Insulin Receptor Gene Polymorphism and Hyperinsulinemia in Hypertensive Patients

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Although insulin resistance often occurs in association with hypertension, considerable variation is observed in the degree of insulin resistance among hypertensive patients. Since there is evidence of a genetic basis in the development of insulin resistance in hypertension, we analyzed the contribution of genetic factors to insulin resistance in hypertensive patients. Sixty-six Japanese hypertensive patients were studied. These patients were divided into two groups (hyperinsulinemia group and normoinsulinemia group) according to plasma insulin response during a 75-g oral glucose tolerance test (75g-OGTT). Insulin receptor gene (INSR) was studied for association with insulin resistance in hypertensive patients. A microsatellite polymorphism in intron-2 of the insulin receptor gene was analyzed by the polymerase chain reaction method. Five alleles were detected in the INSR microsatellite. The frequency of C/C genotype in the hyperinsulinemia group was significantly higher than that in the normoinsulinemia group (73% vs. 43%, $p=0.02$). There was no difference in genotype frequency of INSR between hypertensive patients and control subjects. When the hypertensive patients were divided into two groups, the frequency of C/C genotype in the hyperinsulinemia group was significantly higher than that in the control group (73% vs. 45%, $p=0.014$). There was no significant difference between the normoinsulinemia group and control group. These data suggest that the insulin receptor gene may contribute to insulin resistance in hypertensive patients with hyperinsulinemia. (*Hypertens Res* 1995; 18: 215-218)

Key Words: hypertension, insulin resistance, insulin receptor, hyperinsulinemia

It has been reported that insulin resistance is more frequent in hypertensive patients than in the normal population (1-3). However, since not all hypertensive patients have insulin resistance, hypertension itself is not sufficient for the development of insulin resistance in hypertensive patients. Epidemiological studies suggest that insulin resistance precedes the development of hypertension (4). In addition, normotensive offspring of hypertensive parents have been reported to be insulin resistant (5, 6). These data suggest that there is evidence of a genetic basis for the development of insulin resistance in hypertension, but the putative gene defect is unknown.

The site of insulin resistance in hypertensive patients was reported to be peripheral tissues (mainly skeletal muscle) (3, 7). In peripheral tissues, after insulin binds to the insulin receptor, the insulin receptor plays a critical role in transducing the insulin signal to target cells, resulting in facilitation of glucose transport by the insulin-sensitive glucose transporter (GLUT4) (8). Altered function or expression of the insulin receptor gene (INSR) can cause insulin resistance (9), and therefore this gene was considered to be a candidate gene for insulin resistance. In this study, we investigated the association

of INSR polymorphism with insulin resistance in hypertension to clarify the contribution of genetic factors to insulin resistance.

Subjects and Methods

The study group consisted of 66 patients with essential hypertension. The diagnosis of essential hypertension was defined by the WHO criteria (systolic and diastolic blood pressure above 160 and 95 mmHg, respectively, on two separate occasions after a 10-min rest at our outpatient clinic) and a complete medical workup was carried out to exclude secondary forms of hypertension. The subjects were divided into two groups (hyperinsulinemia group and normoinsulinemia group) according to their insulin levels during 75g-OGTT. Hyperinsulinemia was defined as a peak insulin level higher than 600 pmol/l during 75g-OGTT. Patients who showed a diabetic pattern were excluded from this study. One hundred non-obese healthy subjects served as controls.

DNA was extracted from peripheral blood leukocytes. Blood samples were drawn from the antecubital fossa, placed in heparinized tubes, and stored

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Table 1. Clinical Characteristics of the Hypertensive Patients Studied

Variable	Hyperinsulinemia (peak IRI > 600 pmol/l) (n = 26)	Normoinsulinemia (peak IRI ≤ 600 pmol/l) (n = 40)	Significance
Age (yr)	59 ± 2	55 ± 2	N.S.
BMI (kg/m ²)	23.9 ± 0.7	23.1 ± 0.9	N.S.
sBP (mmHg)	162 ± 4.5	169 ± 4.8	N.S.
dBp (mmHg)	100 ± 2.8	101 ± 2.0	N.S.
Σ GLU (mmol/l per h)	4.2 ± 1.4	3.5 ± 0.9	N.S.
Σ IRI (pmol/l per h)	786 ± 72	333 ± 24	p < 0.001

Values are Mean ± SEM

BMI indicates body mass index; sBP, systolic blood pressure; dBp, diastolic blood pressure; Σ GLU, incremental area of glucose during OGTT; Σ IRI, incremental area of insulin during OGTT.

Fig. 1. Bar graphs show allele frequencies of microsatellite polymorphism in *INSR* of hypertensive patients and control subjects (A-E). Frequency of C allele is significantly higher in the hyperinsulinemia group than in the normoinsulinemia group and control group (86.6% vs. 66.1%, 71.3% **p < 0.01, *p < 0.05).

at -80°C prior to DNA extraction. All patients gave informed consent according to the guidelines of the Osaka University Hospital Ethics Review Committee.

INSR was studied for its possible association with insulin resistance in hypertension. A microsatellite polymorphism located in intron-2 of the *INSR* was detected by the polymerase chain reaction (PCR), as reported previously (10).

Statistical analysis: The difference between groups was tested by χ^2 test with one degree of freedom.

Result

Among the 66 hypertensive patients, 26 were classified as belonging to the hyperinsulinemia group

Fig. 2. Bar graphs show the frequencies of C/C genotype of *INSR* in hypertensive patients. Hatched bar shows the hyperinsulinemia group and white bar shows the normoinsulinemia group. The frequency of C/C genotype in the hyperinsulinemia group is significantly higher than that in the normoinsulinemia group (73% vs. 43%, *p < 0.05).

and 40 to the normoinsulinemia group. There were no differences in age, BMI, blood pressure and incremental area of glucose during 75g-OGTT between the two groups (Table 1). Only the incremental area of insulin during 75g-OGTT was higher in the hyperinsulinemia group than that in the normoinsulinemia group.

Five alleles were detected in the *INSR* microsatellite. The allele frequencies of these subjects are shown in Fig. 1. Frequency of C allele was significantly higher in the hyperinsulinemia group than in the normoinsulinemia group and control group (86.6% vs. 66.1% vs. 71%, $p = 0.007$, $p = 0.023$ respectively). The genotype frequencies of C/C homozygote in these groups are shown in Fig. 2. The frequency of C/C genotype in the hyperinsulinemia group was significantly higher than that in the normoinsulinemia group (73% vs. 43%, $p = 0.02$). Since it was previously reported that *INSR* polymorphism was associated with hypertension (11), we compared genotype frequencies of the *INSR* microsatellite between the hypertensive and control groups. There was no significant difference in the genotype frequency of C/C homozygote between the two groups (Fig. 3). When the hyperten-

Fig. 3. Bar graphs show the frequencies of C/C genotype of INSR. Hatched bar shows the C/C genotype of hypertensive patients and white bar shows the control subjects. There is no significant difference between these two groups.

sive patients were divided into two groups, the frequency of C/C genotype in the hyperinsulinemia group was significantly higher than that in the control group (73% vs. 45%, $p=0.014$) (Fig. 4). There was no significant difference between the normoinsulinemia group and control group.

Discussion

In this study, we investigated the association of INSR with insulin resistance in hypertensive patients. We previously reported that hypertension was associated with glucose intolerance in the Japanese population (12), as reported in the Caucasian population. In this study, considerable variation in the degree of insulin resistance among hypertensive patients was observed even though their age, BMI, degree of hypertension, and incremental area of glucose during 75g-OGTT were similar. Since there is evidence of a genetic basis for the development of insulin resistance in hypertension (5, 6) and several recent studies have suggested that insulin resistance per se is an inherited trait (13), variation in insulin resistance in hypertensive patients may be due to differences in genetic background of the patients. Inherited insulin resistance could be due to various molecular defects, including mutations affecting the function or expression of gene products required for insulin action.

The INSR is an important candidate gene for insulin resistance. The association of the INSR with insulin resistance in the hypertensive patients in the present study suggests that INSR itself may contribute to insulin resistance in hypertension. Although the mechanism responsible for insulin resistance is unclear, one possibility is that the C allele of the INSR microsatellite is in linkage disequilibrium with a mutation in INSR region that could lead to impaired function of INSR (*i.e.*, lower receptor number or altered gene regulation). Another possibility is that the C allele itself contributes to insulin resistance. Since the microsatellite polymorphism used in this study is located in an intron, the C allele

Fig. 4. Bar graphs show the frequencies of C/C genotype of INSR. The frequency of the C/C genotype in the hyperinsulinemia group is significantly higher than that in the control group (73% vs. 45%, $*p<0.05$). There is no significant difference between the normoinsulinemia group and control group.

does not affect the primary structure of INSR molecule, but may affect INSR expression. Recently, a direct contribution of microsatellite polymorphisms to disease has been reported in several inherited diseases, such as fragile X syndrome (14) and myotonic dystrophy (15).

Morris and his colleagues reported that although RsaI polymorphism of INSR was associated with hypertension (11), microsatellite in intron-2 of INSR was not associated with essential hypertension in the Caucasian subject, whether they were obese or not (16). In agreement with their report, it was shown that INSR microsatellite is not associated with hypertension in the Japanese population. However, our data indicate that INSR microsatellite is associated with insulin resistance in hypertension. In our hypertensive patients, there was no significant difference between the normoinsulinemia group and hyperinsulinemia group in body mass index. Since insulin resistance and hyperinsulinemia in hypertensive patients appear to be less severe in Japanese than in the Caucasian population (17), it is reasonable to speculate that INSR polymorphism is primarily associated with insulin resistance or hyperinsulinemia in hypertension, but not with hypertension itself, and that the differential association of INSR polymorphism with hypertension in different ethnic groups may reflect the difference in the frequencies of insulin resistance in hypertensive patients. In populations where insulin resistance is common in hypertension, hypertension as a whole may be associated with INSR polymorphism, but in populations where insulin resistance is less common in hypertensive patients, as in the case of Japanese, association is observed only in a subset of hypertensive patients, *i.e.*, those with insulin resistance. This possibility could be tested by studying the association of INSR polymorphism with hypertension in the Caucasian population by dividing hypertensive patients into insulin resistant and sensitive groups. Determining whether polymorphism at the INSR locus contributes in any way to insulin resistance in

hypertension will require further study.

In conclusion, our data indicate that the INSR is associated with insulin resistance in hypertension, but not with hypertension itself, and suggest that the INSR may contribute to genetic susceptibility to insulin resistance in hypertensive patients.

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References

1. Reaven GM: Role of insulin resistance in human disease. *Diabetes* 1988; **37**: 1595-1607.
2. Reaven GM: Insulin resistance, hyperinsulinemia, hypertriglyceridemia, and hypertension. *Diabetes Care* 1991; **14**: 195-202.
3. Ferrannini E, Buzzigoli G, Bonadonna R, *et al*: Insulin resistance in essential hypertension. *N Engl J Med* 1987; **317**: 350-357.
4. Voors AW, Radhakrishnamurthy B, Srinivasan R, Webber LS, Berenson GS: Plasma glucose level related to blood pressure in 272 children, ages 7-15 years, sampled from a total biracial population. *Am J Epidemiol* 1981; **113**: 347-356.
5. Allemann Y, Horber FF, Colombo M, *et al*: Insulin sensitivity and body fat distribution in normotensive offspring of hypertensive parents. *Lancet* 1993; **341**: 327-331.
6. Ferrari P, Weidmann P, Shaw S, *et al*: Altered insulin sensitivity, hyperinsulinemia, and dyslipidemia in individuals with a hypertensive parent. *Am J Med* 1991; **91**: 589-596.
7. DeFronzo RA, Ferrannini E: Insulin resistance: *Diabetes Care* 1991; **14**: 173-194.
8. Choi WH, O'rahilly S, Buse JB, *et al*: Molecular scanning of insulin-responsive glucose transporter (GLUT4) gene in NIDDM subjects. *Diabetes* 1991; **40**: 1712-1718.
9. Taylor SI, Kadowaki T, Kadowaki H, Accili D, Cama A, Mckeen C: Mutations in insulin-receptor gene in insulin-resistant patients. *Diabetes Care* 1990; **13**: 257-279.
10. Ikegami H, Fukuda M, Kawaguchi Y, Fujioka Y, Ueda H, Ogihara T: A microsatellite polymorphism in the human insulin receptor gene: a highly informative marker for linkage analysis. *Diabetes Res Clin Pract* 1993; **20**: 7-10.
11. Ying L-H, Zee RYL, Griffiths LR, Morris BJ: Association of a RFLP for the insulin receptor gene, but not insulin, with essential hypertension. *Biochem Biophys Res Comm* 1991; **181**: 486-492.
12. Fujioka Y, Ikegami H, Fukuda M, *et al*: Relationship between blood pressure and glucose tolerance in the Japanese population. Prevention and treatment of NIDDM. London, UK: Smith-Gordon; 1992, pp 269-271.
13. Ferrannini E, Haffner SM, Stern MP: Essential hypertension: an insulin-resistant state. *J Cardiovasc Pharmacol* 1990; **15** (suppl 5): S18-S25.
14. Verkerk AJMH, Pieretti M, Sutcliffe JS, *et al*: Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 1991; **65**: 905-914.
15. Brook JD, McCurrach ME, Harley HG, *et al*: Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3' end of a transcript encoding a protein kinase family member. *Cell* 1992; **68**: 799-808.
16. Zee RYL, Lou Y-K, Morris BJ: Insertion variant in intron 9, but not microsatellite in intron-2, of the insulin receptor gene is associated with essential hypertension. *J Hypertens* 1993; **11**: 1283-1288.
17. Yoneda H, Ikegami H, Yamamoto Y, *et al*: Analysis of early-phase insulin responses in nonobese subjects with mild glucose intolerance. *Diabetes Care* 1992; **15**: 1517-1521.