Impact of lipoprotein lipase gene polymorphisms on ulcerative colitis

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METHODS: Peripheral blood was obtained from 131 patients with UC and 106 healthy controls for DNA extraction. We determined LPL gene polymorphisms affecting the enzyme at Ser447stop, as well as HindIII and PvuII polymorphisms using PCR techniques. PCR products were characterized by PCR-RFLP and direct sequencing. Polymorphisms were examined for association with clinical features in UC patients. Genotype frequencies for LPL polymorphism did not differ significantly between UC patients and controls.

CONCLUSION: Ser447stop and HindIII LPL polymorphisms may influence age of onset of UC, while HindIII and PvuII polymorphisms influence serum triglyceride in UC patients.

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Key words: Ulcerative colitis; Lipoprotein lipase; Lipid metabolism; Triglyceride

INTRODUCTION

Inflammatory bowel disease (IBD), characterized by chronic recurrent inflammation of the intestinal tract, includes two common forms: Crohn’s disease (CD) and ulcerative colitis (UC). For both forms, the etiology is unclear, but likely to be multifactorial. Factors that may affect IBD include diet, infantile environment and immune defense abnormalities limited to the intestinal tract. Recently, genetic factors have been examined in IBD, but many studies seeking susceptibility genes for IBD have not produced a consensus. We presently investigated possible influence of the lipoprotein lipase (LPL) gene in UC. LPL plays a critical role in lipid metabolism. Dietary triacylglycerol (TAG) exists in the human circulation as macromol-
ecules (TAG-rich lipoprotein) that are too large to pass through the endothelium of most capillaries. LPL catalyses conversion of TAG-rich lipoprotein to triglyceride (TG), very low-density lipoprotein (VLDL), and chylomicrons (CM), all of which circulate and can enter tissues more readily to serve energy source[1,2]. Our interest in LPL in IBD was provoked by identifying high lipid intake as a risk factor for IBD. Shoda et al[3] reported associated intake of n-6 polyunsaturated fat and animal fat with development of CD in Japanese patients, while Geering et al[4] reported high intake of mono- and polyunsaturated fats to be a risk factor for the UC. Furthermore, the LPL gene has been localized to chromosome 8p22 near N-acetyltransferase2 (NAT2) gene, while Machida et al[5] reported an association between NAT2 gene haplotype NAT2*7B and CD. Considering these various findings, we suspected that LPL gene polymorphisms could influence characteristics and incidence of UC. These issues were examined in the present molecular genetic investigation.

MATERIALS AND METHODS

Subjects
We studied 131 patients with UC (75 males and 56 females) and 106 healthy controls (53 males and 53 females). Diagnosis of UC was based on conventional clinical, radiologic, endoscopic, and pathologic criteria. Characteristics of UC patients are shown in Table 1. We investigated the effect of LPL polymorphism on clinical features as shown in this table. To examine LPL polymorphisms in terms of influence on UC incidence, we compared LPL genotype frequencies in UC patients with those in controls.

DNA extraction
Blood samples were obtained from patients and controls after they had given informed consent to sampling and analyses. This study was approved by the Fujita Health University Ethics Committee. DNA was extracted from blood samples using a PUREGENE DNA isolation kit (Gentra Systems, Inc, Minneapolis, USA).

Genotype
LPL polymorphisms were typed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods to detect Ser447stop, Hind III and Pvu II polymorphisms[6]. The primer sets were as follows: for Ser447stop, 5’-GATGTGGCCTGAGTGTGACAG-3’ (forward) and 5´-GCTAGTGT-3´ (reverse); for Hind III, 5′-GATGTCATCGGTGACGAGCAG-3´ (forward) and 5´-CTTCAGCTAGACATT-GCTAGTG-3´ (reverse); and for Pvu II, 5′-GAGACACAGATCTCTTTAAGAC-3´ (forward) and 5′-ATCAGGGCATGCCGTAGGTAAG-3´ (reverse). PCR was carried out in a 30-μL aliquot containing 50 ng of genomic DNA, 12 pmol of each primer, 3.0 μL of 10 × buffer solution, 20 nmol/μL of dNTP, and 1 U of Taq polymerase. PCR conditions for Ser447stop included initial denaturation at 95°C for 5 min, followed by 35 amplification cycles, each cycle containing denaturation at 95°C for 30 s, primer annealing at 62°C for 30 s, and extension at 72°C for 30 s. PCR conditions for Hind III and Pvu II included initial denaturation at 95°C for 5 min, followed by 35 amplification cycles, each cycle containing denaturation at 95°C for 30 s, primer annealing at 61°C for 30 s, and extension at 72°C for 30 s. PCR products subjected to overnight digestion with Mnl I, Hind III, and Pvu II were examined by electrophoresis on 20 g/L agarose gels. The strategy for the detection of the G allele (Ser447stop) in exon 9 of the lipoprotein lipase by PCR-RFLP analysis. Introduction of G (*1) is in place of C creates a new Mnl I site.

Table 1 Characteristics of patients with ulcerative colitis

| Characteristic | UC (n = 131) |
|---------------|-------------|
| Age of onset (yr) | 36.8 ± 15.3 |
| Colitis duration (yr) | 8.8 ± 7.7 |
| Extension | |
| Proctitis | 22 (16.8%) |
| Left-sided | 59 (45.0%) |
| Pancolitis | 50 (38.2%) |
| Type of clinical course | |
| First episode | 14 (10.7%) |
| Chronic relapse | 79 (60.3%) |
| Chronic persistent | 38 (29.0%) |
| Severity | |
| Mild | 40 (30.5%) |
| Moderate | 61 (46.6%) |
| Severe | 30 (22.9%) |

Figure 1 Strategy for detection of the C/G allele (Ser/stop) in exon 9 of the lipoprotein lipase by PCR-RFLP analysis. Introduction of G (*1) is in place of C creates a new Mnl I site.

G allele

C allele

Asn Lys Lys Stop

Asn Lys Lys Ser

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Tokyo), e.g. Pvu II digestion-cases. Electrophoresed patterns of LPL polymorphisms are shown in Figure 2.

Results of PCR-RFLP were confirmed by direct sequencing. DNA was extracted from agarose gels using an extraction kit (QIAGEN, Hilden, Germany). Then genotype was confirmed by sequence analysis using an auto sequencer (data not shown).

**Statistical analysis**

All data were analyzed by Excel 2000 and STASTISCA software. Clinical features of UC, allele frequency, and genotype distribution were evaluated by using \( \chi^2 \) test. A \( P \) value less than 0.05 was considered statistically significant.

Allelic and genotype frequencies were determined from observed genotype counts, and the extensions of Hardy-Weinberg equilibrium were evaluated by using \( \chi^2 \) test.

### RESULTS

#### LPL polymorphisms and risk of UC

The allele frequency of LPL polymorphism and the genotype distribution are shown in Table 2. The observed genotype data were consistent with Hardy-Weinberg equilibrium (Ser447stop: \( \chi^2 = 0.48, P = 0.49; \) Hind III: \( \chi^2 = 1.73, P = 0.19; \) Pvu II: \( \chi^2 = 0.88, P = 0.35 \)). In the patient group, one patient was not able to identify with genotype of Hind III polymorphism. In the control group, two patients were not able to identify with genotype of Ser447stop polymorphism. The allele frequency of Ser447stop polymorphism was 31.4% in patients and 28.1% in controls. In hind III, the frequency of genotype was 23.2% in patients and 22.7% in controls. In Pvu II, the frequency of genotype was 22.0% in patients and 22.1% in controls. The allele frequency of LPL polymorphism and the genotype data were consistent with Hardy-Weinberg equilibrium.

#### LPL polymorphism and characteristics of UC patients

We sought to identify associations between characteristics of UC patients (age at onset, gender, nature of the clinical course, extent of lesions, severity of colitis) and LPL polymorphisms. The relationship between age of onset of UC patients and LPL polymorphisms is summarized in Table 3. In patients with age at onset of 20 years or younger, more patients had either C/G or G/G genotype for Ser447stop than a C/C genotype (OR = 3.13, 95% CI = 0.95-10.33). In this early-onset group, more patients had either an H+/+, H+/- or H-/- genotype for Hind III polymorphism than an H-/- genotype (OR = 2.51, 95% CI = 0.94-7.45). Other characteristics (gender, nature of...
The LPL gene, located on chromosome 8p22[9], consists of 10 exons. As for the LPL polymorphisms investigated in this study, the Ser447stop polymorphism is within exon 9[9], involving substitution of G for C at nucleotide 1595. The HindIII polymorphism is located in intron 8[10,11], and PvuII in intron 6[12,13]. Abnormalities in LPL function have been associated with various diseases, those linked with LPL polymorphisms are cardiovascular disease[14], cerebrovascular disease[15], chylomicronemia[16], insulin resistance[17], Alzheimer’s disease[18], and various infections[19]. Life styles and habits also can influence many diseases, possibly including UC. We presently sought out the relationship between UC and the LPL gene.

This study is probably the first to report an association between characteristic of UC and LPL polymorphism, representing two important findings. The first finding was that HindIII and PvuII polymorphisms affected serum triglyceride concentrations in UC patients. Some studies reported that Ser447stop polymorphism activated LPL, decreasing triglycerides and increasing HDL cholesterol. As opposed to Ser447stop, HindIII polymorphism lowered LPL activity, and elevated triglycerides, and decreased HDL cholesterol. PvuII polymorphism has similar function[12,13]. Using a cutoff triglyceride value of 150 mg/dL, the above relationship for HindIII and PvuII polymorphisms held true in our UC patients. Patients homozygous for HindIII polymorphism were even more likely to have triglyceride above 150 mg/dL than those homozygous for PvuII polymorphism. HindIII polymorphism, therefore, would appear to suppress LPL activity more strongly than PvuII polymorphism. Among studies of associations between triglyceride in healthy individuals and LPL polymorphism, Ann et al[10] reported that HindIII polymorphism elevated triglycerides, while Chamberlain et al[8] pointed out some subjects with HindIII polymorphism showed normal concentrations. Since associations between elevated triglyceride in healthy individuals and HindIII polymorphism thus are a matter of some disagreement, our finding that UC patients with H<sup>+</sup> genotype for LPL polymorphism were particularly likely to have elevated triglycerides, some part of the difference seen in our UC patients might be specific to UC.

Previous studies have reported that serum triglyceride concentrations did not differ between UC patients and controls[20,21]. In this study, however, patients with H<sup>+</sup> genotype had serum triglyceride concentrations over 150 mg/dL compared to patients with H<sup>+</sup> or H<sup>-/-</sup>, thereby suggesting that HindIII polymorphism may contribute to elevate triglyceride levels directly or may influence other gene-elevated triglyceride concentrations. Although few studies have examined associations between UC and LPL, lipid intake has been reported to influence risk of IBD. Many studies have reported relationship between lipid metabolism and inflammation. During various human inflammatory states, serum triglyceride concentrations increase because some cytokines responsible for inflammatory responses, including tumor necrosis factor (TNF)-α, interferon (IFN)-γ, inhibit LPL activity[22,23]. Other studies reported that lipopolysaccarides derived from Gram-negative bacteria reduced LPL activity in macrophages[23]. LPL also directly induces the expression of the TNF-α gene[24], which synergizes with IFN-γ in stimulating nitric oxide synthetase expression in macrophages[25]. Since LPL is strongly associated with cytokines, it may contribute to development of inflammation. In addition, De Sanctis et al[25] reported an association between natural killer (NK) cells and LPL polymorphism. Some studies have suggested that UC is associated with changes in humoral immunity[26,27] and cellular immunity[28,29], while UC may be associated with loss of immune tolerance in the intestine. Association between LPL and characteristic of UC may shed some light on mechanisms of onset of UC.

The second finding was that Ser447stop polymorphism

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**Table 4 Triglyceride levels of UC patients and LPL polymorphisms**

| Triglycerides (mg/dL) | Genotype  | OR (95%CI) |
|----------------------|-----------|------------|
| Ser447stop           | C/C       | 1/1        |
| ≥ 150 (n = 20)       | C/G + G/G | 4.93 (0.61-40.03) |
| < 150 (n = 68)       | 54        | 14         |
| HindIII had to have HindIII polymorphism. Among studies of associations between triglyceride in healthy individuals and HindIII polymorphism, Ann et al[10] reported that HindIII polymorphism elevated triglycerides, while Chamberlain et al[8] pointed out some subjects with HindIII polymorphism showed normal concentrations. Since associations between elevated triglyceride in healthy individuals and HindIII polymorphism thus are a matter of some disagreement, our finding that UC patients with H<sup>+</sup> genotype for LPL polymorphism were particularly likely to have elevated triglycerides, some part of the difference seen in our UC patients might be specific to UC.

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and Hind III polymorphism might be associated with age of onset of UC patients. In patients with onset at 20 years or younger, more patients had either C/G or G/G genotype at Ser447stop than a C/C genotype, while more patients had either H1 or H2 genotype for Hind III polymorphism than H1/H2 genotype. As for the significance of relatively early onset, the LPL gene might influence onset of disease directly or by acting upon another gene or factor. In addition to genotype, age may affect LPL activity, while lipid metabolism might influence onset of IBD. Hamilton et al. reported that older individuals had inefficient triglyceride metabolism and elevated serum triglycerides because of reduction of LPL activity in postural skeletal muscle during aging. Among UC patients in our study, 10% (2/20) of those aged 20 to 29 years and 15% (4/26) of those aged 30 to 39 years showed triglycerides over 150 mg/dL, while Arai et al. reported that, in a Japanese population sample, the mean triglyceride concentration was 83 mg/dL in the third, and 118 mg/dL in the fourth decade of life. Our finding that younger UC patients appeared more likely to have high triglyceride concentration requires further investigation.

In conclusion, our study indicates that LPL polymorphism influences lipid metabolism in UC patients and age of onset of UC, and might contribute to onset and biologic behavior of UC.

Many studies have considered gene polymorphisms in UC, but few have suggested an influence of polymorphism on metabolism in UC patients. Further study on LPL and other gene polymorphisms in UC remains an important line of investigation.

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