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Effects of gender, hepatic lipase gene polymorphism and type 2 diabetes mellitus on hepatic lipase activity in Chinese

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Abstract

Genetic variation in the hepatic lipase (HL) gene (*LIPC*) promoter is an important determinant of HL activity in Caucasians. As HL activity is increased in patients with type 2 diabetes mellitus, we have investigated whether the − 514 C-to-T polymorphism acted independently of type 2 diabetes to regulate HL activity. The frequency of this polymorphism and its effect on plasma HL activity and lipids were examined in 203 Chinese patients with type 2 diabetes and 205 controls. The frequency of the T allele was 0.343 and 0.376 in male and female diabetic patients, respectively, compared with 0.371 and 0.372 in male and female controls. The effect of *LIPC* genotype on HL activity was similar between men and women, and between diabetic patients and non-diabetic controls, with the lowest HL activity being found in those subjects with the TT genotype. On multivariate analysis, gender, *LIPC* genotype, the presence of type 2 diabetes and body mass index were independent predictors of HL activity, accounting for 22, 9, 5 and 3%, respectively, of the variance in HL activity (whole model adjusted $R^2 = 0.39$, $P < 0.0001$). The T allele was associated with higher high-density lipoprotein in the controls but not in the diabetic patients, and no associations were found between *LIPC* genotype and low-density lipoprotein subfractions in either groups. In conclusion, despite the higher frequency of the T allele in Chinese than in Caucasians, gender was the best predictor for HL activity, with *LIPC* gene polymorphism and type 2 diabetes making relatively smaller contributions to the variation in HL activity. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Hepatic lipase; Diabetes mellitus; Polymorphism; Chinese

1. Introduction

Hepatic lipase (HL) is a glycoprotein belonging to the lipase super family that includes lipoprotein lipase and pancreatic lipase. It hydrolyses phospholipids and triglycerides of plasma lipoproteins and plays an important role in the metabolic processing of high-density lipoprotein (HDL) and low-density lipoprotein (LDL). High HL activity is associated with a more atherogenic lipid profile characterised by reduced HDL_2 [1,2] and increased concentrations of small dense LDL [3,4]. Although the role of HL in lipoprotein metabolism has been studied extensively [5,6], less is known about the factors that determine the activity of this enzyme in vivo. Hepatic lipase activity varies considerably among individuals in the general population and is affected by hormones [7–9] and by adiposity [10]. Recent studies in twins and nuclear families indicate that a significant fraction of inter-individual variation in HL activity is heritable [11,12]. Polymorphisms in the HL gene (*LIPC*) that are associated with changes in HL activity have been identified, and low HL activity is associated with a common C-to-T base substitution at position − 480 or − 514 depending on the nucleotide taken as the transcription start site in the *LIPC* promoter. The − 514 C-to-T polymorphism has been shown to be in complete linkage disequilibrium with three other polymorphism in the HL promoter (− 250 G-to-A, − 710 T-to-C, and − 763 A-to-G) in Caucasian populations and together define a common *LIPC* allele [13–15]. The − 514T allele is associated with a 35–45% decrease in HL activity in Caucasian men and women. It is also associated with increased HDL cholesterol in some

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[13,14,16] but not all reported studies [15,17]. Ethnic differences in the frequency of the −514T allele have been described. The −514T allele is more common in African Americans [18,19] and Japanese Americans [20] than in Caucasian Americans. It has been suggested that this might partly account for the higher HDL cholesterol levels observed in African Americans compared with Caucasian Americans [18,19].

In patients with type 2 diabetes mellitus, an increase in HL activity has been reported and contributes to the dyslipidaemia of these patients [4,21,22]. Baynes et al. demonstrated that insulin insensitivity and hyperinsulinaemia are both associated with higher levels of HL activity in these patients [22], but whether the increase in HL activity in type 2 diabetes mellitus is modulated by genetic factors is not known. The present study is performed to determine whether the *LIPC* genotype acts independently of type 2 diabetes mellitus to regulate HL activity and its impact on plasma lipid and lipoproteins in these patients. In addition, this study will also provide information on the allele frequency of the −514 C-to-T polymorphism in the Chinese population, which has so far not been reported.

2. Methods

Two hundred and three Chinese patients with type 2 diabetes mellitus (102 male, 101 female) with stable glycaemic control (no change in therapy for diabetes in the preceding 3 months), normal renal and liver function and proteinuria <1 g/day were recruited from the diabetic clinics at Queen Mary Hospital. All subjects were non-smokers, and 81% were on oral hypoglycaemic agents, 7% were diet-controlled and 12% were on a combination of insulin and biguanide. None of the patients were on lipid-lowering agents. Two hundred and five non-smoking Chinese controls (99 male, 106 female) were recruited from hospital personnel and from the local community by advertisement. None of the post-menopausal women in either the diabetic ($n=39$) or control groups ($n=32$) were taking hormone replacement therapy. In all subjects, fasting blood was taken for the measurement of lipids and lipoproteins. Plasma was separated immediately and stored at 4°C until lipoprotein preparations were commenced. A second fasting blood sample was taken 15 min after 100 U/kg heparin was administered intravenously for the measurement of HL activities. All subjects gave informed consent and the protocol was approved by the Ethics Committee of the University of Hong Kong.

Plasma total cholesterol and triglyceride were determined enzymatically (Boehringer Mannheim, Mannheim, Germany) on a Hitachi 717 analyzer (Boehringer Mannheim, GmbH, Germany). HDL cholesterol was measured by the same method after precipitation of apolipoprotein B-containing lipoproteins with PEG 6000. LDL cholesterol was calculated by the Friedewald equation. Apolipoproteins AI and B were measured by rate nephelometry using the Beckman Array System (Beckman Instruments). HbA1c was measured in whole blood using ion-exchange high-performance liquid chromatography by Bio-Rad Variant Analyser System (Bio-Rad Laboratories Inc., California, USA). Total lipolytic activity in post-heparin plasma was measured using an emulsion of triolein and gum arabic as substrate. Hepatic lipase activity was determined as the activity of the salt-resistant lipase in the presence of 1 M NaCl [4].

LDL subfractionation was achieved by density gradient ultracentrifugation using a six-step discontinuous salt gradient as previously described [4]. In brief, fresh plasma was fractionated into three distinct LDL subfractions after 24 h centrifugation in a Beckman SW40 rotor (40000 r.p.m., 23°C). The gradient containing the separated LDL fractions was displaced from the tube by upward displacement and identified by absorbance at 280 nm. The elution times of the first, least dense, LDL fraction and the appearance of plasma proteins were reproducible and provided references for the identification of LDL subfractions. Major LDL subfractions were identified by peak maxima that occurred between hydrated density intervals of 1.025–1.034 g/ml (LDL-I), 1.034–1.044 g/ml (LDL-II), or 1.044–1.060 g/ml (LDL-III). The individual subfraction areas beneath the LDL profiles were quantified and the total LDL mass (all protein and lipid components) was then subdivided in proportion to the percentage area. This gave rise to concentration values for each LDL subfraction in milligrams of lipoprotein per 100 ml plasma.

The −514 C-to-T polymorphism was assayed as described by Guerra et al. with minor modification [13]. The C-to-T substitution 514 base pair (bp) upstream of the transcription initiation site created a NlaIII restriction site (5′-CATG-3′). To assay the polymorphism, a 299 bp fragment containing the restriction site was polymerase chain reaction (PCR) amplified. The fragments were digested by adding 10 U NlaIII in 30 μl NEB buffer 4 (New England Biolabs) to the PCR reaction and electrophoresed on a 3% metaphor gel (FMC BioProducts, Rockland, ME).

Results in this study are expressed as the means and standard deviations, or as median and interquartile range if the distribution of the data were skewed. Triglyceride was logarithmically transformed before analyses were made because of the skewed distribution. The difference in allele frequency of the C-to-T polymorphism between control subjects and patients with diabetes was tested using the χ^2 test. Associations between different parameters were determined by Pearson correlation coefficients. Multiple stepwise linear regression analysis was used to assess the relationships be-

tween HL activity and various variables simultaneously. The statistical package SPSS (version 7.5; SPSS Inc., Chicago, IL, USA) was used for data analysis.

3. Results

There were no significant differences in age and body mass index (BMI) between male controls and male diabetic patients, but the female controls were significantly younger and had lower BMI than female diabetic patients (Table 1). Fasting glucose and HbA1c were higher in patients with diabetes, as expected. Both male and female diabetic patients had significantly higher plasma triglyceride, lower HDL cholesterol and apolipoprotein AI than non-diabetic controls (Table 1). LDL-I concentration was lower only in female diabetic patients, whereas LDL-III was increased in both male and female diabetic patients compared with their counterparts (Table 2). Hepatic lipase activity was also significantly higher in the diabetic patients (Table 2). As the female controls were younger and had lower BMI than the female diabetic patients, plasma lipids, lipoproteins and HL activity were also compared after adjustment for age and BMI, and the differences already described between the two groups remained significant (data not shown).

Weak univariate correlations were seen between HL activity and plasma HDL and LDL subfractions. Hepatic lipase activity correlated inversely with HDL both in the controls ($r = -0.50$, $P < 0.001$) and in the diabetic patients ($r = -0.29$, $P < 0.01$). In the controls, HL activity correlated with LDL-I ($r = -0.30$, $P < 0.001$), LDL-II ($r = 0.35$, $P < 0.001$) and LDL-III ($r = 0.32$, $P < 0.001$), whereas in the patients with diabetes HL activity correlated only with LDL-III ($r = 0.24$, $p < 0.01$). There were no correlations between HL activity and HbA1c in either the controls or the diabetic patients.

The frequency of the −514 C-to-T polymorphism is shown in Table 3. The genotype frequencies did not deviate significantly from those predicted by the Hardy–Weinberg equation and there was no difference in the allele frequency between non-diabetic and diabetic subjects. The effect of the −514 C-to-T polymorphism on HL activity is shown in Fig. 1. The relationship between *LIPC* genotype and HL activity was similar between men and women, and between diabetic patients and non-diabetic controls. Subjects with the TT genotype had the lowest HL activity. Although no significant differences were found when diabetic patients were compared with controls matched for gender and genotype, there was a trend that both male and female diabetic patients with CC genotype had higher HL activity than their respective controls. Since the effect of *LIPC* genotype was the same in male and female subjects, we have also analysed data from male and female subjects together as a group. A significant difference was seen in the CC genotype with the diabetic patients having higher HL activity than non-diabetic controls (26.4 ± 9.9 versus 20.8 ± 8.2 μmol free fatty acid released/ml/h; $P < 0.01$).

To determine the relative importance of the *LIPC* genotype and diabetes mellitus in determining plasma HL activity, data from all subjects were analysed together. In stepwise linear regression, gender, *LIPC* genotype, diabetes mellitus and BMI were found to be significant independent predictors of HL activity, contributing 22, 9, 5 and 3%, respectively, to the variance in HL activity (adjusted R^2 of the whole model = 0.39, $P < 0.0001$). As gender had such an important effect on HL, we also analysed male and female subjects separately in order to evaluate the impact of other factors

Table 1
Clinical characteristics, fasting lipids and apolipoproteins of controls and diabetic patients[a]

	Male		Female	
	Controls ($n = 99$)	Diabetes ($n = 102$)	Controls ($n = 106$)	Diabetes ($n = 101$)
Age (years)	48.5 ± 9.7	49.9 ± 9.2	48.8 ± 9.0	51.4 ± 9.2*
BMI (kg/m^2)	24.9 ± 3.0	24.9 ± 3.2	24.2 ± 3.7	25.4 ± 3.9*
Duration of diabetes (years)	–	8.7 ± 5.9	–	8.6 ± 6.5
Fasting glucose (mmol/l)	5.2 ± 0.4	8.5 ± 1.9**	5.0 ± 0.4	8.1 ± 2.1**
HbA1c (%)	5.7 ± 0.5	7.6 ± 1.2**	5.6 ± 0.5	7.6 ± 1.5**
TC (mmol/l)	5.47 ± 0.90	5.26 ± 1.0	5.41 ± 0.81	5.48 ± 1.12
TG (mmol/l)	1.10 (0.90–1.60)	1.50 (0.80–2.00)*	0.90 (0.70–1.30)	1.50 (0.90–2.10)**
LDL (mmol/l)	3.64 ± 0.80	3.51 ± 0.84	3.41 ± 0.74	3.45 ± 0.90
HDL (mmol/l)	1.21 ± 0.21	1.05 ± 0.25*	1.52 ± 0.35	1.20 ± 0.37**
Apo AI (g/l)	1.42 ± 0.24	1.31 ± 0.27*	1.58 ± 0.38	1.35 ± 0.19**
Apo B (g/l)	1.10 ± 0.25	1.05 ± 0.27	0.95 ± 0.22	1.04 ± 0.27

[a] Values are presented as mean ± S.D. or median (interquartile range). * $P<0.05$, ** $P<0.001$ versus controls. TC, Total cholesterol; TG, triglyceride; apo, apolipoprotein.

Table 2
LDL subfractions and HL activities in controls and diabetic patients[a]

	Male		Female	
	Controls ($n=99$)	Diabetes ($n=102$)	Controls ($n=106$)	Diabetes ($n=101$)
LDL-I (mg/dl)	55.5 ± 30.0	48.5 ± 25.7	72.4 ± 38.4	52.7 ± 31.6**
LDL-II (mg/dl)	168.2 ± 49.1	155.4 ± 49.9	148.3 ± 42.0	154.7 ± 50.5
LDL-III (mg/dl)	100.5 ± 42.3	115.3 ± 41.8*	80.4 ± 46.2	103.4 ± 40.9†
HL (μmol free fatty acid released/ml/h	21.2 ± 7.2	23.6 ± 9.5*	14.5 ± 6.0	17.2 ± 7.6**

[a] Values are presented as mean ± S.D. * $P<0.05$, ** $P<0.01$, † $P<0.001$ versus controls.

on HL activity. In men, *LIPC* genotype and diabetes mellitus accounted for 8 and 5% of the variance in HL activity, whereas in women *LIPC* genotype, diabetes mellitus and BMI accounted for 9, 4 and 3% of the variance in HL activity.

The effect of the *LIPC* genotype on plasma lipids was evaluated in both groups of subjects. There were no significant differences in plasma total cholesterol, triglyceride and LDL cholesterol between the different genotypes in either the controls or diabetic patients. HDL cholesterol was significantly lower in female controls with the CC genotype (Fig. 2) and remained significant after adjusting for age and BMI. A trend was also observed in the male controls (Fig. 2). However, this effect was only found in the controls. In the diabetic patients, *LIPC* genotype did not appear to have any effect on HDL cholesterol even after adjusting for possible confounding factors (Fig. 2). Despite the associations between HL activity and LDL subfractions, we did not find any significant difference in LDL subfractions between subjects with the different genotype in either the controls or diabetic patients.

4. Discussion

The frequency of the less common *LIPC* promoter allele in American and European Caucasians ranges from 15 to 25% and is up to 52% in African Americans [13–16,18,19]. There is very little reported data on the allele frequency in oriental subjects. Zambon et al. reported that the allele frequency of the −250 G-to-A polymorphism is 47% in a small group of Japanese Americans, and this promoter variant is in strong but not complete linkage disequilibrium with other *LIPC* promoter variants [20]. We have demonstrated in the present study that, in the Chinese population, the allele frequency of the −514 C-to-T polymorphism is around 37%. Similar to previous studies in other populations, the rare T allele is associated with lower HL activity in Chinese subjects. However, in our population, the most important determinant of HL activity appears to be gender, accounting for nearly one-quarter of the variation in HL activity, and the *LIPC* genotype accounts for just under 10% of the variation in both sexes. Despite the higher frequency of the T allele in Chinese than Caucasians, gender has a greater impact than *LIPC* gene polymorphism on HL activity. In contrast, *LIPC* gene polymorphism has been shown to have a much stronger effect on HL activity in Caucasians. Zambon et al. reported that variability in the *LIPC* promoter region accounted for 20 and 30% of the variance in HL activity among normal subjects and patients with CHD, respectively [20]. De Oliveira e Silva et al. demonstrated that *LIPC* promoter genotype accounted for 38% of the variation in HL activity in normolipidaemic Caucasian females [23]. The molecular mechanism(s) responsible for the decreased HL activity associated with the −514T allele is still unclear. Since no functional mutations have been found in the coding region of *LIPC* that could account for the observed association between the polymorphism and plasma HL activity, it has been suggested that the promoter polymorphism might be in linkage disequilibrium with other variants located either within *LIPC* itself or within an adjacent gene encoding a protein that regulates *LIPC* expression [14–16]. Alternatively, the decrease in HL activity might reflect a decrease in HL gene expression. Deeb and Peng recently reported that the −514T promoter had approximately 30% lower activity than −514C promoter in transient transfection studies [24].

Hepatic lipase activity is a multifactorial trait, determined in part by polymorphisms within the *LIPC* gene as well as hormonal, metabolic and environmental factors. Hepatic lipase is sex-steroid sensitive, and its activity is increased by androgens and decreased by oestrogens [7,25,26]. Our results show that the effects of gender and *LIPC* gene polymorphism on HL activity are independent. This is in agreement with the findings of Vega et al., and the androgen-mediated stimulation of HL activity is not influenced by the −514 C-to-T polymorphism [26]. Because of the much larger impact of gender on HL activity in our population, this would suggest that sex steroids may be a more important regulator of HL activity in Chinese.

Other than hormonal factors like thyroid hormone and sex steriods [7,9,25,26], recent studies have also shown that *LIPC* promoter polymorphism and BMI

Table 3
Allele frequency and genotype frequency of −514 C-to-T polymorphism

	Male		Female	
	Controls ($n=99$)	Diabetes ($n=102$)	Controls ($n=106$)	Diabetes ($n=101$)
−514T	0.371	0.343	0.372	0.376
−514C	0.629	0.657	0.628	0.624
−514T/T	0.157	0.137	0.148	0.168
−514C/T	0.427	0.412	0.447	0.416
−514C/C	0.416	0.451	0.405	0.416

jointly influence HL activity [27], and *LIPC* promoter polymorphism attenuates the increase in HL activity associated intra-abdominal adiposity [28]. A new finding in the present study is that there is an independent additive effect between type 2 diabetes mellitus and the *LIPC* genotype on HL activity. The effect of type 2 diabetes mellitus on HL activity is most marked in those diabetic patients with the CC genotype. It is of interest to note that Jansen et al. reported in their study an interaction between insulin levels and *LIPC* polymorphism in those subjects with the C allele [14]. They suggested that the expression of the C-to-T polymorphism on HL activity might be influenced by insulin concentration, and induction of HL activity by insulin was blunted in carriers of the T allele. It has been shown that HL activity is associated with the degree of insulin insensitivity in both patients with type 2 diabetes mellitus [22,29] and in non-diabetic subjects [30]. Whether the interaction between type 2 diabetes and *LIPC* polymorphism on HL activity is mediated by insulin resistance and/or hyperinsulinemia remains to be proven.

Genetic variation in HL activity has been demonstrated to contribute to variation in plasma lipoprotein concentrations. The -514T allele is associated with increased HDL cholesterol in the Finnish participants of the EARS study [16] and in the Dutch CHD patients from the REGRESS study [14]. Jansen et al. have shown that the −514T allele is associated with increased fasting lipid and HDL, and is an increase in postprandial LpCIII:B [31]. Zambon et al. have shown that the −250 polymorphism was associated with an increase in HDL_2 and buoyant LDL [20]. The presence of the T allele therefore seems to confer some benefit to the lipid profile. However, we have shown that this benefit is lost in patients with type 2 diabetes. The *LIPC* genotype is only associated with HDL cholesterol level in our non-diabetic Chinese subjects but not in our diabetic patients. This would suggest that, although a genetic reduction in HL activity predisposes an individual to high plasma HDL cholesterol concentration, this might not be sufficient to confer high HDL concentration in the presence of a HDL lowering factor like type 2 diabetes mellitus. Unlike the findings of Zambon et al., we did not find an association between LDL subfractions and LIPC genotype. This might be due to the fact that HL activity, although an independent determinant of LDL subfraction profiles, plays a relatively small role compared with other factors in determining LDL subfractions in our population. We have previously shown that HL activity only accounts for about 5% of the variability of LDL-III in non-diabetic Chinese subjects [4].

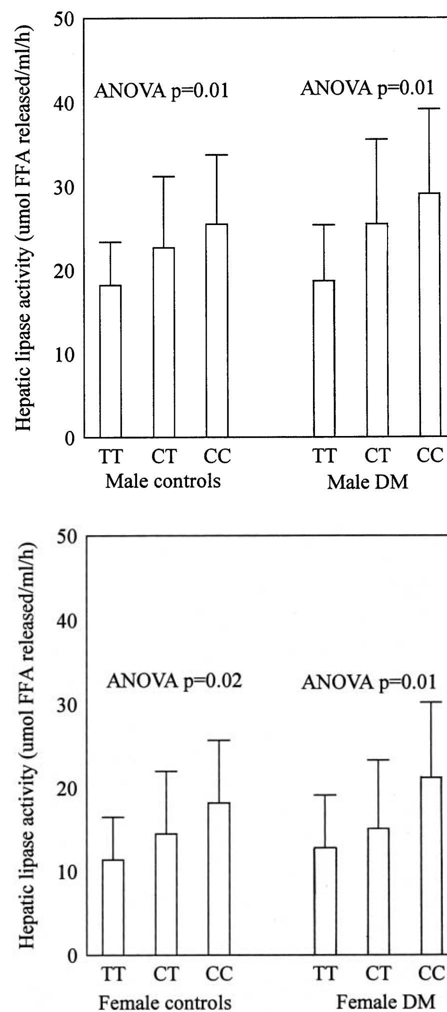

Fig. 1. Hepatic lipase activity in male and female controls and diabetic patients according to *LIPC* genotype. Values are presented as mean ± S.D.

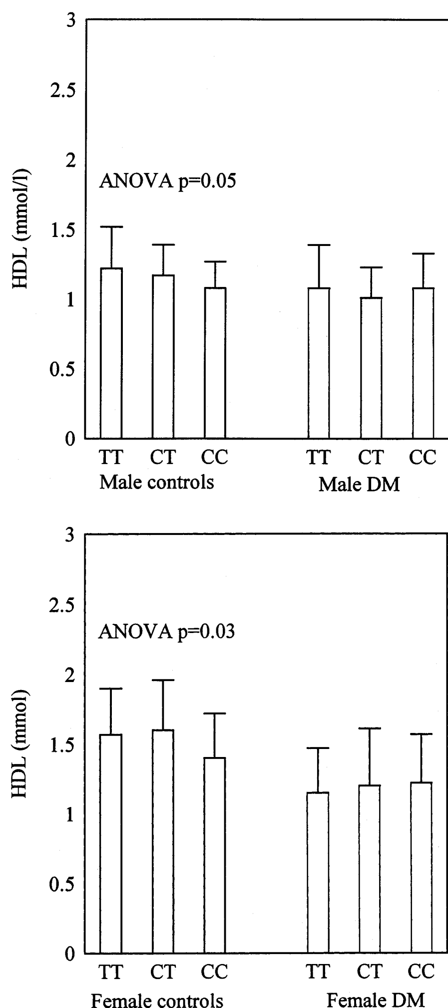

Fig. 2. HDL in male and female controls and diabetic patients according to *LIPC* genotype. Values are presented as mean ± S.D.

In conclusion, the frequency of the rare T allele of the −514 C-to-T polymorphism is higher in Chinese subjects than that reported in Caucasians and the T allele is associated with lower HL activity. Despite the higher frequency of the T allele, gender is the best predictor for HL activity, with *LIPC* gene polymorphism and type 2 diabetes making relatively smaller contributions to the variation in HL activity.

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