patients, the glucose infusion rate in type 2 diabetic patients with the sEH gene 860A allele (GA + AA) was significantly lower than those without the 860A allele (GG). This is the first report showing the association of sEH and insulin resistance, implying that sEH and EDHF play some important role in the pathogenesis of insulin resistance found in type 2 diabetes.

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References

[1] A.D. Baron, H.O. Steinberg, H. Chaker, R. Leaming, A. Johnson, G. Brechtel, Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans, J. Clin. Invest. 96 (1995) 786–792.

[2] H.O. Steinberg, H. Chaker, R. Leaming, A. Johnson, G. Brechtel, A.D. Baron, Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance, J. Clin. Invest. 97 (1996) 2601–2610.

[3] J.H. Capdevila, J.R. Falck, R.W. Estabrook, Cytochrome P450 and the arachidonate cascade, FASEB J. 6 (1992) 731–736.

[4] B. Fisslthaler, R. Popp, L. Kiss, M. Potente, D.R. Harder, I. Fleming, R. Busse, Cytochrome P450 2C is an EDHF synthase in coronary arteries, Nature 401 (1999) 493–497.

[5] D.L. Kroetz, D.C. Zeldin, Cytochrome P450 pathways of arachidonic acid metabolism, Curr. Opin. Lipidol. 13 (2002) 273–283.

[6] N.L. Weintraub, X. Fang, T.L. Kaduce, M. VanRollins, P. Chatterjee, A.A. Spector, Potentiation of endothelium-dependent relaxation by epoxyeicosatrienoic acids, Circ. Res. 81 (1997) 258–267.

[7] C.L. Oltman, N.L. Weintraub, M. VanRollins, K.C. Dellsperger, Epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids are potent vasodilators in the canine coronary microcirculation, Circ. Res. 83 (1998) 932–939.

[8] K. Node, Y. Huo, X. Ruan, B. Yang, M. Spiecker, K. Ley, D.C. Zeldin, J.K. Liao, Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids, Science 285 (1999) 1276–1279.

[9] Z. Yu, F. Xu, L.M. Huse, C. Morisseau, A.J. Draper, J.W. Newman, C. Parker, L. Graham, M.M. Engler, B.D. Hammock, D.C. Zeldin, D.L. Kroetz, Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids, Circ. Res. 87 (2000) 992–998.

[10] K. Node, X.L. Ruan, J. Dai, S.X. Yang, L. Graham, D.C. Zeldin, J.K. Liao, Activation of G αs mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids, J. Biol. Chem. 276 (2001) 15983–15989.

[11] J. Sun, X. Sui, J.A. Bradbury, D.C. Zeldin, M.S. Conte, J.K. Liao, Inhibition of vascular smooth muscle cell migration by cytochrome p450 epoxygenase-derived eicosanoids, Circ. Res. 90 (2002) 1020–1027.

[12] M. Potente, U.R. Michaelis, B. Fisslthaler, R. Busse, I. Fleming, Cytochrome P450 2C9-induced endothelial cell proliferation involves induction of mitogen-activated protein (MAP) kinase phosphatase-1, inhibition of the c-Jun N-terminal kinase, and up-regulation of cyclin D1, J. Biol. Chem. 277 (2002) 15671–15676.

[13] U.R. Michaelis, B. Fisslthaler, M. Medhora, D. Harder, I. Fleming, R. Busse, Cytochrome P450 2C9-derived epoxyeicosatrienoic acids induce angiogenesis via cross-talk with the epidermal growth factor receptor (EGFR), FASEB J. 17 (2003) 770–772.

[14] M. Spiecker, H. Darius, T. Hankeln, M. Soufi, A.M. Sattler, J.R. Schaefer, K. Node, J. Borgel, A. Mugge, K. Lindpaintner, A. Huesing, B. Maisch, D.C. Zeldin, J.K. Liao, Risk of coronary artery disease associated with polymorphism of the cytochrome P450 epoxygenase CYP2J2, Circulation 110 (2004) 2132–2136.

[15] M. Sandberg, C. Hassett, E.T. Adman, J. Meijer, C.J. Omiecinski, Identification and functional characterization of human soluble epoxide hydrolase genetic polymorphisms, J. Biol. Chem. 275 (2000) 28873–28881.

[16] P.K. Srivastava, V.K. Sharma, D.S. Kalonia, D.F. Grant, Polymorphisms in human soluble epoxide hydrolase: effects on enzyme activity, enzyme stability, and quaternary structure, Arch. Biochem. Biophys. 427 (2004) 164–169.

[17] M. Fornage, E. Boerwinkle, P.A. Doris, D. Jacobs, K. Liu, N.D. Wong, Polymorphism of the soluble epoxide hydrolase is associated with coronary artery calcification in African-American subjects, Circulation 109 (2004) 335–339.