Analysis of Candidate Genes for Insulin Resistance in Essential Hypertension

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To clarify the genetic basis of insulin resistance in hypertension, case-control association studies were performed to examine candidate genes for insulin resistance in hypertension. Since the main site of insulin resistance in hypertension is glycogen synthesis in skeletal muscle, genes that encode molecules involved in this pathway, i.e., insulin receptor (INSR), insulin-responsive glucose transporter (GLUT4) and glycogen synthase (GSY), were studied. In addition, since recent studies suggest the contribution of $\beta_3$ adrenergic receptor to the insulin resistance syndrome, the gene encoding $\beta_3$ adrenergic receptor (ADRB3) was also studied. Frequency of homozygotes for common C allele of a microsatellite polymorphism in the INSR gene was higher in the hyperinsulinemia group, but not in the normoinsulinemia group of hypertensive patients than in normotensive control subjects. Insulin sensitivity, however, was not significantly different between hypertensive patients with C/C genotype and those without this genotype. No significant differences were observed in the distribution of alleles or genotypes of the GLUT4, GSY and ADRB3 genes between hyperinsulinemia and normoinsulinemia groups of hypertensive patients or between these groups and the control group. These data suggest that the INSR polymorphism is associated with hyperinsulinemia, but not with insulin resistance, in hypertension. (Hypertens Res 1996; 19 Suppl. I: S31-S34)

Key Words: hypertension, insulin resistance, genetics, candidate gene, association

Hypertensive patients have been reported to be resistant to insulin-mediated glucose uptake when compared with normotensive control subjects (1). Considerable variation, however, is observed in the degree of insulin resistance among hypertensive patients, suggesting that insulin resistance and hypertension are not directly related, but there may be other factors that link these two characteristics. Since normotensive offspring of hypertensive patients have been reported to be insulin resistant (2, 3), genetic factors appear to contribute to the development of insulin resistance in hypertension. To clarify the genetic basis of insulin resistance in hypertension, we studied the association of candidate genes with insulin resistance in hypertension. Since the site of insulin resistance in hypertension is reported to be the non-oxidative pathway (i.e. glycogen synthesis) in skeletal muscle (1, 4), genes that encode molecules involved in this pathway are candidate genes for insulin resistance in essential hypertension (Fig. 1). Therefore, genes for the insulin receptor (INSR), the insulin-responsive facilitative glucose transporter (GLUT4) and glycogen synthase (GSY) were studied. In addition, since recent studies suggest the contribution of the $\beta_3$ adrenergic receptor to the insulin resistance syndrome (5), the gene encoding $\beta_3$ adrenergic receptor (ADRB3) was also studied.

Methods

The subjects studied were 97 normotensive control subjects and 126 patients with essential hypertension. Informed consent was obtained from all the subjects. Patients with NIDDM, diagnosed according to the guidelines of WHO, were excluded. Hypertensive patients whose insulin levels during 75g OGTT were available (n=98) were subdivided into two groups according to peak insulin level during 75g OGTT. Peak insulin levels less than 600 pmol/l (100 µU/ml) were classified as normoinsulinemia (n=33), and those equal to or higher than 600 pmol/l were classified as hyperinsulinemia (n=65). In a subgroup of subjects (n=50) randomly selected from the hypertensive patients, insulin sensitivity was evaluated by the 2-h euglycemic hyperinsulinemic glucose clamp test (6). M value (mg/kg/min), an index of insulin sensitivity, was calculated from the glucose disposal rate during the time 90-120 min of the clamp.

A microsatellite polymorphism located in intron 2 of the INSR gene was detected by the polymerase chain reaction (PCR) as reported previously (7). C to T substitution in exon 4a of the GLUT4 gene...
was detected by hybridization of PCR products with allele specific oligonucleotide (ASO) probes (8, 9). PCR-primers and ASO probes were as follows: forward primer (GLUT4F): 5'-TCACAGCCTCACTCTGTCTG-3', reverse primer (GLUT4R): 5'-GCGAGGCGTGTGGTGC-3', ASO probes for T allele (GLUT4T): 5'-GGCAGCAGCATTGGGACGG-3', ASO probes for C allele (GLUT4C): 5'-3CCTGGCCAACGCTGCTGC-3', Xba I polymorphism in the GS gene was detected by PCR as reported previously (10). A microsatellite polymorphism in the GS gene was detected by primer asing a sensitive fluorescence detection method as reported previously (11). Tryptophan to arginine substitution at codon 64 (Trp64Arg) of the ADRB3 gene was detected by PCR-RFLP method as reported previously (12) (Fig. 2).

Results are given as mean ± SD. Chi-square test or Fisher's exact test was used to compare frequencies. Unpaired Student's t-test was used to test differences in variables between groups.

Results

In a smaller number of subjects, a significant association of C/C genotype of a microsatellite polymorphism in the INS gene with hyperinsulinemia in hypertension was previously suggested (13). With the larger number of subjects in this study, the frequency of C/C genotype was significantly higher in hypertensive patients with hyperinsulinemia than in normotensive control subjects (72% vs. 45%, p < 0.01). Frequency of C/C genotype tended to be higher in hypertensive patients with hyperinsulinemia than in those with normoinsulinemia (72% vs. 55%, p = 0.09). No significant difference, however, was observed in M value between hypertensive patients with and without C/C genotype (4.5 ± 1.9 vs. 3.6±1.9 mg/kg/min).

Genotype frequencies of GLUT4 polymorphism were similar and no significant difference was observed between hypertensive patients with hyperinsulinemia and those with normoinsulinemia (C/C: 61% vs. 48%, C/T: 29% vs. 29%, T/T: 10% vs. 23%, respectively).

In a previous study in the Finnish population, a rare A2 allele of the Xba I polymorphism in the GS gene was reported to be associated with NIDDM and a high prevalence of hypertension (14). None of the subjects in this study, however, had A2 allele, indicating that the frequency of A2 allele is very low, if not absent, in the Japanese population and that this marker is not informative in this population. To further study the association of the GS gene with insulin resistance in hypertension, we used a microsatellite polymorphism in the GS gene (11). Nine different alleles were detected in the subjects studied, indicating the highly polymorphic nature of this marker. No significant difference, however, was observed in the distribution of alleles between hyperinsulinemia and normoinsulinemia groups of hypertensive patients or between these groups and the control group.

Trp64Arg mutation of the ADRB3 gene is reported to be associated with an increased capacity to gain weight, an earlier onset of NIDDM and clinical features of the insulin resistance syndrome (5, 15, 16). In our previous study (12), the mutation was observed with higher frequency in Japanese than in other ethnic groups, except for Pima Indians. The subjects with mutation tended to have larger body mass index and an earlier onset of NIDDM, as previously reported in other ethnic groups (13). No significant difference, however, was observed in the frequency of the mutation between hypertensive patients (17%) and control subjects (19%). No significant difference was observed in systolic and diastolic blood pressure, plasma insulin levels during OGTT, and insulin sensitivity as expressed by M value between the patients with and without the mutated allele.

Discussion

To clarify the genetic basis of insulin resistance in essential hypertension, case-control association studies with candidate genes for insulin resistance were
performed in the genetically homogenous Japanese population. In a previous study with a smaller number of subjects, INSR polymorphism was suggested to be associated with hyperinsulinemia in hypertensive patients, but not with normoinsulinemia in hypertensive patients (13). In the present study with a larger number of subjects, the C/C genotype was associated with hyperinsulinemia in hypertensive patients, but not with insulin resistance as assessed by euglycemic hyperinsulinemic glucose clamp test. These data suggest that the INSR polymorphism contributes to hyperinsulinemia, but not to insulin resistance in hypertension. Since only a small number of patients was tested for insulin sensitivity in this study, further studies with larger number of subjects are needed to clarify the association of the INSR polymorphism with insulin resistance.

Insulin resistance in hypertension is reported to be mainly observed in non-oxidative pathway of glucose utilization (i.e. glycogen synthesis) in skeletal muscle. Glycogen synthase, a key enzyme in this pathway, is therefore a candidate gene for insulin resistance in hypertension. In fact, a rare A2 allele of the XbaI polymorphism in the GS gene is reported to be associated with NIDDM in which insulin resistance is a characteristic feature and with a high prevalence of hypertension in the Finnish population (14). In the present study in Japanese, however, none of the subjects had this allele. A similar finding was reported in another Japanese study (17), indicating that the frequency of A2 allele is extremely low, if not absent, in Japanese, and the XbaI RFLP is not an informative marker in genetic analysis in the Japanese population. Therefore, we used another polymorphism, a microsatellite polymorphism in the GS gene. Although this marker is highly polymorphic and informative in Japanese, no significant difference in the distribution of alleles was observed between the hyperinsulinemia and normoinsulinemia groups of hypertensive patients or between these groups and the control group (11). These data suggest that the GS gene is not associated with insulin resistance in hypertension.

The β3-adrenergic receptor is predominantly expressed in adipose tissue and plays an important role in lipid metabolism and metabolic rate (18, 19). Thus, molecular abnormalities of β3 adrenergic receptor are expected to lead to obesity and insulin resistance (18, 19), and therefore, the ADRB3 gene is a strong candidate gene for insulin resistance in hypertension. In fact, a Trp64Arg mutation in the ADRB3 gene has been suggested to be associated with higher diastolic blood pressure as well as with clinical features of the insulin resistance syndrome (5). No significant difference, however, was observed in the frequency of the mutated allele between hypertensive patients and control subjects, nor between hypertensive subjects with and without insulin resistance. These data suggest that the ADRB3 gene is not associated with hypertension itself or insulin resistance in hypertension.

Apart from the weak association of the INSR gene polymorphism with hyperinsulinemia in hyper-

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References