Detection of an Amino Acid Polymorphism in Hormone-Sensitive Lipase in Japanese Subjects

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Hormone-sensitive lipase (HSL) plays an important role in energy metabolism by controlling the hydrolysis of triglycerides stored in adipose tissue. To investigate whether mutations in the HSL gene are associated with non–insulin-dependent diabetes mellitus (NIDDM), we screened for mutations of this gene using single-stranded conformation polymorphism (SSCP) in 35 Japanese subjects with NIDDM. SSCP analysis identified a variant pattern in exon 4, and the sequence showed that this variant pattern resulted from amino acid polymorphism (Arg309Cys). Subsequent study showed that this polymorphism was found in 18 of 151 NIDDM patients and 10 of 97 nondiabetic subjects, but allele frequency was not significantly different between the two groups ($P = .7$). Body mass index, serum triglyceride, and high-density lipoprotein (HDL) cholesterol were not different in subjects with and without the polymorphism. But serum total cholesterol was higher in subjects with the polymorphism than in subjects without it ($P = .0005$). These data indicate that this HSL polymorphism is not associated with NIDDM, obesity, and serum triglyceride level. However, an effect of the polymorphism to elevate serum total cholesterol has not been excluded, although further study is necessary to resolve its association with cholesterol metabolism.

GENETIC FACTORS play an important role in the development of non–insulin-dependent diabetes mellitus (NIDDM), a heterogeneous disorder characterized by defects in insulin action and insulin secretion.[1] Mutations associated with NIDDM have been identified in insulin,[2] insulin receptor,[3,4] glucokinase,[5-7] and mitochondrial genes.[8,9] This suggests that direct screening of other candidate genes for mutations may lead to identification of other diabetes-susceptibility loci.

Hormone-sensitive lipase (HSL) has a critical role in the metabolism of free fatty acid (FFA) by controlling the rate of lipolysis of triglycerides stored in adipose tissue. HSL also has catalytic activity toward cholesteryl ester.[10] As part of this key role of HSL in energy metabolism, HSL may play some role in abnormal lipid metabolism or the development of obesity. Since obesity is one of the risk factors for development of NIDDM, studies on genetic variations in the coding region of the HSL gene in NIDDM would be of particular importance. To investigate the prevalence of potential mutations in the gene encoding HSL, we screened 35 unrelated NIDDM Japanese subjects for mutations of the HSL gene using single-stranded conformation polymorphism (SSCP) analysis.

SUBJECTS AND METHODS

Subjects

Thirty-five subjects with NIDDM were first screened for mutations throughout the coding region of HSL. We tested 116 additional subjects with NIDDM for the amino acid substitution observed in the first screening group. Subjects with thyroid dysfunction or nephrotic syndrome and subjects treated with lipid-lowering agents were excluded from the study (Table 1). The 97 nondiabetic subjects screened had no family history of diabetes and had normal glucose tolerance as determined by a 75-g oral glucose tolerance test. None of the nondiabetic subjects were taking medications known to influence lipid metabolism. The diagnosis of NIDDM was based on World Health Organization criteria.[11] All patients were informed of the purpose of the study, and informed consent was obtained from each. The study was approved by the ethics committee of Chiba University Hospital.

Biochemical Studies

After a 12-hour overnight fast, morning blood samples were drawn. Plasma concentrations of total cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol were determined using standard methods.

Methods

DNA was isolated from human lymphocytes. The nine exons and adjacent introns of HSL[12] were amplified using the polymerase chain reaction (PCR) and primers specific for each exon (Table 2). We could not obtain extended sequences from the 5′ splice donor of intron 5 and the 3′ splice acceptor of intron 6, because these sequences are found at the ends of cloning fragments and we designed the downstream primer of exon 5 and the upstream primer of exon 7 according to the sequence of exon-intron junctions. Standard PCR conditions were used with ^{32}P-labeled primers. PCR products were diluted 15-fold with formamide buffer

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Table 1. Clinical Characteristics of Study Subjects

Characteristic	NIDDM Patients	Nondiabetic Subjects
No. (male/female)	151 (59/92)	97 (65/32)
Age (yr)	56 ± 1	51 ± 1
Body mass index (kg/m^2)	22.9 ± 0.3	22.8 ± 0.3
Age at diagnosis (yr)	46 ± 1	—
Family history of diabetes (%)	33.1	0
Treatment (%)		
Diet	39.7	
Oral agent	43.7	
Insulin	16.6	

Table 2. Sequence of Primer Pairs for PCR-SSCP Analysis of Human HSL

Exon	Upstream Primer	Downstream Primer	Product (bp)
1	5′-AACAGGCCTCCCCACCTGCC-3′	5′-CGGGATTTGTGCAGGAGGTG-3′	314
	5′-CGGGATTTGTGCAGGAGGTG-3′	5′-GCCTTCATTGTGGGCCAGAG-3′	348
2	5′-CATCCCTCTCTTGAGCGGTG-3′	5′-CCAGTGGGTCAGGCTGCTTG-3′	217
3	5′-CAAGCAGCCTGACCCACTGG-3′	5′-CCTCAGATGAGTCTCTGGGC-3′	246
4	5′-ACCCCTGCAGGCAGACCTTC-3′	5′-CCACGCTCCTCGGCTCTGTC-3′	285
5	5′-AGCTCTCCCCAACCTCACAC-3′	5′-AGGCTCACAGGAGGGCGCAG-3′	346
6	5′-TCTGCCCCTGCCAGGTTGTC-3′	5′-AGTCAGACATCCATGCAGTC-3′	323
7	5′-GTCGACCTGCAGGTGCAAAG-3′	5′-AGGCTGTCCCTCCTGCCCAC-3′	244
8	5′-ACCAAACTAACGGAGCCAGG-3′	5′-CAGCTCATTTTTGGCCTCAG-3′	285
	5′-GACACTTAGCCCCTCCACAC-3′	5′-AGGTGTACCGTGCCCGGTCC-3′	356
9	5′-ACCCTCTCTCCACGTCCCTC-3′	5′-ACGAGGCGGATGCGCTCCAC-3′	215
	5′-TAGCGGCGCTGTGCCGAGAC-3′	5′-TGGCGAGGGTCTCAGCTTTC-3′	281

(95% formamide, 0.05% bromphenol blue, and 0.05% xylene cyanol) and heated at 95°C for 3 minutes, and 1.5 μL of each sample was loaded onto a 5% nondenaturing polyacrylamide gel (30 × 40 × 0.03 cm; ratio of acrylamide to *N,N′*-methylene-bis-acrylamide, 49:1). Each sample was analyzed on four gels containing 0% or 5% glycerol at room temperature or 4°C. The gels were then transferred to paper, dried, and exposed to film (XAR-5; Kodak, Rochester, NY) with an intensifying screen for 12 hours at −70°C. Sequences of novel bands observed by SSCP were determined directly.

Statistical Analysis

The association of polymorphism with NIDDM was analyzed by 2 × 2 contingency tables, and the significance of differences was tested by Fisher's exact test. Differences between groups were tested by Student's *t* test.

RESULTS

All nine exons of the HSL gene of 35 NIDDM patients were amplified and analyzed by SSCP. A variant pattern was noted in SSCP of exon 4, and the sequence showed that this variant pattern resulting from a mutation in codon 309 resulted in replacement of Arg (CGT) by Cys (TGT) (Fig 1). Then we tested 116 additional subjects with NIDDM and 97 nondiabetic subjects. Eighteen of 151 NIDDM subjects and 10 of 97 nondiabetic subjects were heterozygous for Arg309Cys polymorphism. The allele frequency of this polymorphism among NIDDM patients (6.0%) and among nondiabetic subjects (5.2%) was not significantly different ($P = .7$).

These two groups were combined, and clinical data of subjects with and without the polymorphism were compared. Age, body mass index, serum triglyceride, and HDL cholesterol were not significantly different between subjects with and without the polymorphism (Table 3). However, serum total cholesterol was higher in subjects with the polymorphism than in those without it ($P = .0005$). This difference was more pronounced in NIDDM subjects with (216 ± 7 mg/dL) and without (188 ± 3 mg/dL) the polymorphism ($P = .0002$), but it was not significant between nondiabetic subjects with (203 ± 12 mg/dL) and without (191 ± 4 mg/dL) the polymorphism ($P = .31$). Plasma FFA levels of most of the subjects were not measured, and among subjects whose FFA data were available, FFA levels were not significantly different between those with the polymorphism (175 ± 40 mg/L, n = 3) and those without it (144 ± 11 mg/L, n = 20).

DISCUSSION

HSL has a critical role in the control of energy homeostasis and adipose tissue lipolysis. Therefore, variations in the structure of HSL may be related to abnormal lipid metabolism, obesity, or NIDDM if the enzymatic activity is impaired. In this study, codon 309 polymorphism in HSL was identified, but the functional significance could not be established. The complete sequence of human HSL has now been obtained,[12] and site-directed mutagenesis can be used to test whether this amino acid change has any effect on the function of the enzyme. Codon 309 is conserved in rat and mouse HSL,[10,13] indicating probable functional importance. Amino acid substitutions from polar amino acids with the charged side chain arginine to cysteine create a new disulfate-bound–forming residue and may cause

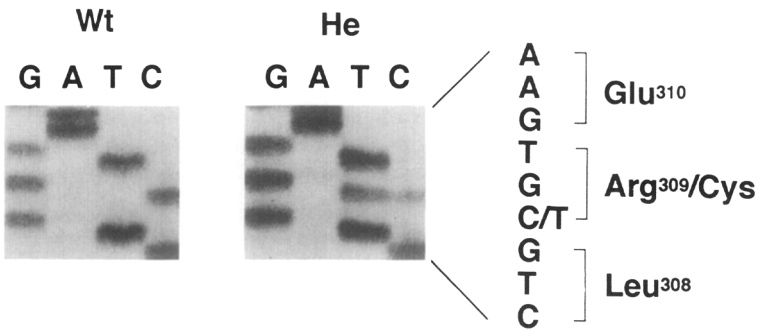

Fig 1. Nucleotide sequence of exon 4 from subjects who were heterozygous (He) and wild-type (Wt) for codon 309 polymorphism. Exon 4 was amplified by PCR and directly sequenced.

Table 3. Clinical Characteristics of Subjects With and Without the Polymorphism

Characteristic	NIDDM Subjects		Nondiabetic Subjects		Combined Groups	
	With	Without	With	Without	With	Without
No. (male/female)	18 (6/12)	133 (53/80)	10 (6/4)	87 (59/28)	28 (12/16)	220 (112/108)
Age (yr)	53 ± 3	57 ± 1	49 ± 3	51 ± 1	51 ± 2	54 ± 1
Age at onset (yr)	42 ± 3	47 ± 1				
Body mass index (kg/m^2)	22.9 ± 0.6	22.9 ± 0.3	23.2 ± 0.7	22.7 ± 0.3	23.0 ± 0.5	22.8 ± 0.2
Fasting serum total cholesterol (mg/dL)	216 ± 7*	188 ± 3	203 ± 12	191 ± 4	211 ± 6†	189 ± 2
Fasting serum triglyceride (mg/dL)	159 ± 30	135 ± 8	110 ± 25	109 ± 6	142 ± 21	125 ± 5
Fasting serum HDL cholesterol (mg/dL)	56 ± 5	49 ± 2	51 ± 4	53 ± 2	53 ± 3	51 ± 1
Hemoglobin A_{1c} (%)	8.9 ± 0.5	8.5 ± 0.2	ND	ND		

Abbreviation: ND, not determined.

*P = .0002.

†P = .0005.

alteration of enzymatic activity. Serum total cholesterol in subjects with the Arg309Cys polymorphism was higher than in those without it. HSL also has cholesteryl ester hydrolase activity and triglyceride lipase activity, and HSL mRNA is expressed in a variety of tissues, including adipose tissue, adrenal gland, ovary, testis, placenta, and heart and skeletal muscle, although its role in muscle is unknown.[10] The polymorphism may cause a defect in enzymatic activity of HSL. Decreased cholesteryl ester hydrolase activity may result in the accumulation of cholesteryl ester in those cells and affect serum total cholesterol through suppression of cholesterol intake into the cells. On the other hand, we must also consider the opposite possibility that the polymorphism has no effect on the enzymatic activity of HSL. The difference in serum total cholesterol between subjects with and without the polymorphism was much more distinct in NIDDM subjects. The reason for this is unknown, but NIDDM subjects may have some genetic or environmental factors that elevate the total cholesterol level, and these factors might interact with the polymorphism and make the difference more pronounced. We cannot exclude the possibility that some factors such as exercise and diet had effects on serum total cholesterol, because subjects with and without the polymorphism were not perfectly matched in this study, although age, age at onset, body mass index, and hemoglobin A_{1c} were not different between these subjects. If the amino acid change causes a defect in the lipolytic activity of the triglyceride, it may result in a decrease in plasma FFA, which was not confirmed in this study.

In summary, this polymorphism of HSL is not associated with NIDDM or obesity, and HSL is not a major determinant of NIDDM, although a rare mutation cannot be ruled out. Nevertheless, Arg309Cys polymorphism seems to be associated with moderate hypercholesterolemia especially in NIDDM through its interaction with other genetic and/or environmental factors. Further study will be required to establish whether the HSL gene has any role in the abnormality of cholesterol metabolism.

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