Effect of Hepatic Lipase −514C→T Polymorphism and Its Interactions With Apolipoprotein C3 −482C→T and Apolipoprotein E Exon 4 Polymorphisms on the Risk of Nephropathy in Chinese Type 2 Diabetic Patients

Larry Baum, phd[1]
Maggie C.Y. Ng, phd[1]
Wing-Yee So, mbchb, mrcp[1]
Vincent K.L. Lam, mphil[1]
Ying Wang, phd[1]
Emily Poon, mphil[1]
Brian Tomlinson, md, frcp[1]
Suzanne Cheng, phd[2]
Klaus Lindpaintner, md[3]
Juliana C.N. Chan, md, frcp[1]

OBJECTIVE — Triglyceride-rich lipoprotein particles may promote the progression of diabetic nephropathy. Patients with diabetic nephropathy have increased plasma triglycerides and reduced activity of hepatic lipase (HL), which hydrolyzes triglycerides. We hypothesized that the HL −514C→T polymorphism, which reduces HL expression, and its interactions with polymorphisms in apolipoprotein (apo) E and apoC3 increase the risk of diabetic nephropathy.

RESEARCH DESIGN AND METHODS — In a case-control study involving 374 Chinese type 2 diabetic patients with and 392 without diabetic nephropathy, we genotyped the HL −514C→T, apoE exon 4, and apoC3 −482C→T polymorphisms.

RESULTS — HL −514T−containing genotypes (T+) were associated with diabetic nephropathy (OR = 1.7, $P = 0.0009$). Adjustment by multiple logistic regression for hypertension, triglycerides, sex, non-HDL cholesterol, BMI, smoking, and alcohol intake did not diminish the association (OR = 1.8, $P = 0.003$). The association between HL T+ genotypes and diabetic nephropathy appeared stronger in diabetic patients with apoC3 −482 non-TT genotypes (OR = 1.9, $P = 0.003$) or apoE ε2 or ε4 alleles (OR = 2.2, $P = 0.005$). Subjects with HL TT exhibited trends toward increased triglyceride and non-HDL cholesterol levels compared with CC carriers.

CONCLUSIONS — HL T+ genotypes might increase the risk of developing diabetic nephropathy by slowing clearance of triglyceride-rich remnant lipoproteins. In concert with other risk factors (e.g., hyperglycemia), lipid abnormalities may damage the kidneys and endothelium, where reduced binding sites for lipases may precipitate a vicious cycle of dyslipidemia, proteinuria, and nephropathy.

Diabetes Care 28:1704–1709, 2005

From the [1]Department of Medicine and Therapeutics, Chinese University of Hong Kong, Shatin, Hong Kong; the [2]Department of Human Genetics, Roche Molecular Systems, Alameda, California; and the [3]Roche Center for Medical Genomics, F. Hoffmann-La Roche, Basel, Switzerland.

Address correspondence and reprint requests to Prof. Juliana C.N. Chan, Department of Medicine and Therapeutics, Chinese University of Hong Kong, Shatin, Hong Kong. E-mail: jchan@cuhk.edu.hk.

Received for publication 11 January 2005 and accepted in revised form 26 March 2005.

Abbreviations: ACR, albumin-to-creatinine ratio; AER, albumin excretion rate; apo, apolipoprotein; FPG, fasting plasma glucose; HL, hepatic lipase; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Diabetic nephropathy is a major cause of end-stage renal disease. Epidemiological and family-based studies suggest there is a genetic predisposition to diabetic nephropathy (1). Identifying the genetic risk factors for diabetic nephropathy may allow vulnerable type 2 diabetic patients to be screened for the disease and preventive treatment to be developed; in addition, elucidating the pathogenesis of the disease may aid in the design of better treatment.

There is evidence to suggest that triglyceride-rich lipoprotein particles containing predominantly apolipoproteins (apos) E, C, and B may be major promoters of diabetic nephropathy (2,3). In diabetic nephropathy patients, the increase in plasma triglycerides may in part be due to reduced activity of hepatic lipase (HL), which hydrolyzes triglycerides (4). The HL promoter polymorphism −514C→T, alternatively named −480C→T, belongs to a haplotype that alters HL expression (5). Interactions among HL −514C→T, apoE exon 4, and apoC3 −482C→T polymorphisms in patients with young-onset type 2 diabetes or cardiovascular diseases have been reported (6,7). Given the importance of lipid metabolism in these related diseases, we hypothesized that HL −514C→T may interact with apoE and apoC polymorphisms to induce nephropathy in diabetic patients. We tested this hypothesis in a large case-control study involving Chinese type 2 diabetes patients with or without diabetic nephropathy.

RESEARCH DESIGN AND METHODS

— The Prince of Wales Hospital is the teaching hospital of the Chinese University of Hong Kong. It serves a population of >1.2 million. Since

Table 1—*Clinical and biochemical characteristics of Chinese type 2 diabetic patients with or without nephropathy*

	DN−	DN+	*P*
n	392	374	—
Sex (% men)	29.3	55.1	<0.001
Age (years)	58.3 ± 10.2	60.3 ± 11.2	0.007
Diabetes duration (years)	16.1 ± 4.7	7.9 ± 4.5	<0.001
Past or current smoker (%)	19.9	38.5	0.19
Past or current alcohol drinker (%)	13.5	26.2	0.38
Past or current smoker or alcohol drinker (%)	23.2	44.4	0.09
Retinopathy (%)	28.1	54.0	<0.001
Sensory neuropathy (%)	19.1	42.8	<0.001
Coronary heart disease (%)	7.1	11.0	0.11
Peripheral vascular disease (%)	3.3	15.5	<0.001
Stroke (%)	3.6	9.6	0.006
BMI (kg/m^2)	24.0 ± 3.3	25.6 ± 4.1	<0.001
Obesity (BMI ≥25 kg/m^2) (%)	37.0	49.6	<0.001
WHR	0.87 ± 0.06	0.91 ± 0.07	<0.001
Men	0.91 ± 0.07	0.93 ± 0.06	0.002
Women	0.86 ± 0.06	0.89 ± 0.07	<0.001
Hypertension or treatment (%)	47.2	82.1	<0.001
Systolic blood pressure (mmHg)	132.6 ± 17.8	152.2 ± 23.0	<0.001
Diastolic blood pressure (mmHg)	72.6 ± 9.8	82.1 ± 12.1	<0.001
Dyslipidemia or treatment (%)	51.3	83.4	<0.001
Triglycerides (mmol/l)	1.13 (1.07–1.19)	1.88 (1.76–2.01)	<0.001
Cholesterol (mmol/l)			
Total	5.22 ± 1.01	5.86 ± 1.49	<0.001
HDL	1.38 ± 0.38	1.17 ± 0.33	<0.001
LDL	3.24 ± 0.88	3.67 ± 1.20	<0.001
Non-HDL	3.83 ± 0.99	4.62 ± 1.36	<0.001
A1C (%)	7.7 ± 1.4	8.0 ± 1.8	0.05
FPG (mmol/l)	8.4 ± 2.9	9.0 ± 3.9	0.02
Plasma creatinine (μmol/l)	69.4 (67.7–70.6)	125 (118–133)	<0.001
Urinary ACR (mg/mmol)	1.25 (1.14–1.36)	141 (124–158)	<0.001
Urinary AER (μg/min)	7.5 (6.9–8.0)	814 (733–904)	<0.001

Data are means ± SD or geometric mean (95% CI), unless otherwise noted. *P* values are adjusted for age and sex. Retinopathy was defined by the presence of characteristic changes, including hemorrhages, exudates, laser marks, and fibrous proliferation, as detected by direct ophthalmoscopy through dilated pupils by a diabetologist or ophthalmologist, or a history of vitrectomy. Sensory neuropathy was defined as the presence of two of the following three features: reduced sensation to monofilament or graduated tuning fork or symptoms of altered sensation in lower limbs. Peripheral vascular disease was defined as absent foot pulse and an ankle-to-brachial ratio <0.9. Hypertension was defined as blood pressure ≥140/90 mmHg or treatment with antihypertensive drugs. Dyslipidemia was defined as triglycerides ≥1.7 mmol/l and/or HDL cholesterol <1.1 mmol/l in men and <1.3 in women and/or LDL cholesterol >2.6 mmol/l or treatment with lipid-lowering drugs. DN, diabetic nephropathy.

1995, as part of a continuous quality improvement program, all newly referred patients to the clinic have undergone a comprehensive assessment of diabetes-associated complications and risk factors based on the European DiabCare protocol. Clinical assessments included measurement of BMI, waist-to-hip ratio (WHR), and blood pressure, as well as documentation of visual acuity and examination by fundoscopy through dilated pupils. For the foot examination, we used monofilament and graduated tuning forks to assess sensory neuropathy. Fasting blood samples were taken for the measurement of plasma glucose, HbA_{1c} (A1C), lipids (total cholesterol), HDL cholesterol, triglycerides, and plasma creatinine. A sterile, random spot urine sample was used to measure the albumin-to-creatinine ratio (ACR), followed by a timed collection (4 or 24 h) for the albumin excretion rate (AER).

Between 1995 and 1998, 3,318 type 2 diabetic patients were recruited, 1,533 (46.2%) of whom were normoalbuminuric and 374 (11.3%) of whom were identified as having nephropathy (DN+). Diabetic nephropathy was defined as plasma creatinine ≥150 μmol/l or an average ACR from the timed and spot urine collections ≥25 mg/mmol. Patients with microscopic hematuria, urinary tract infection, microalbuminuria (mean ACR 3.5–25 mg/mmol), or a history of non−diabetes-related renal disease, such as obstructive uropathy, were excluded. Within the normoalbuminuric group, 392 control subjects (DN−) were identified who had a long duration of diabetes (≥10 years) yet retained normal renal function, defined as plasma creatinine <100 μmol/l and ACR <3.5 mg/mmol; these were included because a long diabetes duration in control subjects increases the power of case-control studies of diabetic complications (8). Patients with type 1 presentation, defined as diabetic ketoacidosis, acute presentation with heavy ketonuria (>3+), or continuous requirement of insulin within 1 year of diagnosis, were excluded. Another 200 normal (nondiabetic) control subjects with normal glucose tolerance on a 75-g oral glucose tolerance test (1998 World Health Organization criterion) were recruited from a community health screen of cardiovascular risk factors.

Genotyping and laboratory assays

DNA was extracted from whole blood. HL −514C→T, ApoE exon 4, and ApoC3 −482T→C genotypes were obtained using strips from Roche Molecular Systems, as previously described (9,10). In brief, multiplex PCR with biotinylated primers amplified products containing 64 cardiovascular disease–related single nucleotide polymorphisms. The PCR products were then hybridized against allele-specific oligonucleotide probes immobilized on nylon membranes and visualized by conjugation with horseradish peroxidase and development with 3,3′,5,5′-tetramethylbenzidine. Genotypes were independently determined from the strips by two investigators. The laboratory assay details have been previously described (3).

Statistical analysis

Data are expressed as means ± SD or geometric means (95% CI). Plasma triglyceride levels and urinary ACR and AER were

Table 2—*Association of hepatic lipase −514T allele and risk of diabetic nephropathy or type 2 diabetes*

	Genotypes				Alleles		
	n	CC	CT	TT	HWE	*n*	C	T
Diabetic subjects								
DN−	390	159 (40.8)	181 (46.4)	50 (12.8)	1.00	780	499 (64.0)	281 (36.0)
DN+	366	107 (29.2)	198 (54.1)	61 (16.7)	0.40	732	412 (56.3)	320 (43.7)
P	—	All: 0.004	T+: 0.0009	TT: 0.14	—	—	—	T: 0.002
Odds ratio (95% CI)	—	—	1.7 (1.2–2.3)	1.4 (0.9–2.1)	—	—	—	1.4 (1.1–1.7)
Nondiabetic subjects	200	70 (35.0)	104 (52.0)	26 (13.0)	0.67	400	244 (61.0)	156 (39.0)
P vs. DN−	—	All: 0.37	T+: 0.17	TT: 0.95	—	—	—	T: 0.32
P vs. DN+	—	All: 0.27	T+: 0.16	TT: 0.25	—	—	—	T: 0.12

Data are *n* (%), unless otherwise noted. "All," comparison of all genotypes; DN, diabetic nephropathy; HWE, *P* value for Hardy-Weinberg equilibrium; T+, CT/TT vs. CC; TT, TT vs. CC/CT.

logarithmically transformed. Continuous variables were compared by Student's *t* test. Categorical variables, including genotype distributions (grouped into 2 × 2 tables), were compared by a χ^2 test. Multiple logistic regression analysis was used to control for confounding factors for diabetic nephropathy. Odds ratios (ORs) with 95% CI were calculated using Epi6 software (World Health Organization, Geneva, Switzerland). Other calculations were performed using SPSS 11.5 (SPSS, Chicago, IL). *P* values <0.05 were considered significant.

RESULTS — We selected 374 type 2 diabetic patients with diabetic nephropathy (DN+) and 392 without diabetic nephropathy (DN−). We also chose 200 nondiabetic control subjects (54% male, mean age 42.9 ± 8.3 years, geometric mean plasma creatinine 75.6 [CI 72.3–78.9] μmol/l, geometric mean ACR 0.79 [CI 0.67–0.92] mg/mmol). DN− patients had diabetes longer than DN+ patients (Table 1) due to the study design. Men were more likely to have diabetic nephropathy. Retinopathy, sensory neuropathy, and cardiovascular complications were associated with diabetic nephropathy. DN+ patients had higher BMI and WHR, more adverse lipid and glucose profiles, and more hypertension and dyslipidemia than DN− patients. Because men have a greater WHR than women, sex might confound the observed relation between WHR and nephropathy. To evaluate this possibility, the relation between WHR and nephropathy was stratified by sex, but it remained significant in both women and men (Table 1). In nondiabetic subjects, triglyceride levels (1.03 [CI 0.95–1.11] mmol/l) were 9% lower than in DN− ($P = 0.04$) and 45% lower than in DN+ ($P < 0.001$) patients.

HL −514 genotype and allele distributions in nondiabetic control subjects were not different from that seen in DN+ or DN− patients (Table 2). HL genotypes were in Hardy-Weinberg equilibrium in all three subject groups. HL T alleles and T-containing genotypes (HL T+) were increased in DN+ patients, with a similar OR but falling short of significance for TT genotypes, thereby suggesting a dominant genetic model.

Variables that significantly differed between DN+ and DN− patients were entered into a logistic regression model to identify independent diabetic nephropathy predictors. Age, WHR, A1C, and fasting plasma glucose (FPG) levels were not selected; hypertension, triglyceride levels, sex, non-HDL cholesterol (total cholesterol minus HDL cholesterol), and BMI were independently associated ($P < 0.01$) with diabetic nephropathy. Current or past smoking or alcohol intake was weakly associated with diabetic nephropathy ($P = 0.11$ before adjustment). After adjusting for these six variables, the HL T+ OR for diabetic nephropathy remained essentially unchanged (1.8 [CI 1.2–2.6], $P = 0.003$). Alternatively, after adjusting for 15 variables (hypertension; systolic and diastolic blood pressure; triglyceride levels; total, HDL, and LDL cholesterol; sex; age; BMI; WHR; A1C; FPG levels; past or current smoking; and past

Table 3—*Absence of apolipoprotein E 33 or apolipoprotein C3 −482TT genotype enhances effect of hepatic lipase −514T genotypes on risk of diabetic nephropathy*

	DN−	DN+	OR (95% CI)	*P*
ApoE				
33				
CC	115 (29.8)	77 (21.0)	1	—
T+	167 (43.3)	160 (43.7)	1.4 (1.0–2.1)	0.05
Non-33				
CC	42 (10.9)	30 (8.2)	1.1 (0.6–1.9)	0.82
T+	62 (16.1)	99 (27.0)	2.4 (1.5–3.8)	0.00006
ApoC3				
Non-TT				
CC	125 (32.2)	80 (21.9)	1	—
T+	185 (47.7)	222 (60.7)	1.9 (1.3–2.7)	0.0003
TT				
CC	33 (8.5)	27 (7.4)	1.3 (0.7–2.4)	0.41
T+	45 (11.6)	37 (10.1)	1.3 (0.7–2.2)	0.34

Data are *n* (%), unless otherwise noted. Odds ratio (OR) was determined using the combination of HL CC and apoE 33 or HL CC and apoC3 non-TT as the basis for comparison. Bonferroni correction for three possible ways to stratify by apoE genotypes (33 vs. non-33, 2+ vs. non-2+, or 4+ vs. non-4+) would set a significance level at $P < 0.017$; correction for two ways to stratify by apoC3 genotypes (TT vs. non-TT or CC vs. non-CC) would set a significance level at $P < 0.025$. DN, diabetic nephropathy.

Table 4—*Dyslipidemia status of subjects without apoC3 −482TT divided according to their hepatic lipase genotypes and diabetes or diabetic nephropathy status*

	Nondiabetic				DN−				DN+			
	CC	CT	TT	*P*	CC	CT	TT	*P*	CC	CT	TT	*P*
n	57	87	18	—	125	148	37	—	80	166	56	—
Lipid-lowering drugs (%)	—	—	—	—	8.0	11.5	8.1	—	11.3	15.7	7.1	—
Triglycerides (mmol/l)	1.03 (0.90–1.19)	1.00 (0.90–1.11)	1.10 (0.78–1.54)	0.69	1.09 (0.99–1.19)	1.08 (0.99–1.18)	1.16 (1.00–1.35)	0.50	1.85 (1.60–2.14)	1.83 (1.66–2.03)	2.14 (1.79–2.56)	0.20
Cholesterol (mmol/l)												
Non-HDL	3.47 ± 1.03	3.58 ± 0.94	3.63 ± 0.89	0.58	3.77 ± 0.86	3.74 ± 1.02	3.92 ± 1.12	0.45	4.59 ± 1.33	4.62 ± 1.48	4.81 ± 1.32	0.34
LDL	2.97 ± 0.92	3.06 ± 0.82	2.98 ± 0.58	0.95	3.20 ± 0.79	3.15 ± 0.90	3.34 ± 1.03	0.45	3.68 ± 1.20	3.62 ± 1.23	3.82 ± 1.29	0.56
HDL	1.60 ± 0.40	1.56 ± 0.39	1.69 ± 0.53	0.47	1.31 ± 0.38	1.44 ± 0.39	1.37 ± 0.31	0.42	1.17 ± 0.29	1.16 ± 0.32	1.17 ± 0.35	0.97

Data are means ± SD or log-transformed geometric means (95% CI). *P* values given for TT vs. CC genotypes.

or current alcohol intake, the HL T+ OR for diabetic nephropathy also remained essentially unchanged (1.9 [CI 1.2–2.8], $P = 0.002$).

In diabetic patients with apoE ε2 or ε4 alleles, the association between HL T+ and diabetic nephropathy appeared stronger. The effects of ε2 and ε4 on diabetic nephropathy were similar; thus patients with either allele were combined as apoE non-33 carriers, as in a previous study of apoE and diabetic nephropathy (11). HL T+ was more strongly associated with diabetic nephropathy in non-33 carriers (OR = 2.2, CI 1.2–4.1, $P = 0.005$) than in 33 carriers (OR = 1.4, CI 1.0–2.1, $P = 0.05$). Bonferroni correction for three possible ways to stratify by apoE genotypes would set significance at $P < 0.017$. For apoC3, only −482 non-TT carriers displayed HL T+ association with diabetic nephropathy (Table 3).

On multiple logistic regression analysis using the aforementioned six significant independent variables, the addition of these two interactions as well as apoE 33 and apoC3 TT genotypes revealed no significant main effect of individual genotypes. However, after removing individual genotypes, a weak association for combined apoE non-33 and HL T+ (adjusted OR = 1.3, CI 0.8–2.1, $P = 0.28$) and a significant association for apoC3 non-TT and HL T+ (adjusted OR = 1.9, CI 1.3–2.8, $P = 0.001$) remained. Excluding the apoE/HL interaction gives the apoC3/HL interaction an adjusted OR = 2.0 (CI 1.4–2.9, $P < 0.001$). When adjusting instead for the above-listed 15 independent variables, the results were almost identical. Among apoC3 non-TT carriers with or without diabetes or diabetic nephropathy, there were nonsignificant trends toward higher triglyceride (7–16%) and non-HDL cholesterol (4–5%) levels in HL TT than in CC carriers (Table 4). The above values were 7–10% for triglycerides and 3–7% for non-HDL cholesterol after excluding patients treated with lipid-lowering agents (mainly statins).

CONCLUSIONS — In type 2 diabetes, lipotoxicity and glucotoxicity increase the risk of vascular complications (12). This is the first study to examine the association of an HL polymorphism with diabetic nephropathy. We found a strong association between the HL T+ genotype and diabetic nephropathy, both before and after adjusting for confounders. Further studies are needed to confirm this association and determine whether the association is causative. Although triglyceride levels tend to be increased in diabetic nephropathy, definitive studies such as intervention trials and experimental models are needed to confirm causality in the relation between dyslipidemia and diabetic nephropathy (3,4). Short of such definitive evidence, there are data to support hypothesized mechanisms for this association. Increased triglyceride levels and oxidized remnant lipoprotein particles may exacerbate nephritis by inducing mesangial cells to proliferate and secrete cytokines, including transforming growth factor-β (13). In diabetic nephropathy, urinary loss of lipoprotein particles may enhance their hepatic synthesis. These particles bind to glomerular basement membranes during filtration and renal tubular cells upon reabsorption, which may cause renal damage, perhaps instituting a vicious cycle of proteinuria and dyslipidemia (12). Indeed, in kidney damage, whether type 2 diabetes–related or not, triglycerides and remnants are often increased and HL activity is decreased (4). Because HL −514T reduces HL expression (5,14–16), perhaps this polymorphism contributes to diabetic nephropathy by altering lipids. We did observe trends toward higher triglyceride and non-HDL cholesterol levels in HL TT than in CC carriers. However, −514T usually increases HDL cholesterol, which might have beneficial effects (17,18). Yet, when fat intake is high, the TT genotype may decrease HDL cholesterol (17). In this regard, diabetes could be equivalent to a state of high fat intake, thus making −514T detrimental and increasing triglyceride-rich lipoproteins. In agreement with most reports (16–18), we found no association of the HL −514 genotypes with type 2 diabetes. However, one report associated impaired glucose tolerance with the HL T+ and apoC3 non-TT combination (7), and an intervention study linked another HL polymorphism and type 2 diabetes (19).

Besides the effects of lipotoxicity on the kidney, incipient diabetic nephropathy patients had vascular endothelial damage evidenced by increased von Willebrand factor and decreased heparin-releasable lipoprotein lipase levels (4). Reduced lipase binding to the damaged endothelium might further increase tri-

glycerides in diabetic nephropathy. In patients with reduced HL activity, the slow clearance of triglyceride-rich lipoprotein remnants might allow more of them to bind and damage endothelium. Although these associations do not prove that endothelial damage contributes to diabetic nephropathy, this hypothesis is supported by our association of HL −514T with diabetic nephropathy, by observations that healthy subjects with −514T had impaired vascular function (reduced coronary vascular reactivity and vascular smooth muscle relaxation), and by greater arterial calcification in −514T+ than non-T type 2 diabetic patients (20,21). Type 2 diabetic patients might be susceptible to endothelial dysfunction by many mechanisms: nitric oxide depletion, aldose reductase activation, advanced glycation end product generation, reactive oxygen intermediate formation, adiponectin reduction, metal dysregulation, and protein kinase C activation (22,23). The loss of endothelial binding sites for lipases in type 2 diabetes might increase triglyceride-rich lipoprotein particle and remnant levels, thus initiating a vicious cycle of dyslipidemia, proteinuria, endothelial dysfunction, and cardiorenal complications, especially in genetically predisposed patients.

ApoE non-33 genotypes are associated with higher levels of apoE-rich remnant particles and thus may act synergistically with HL T+ genotypes to increase diabetic nephropathy risk (24). Alternatively, HL processing of remnant lipids usually exposes apoE, thereby facilitating binding to apoE receptors for clearance from circulation (25). Among healthy apoE ε4 carriers, HL −514T carriers had 26% higher levels of small, dense LDL cholesterol than did HL −514 CC carriers (15). In one interesting finding in our study, apoE non-33/HL T+ patients had the highest diabetic nephropathy risk.

ApoC3 is a major triglyceride-rich lipoprotein and chylomicron component. It can increase triglycerides by inhibiting lipases (26). Insulin reduces apoC3 expression and induces HL expression (14,27) to help remove postprandial triglycerides (26). The −482C→T polymorphism lies in an apoC3 gene promoter insulin response element, and functional studies have demonstrated that the −482T allele reduces the inhibitory effect of insulin on apoC3 expression (26). Therefore, subjects with non-TT genotypes may respond to insulin with reduced postprandial apoC3 expression and thus increased HL activity. It is notable that the apoC3 non-TT/HL CC carriers, who would be expected to have low apoC3 and high HL activity, had the lowest OR for diabetic nephropathy in our study. However, we observed no additive effect between HL T+ and apoC3 TT genotypes, perhaps due to the small sample size.

Our study has potential limitations. The development of proteinuria and nephropathy may be confounded by other factors, including blood pressure, glucose levels, and metabolic control as well as treatment received. These factors may partly explain the relatively low frequency of patients having diabetic retinopathy in our cohort given their fair metabolic control as compared with other studies. In addition, it is well recognized that there is potential discordance between retinopathy and nephropathy (28,29). Furthermore, a case-control study has inherent difficulties, including potential survival bias with possible premature mortality due to heart disease. However, we were able to find a strong association between the HL T+ genotype and diabetic nephropathy, both before and after adjusting for confounders.

In conclusion, in Chinese type 2 diabetic patients, HL −514T+ genotypes were associated with increased risk of developing diabetic nephropathy. This association and its interactions with apoE and apoC3 polymorphisms should be confirmed in other patient populations.

Acknowledgments— This study was supported by a Chinese University of Hong Kong Strategic Grant, Hong Kong Research Grant Committee Earmarked Grants, and the Hong Kong Innovation and Technology Fund (ITS/033/00).

We thank Stanley Ho for technical support, Dr. William Goggins for statistical advice, and Kevin H.M. Yu and Patricia Pinna for managing the Prince of Wales Hospital (PWH) Diabetes Registry. We are most grateful to the PWH Diabetes Centre staff for recruiting and managing patients. We thank all patients for kindly donating their DNA to improve our understanding of diabetes.

References

1. Seaquist ER, Goetz FC, Rich S, Barbosa J: Familial clustering of diabetic kidney disease: evidence for genetic susceptibility to diabetic nephropathy. *N Engl J Med* 320: 1161–1165, 1989
2. Ravid M, Brosh D, Ravid-Safran D, Levy Z, Rachmani R: Main risk factors for nephropathy in type 2 diabetes mellitus are plasma cholesterol levels, mean blood pressure, and hyperglycemia. *Arch Intern Med* 158:998–1004, 1998
3. Wang Y, Ng MC, Lee SC, So WY, Tong PC, Cockram CS, Critchley JA, Chan JC: Phenotypic heterogeneity and associations of two aldose reductase gene polymorphisms with nephropathy and retinopathy in type 2 diabetes. *Diabetes Care* 26:2410–2415, 2003
4. Hirano T: Lipoprotein abnormalities in diabetic nephropathy. *Kidney Int* (Suppl. 71): S22–S24, 1999
5. Guerra R, Wang J, Grundy SM, Cohen JC: A hepatic lipase (LIPC) allele associated with high plasma concentrations of high density lipoprotein cholesterol. *Proc Natl Acad Sci U S A* 94:4532–4537, 1997
6. Andersen RV, Wittrup HH, Tybjaerg-Hansen A, Steffensen R, Schnohr P, Nordestgaard BG: Hepatic lipase mutations, elevated high-density lipoprotein cholesterol, and increased risk of ischemic heart disease: the Copenhagen City Heart Study. *J Am Coll Cardiol* 41:1972–1982, 2003
7. Jansen H, Waterworth DM, Nicaud V, Ehnholm C, Talmud PJ: Interaction of the common apolipoprotein C-III (APOC3 −482C→T) and hepatic lipase (LIPC −514C→T) promoter variants affects glucose tolerance in young adults: European Atherosclerosis Research Study II (EARS-II). *Ann Intern Med* 65:237–243, 2001
8. Rogus JJ, Warram JH, Krolewski AS: Genetic studies of late diabetic complications: the overlooked importance of diabetes duration before complication onset. *Diabetes* 51:1655–1662, 2002
9. Cheng S, Grow MA, Pallaud C, Klitz W, Erlich HA, Visvikis S, Chen JJ, Pullinger CR, Malloy MJ, Siest G, Kane JP: A multilocus genotyping assay for candidate markers of cardiovascular disease risk. *Genome Res* 9:936–949, 1999
10. Ng MC, Wang Y, So WY, Cheng S, Visvikis S, Zee RY, Fernandez-Cruz A, Lindpaintner K, Chan JC: Ethnic differences in the linkage disequilibrium and distribution of single-nucleotide polymorphisms in 35 candidate genes for cardiovascular diseases. *Genomics* 83:559–565, 2004
11. Orchard TJ, Chang YF, Ferrell RE, Petro N, Ellis DE: Nephropathy in type 1 diabetes: a manifestation of insulin resistance and multiple genetic susceptibilities? Further evidence from the Pittsburgh Epidemiology of Diabetes Complication Study. *Kidney Int* 62:963–970, 2002
12. Cooper ME: Pathogenesis, prevention,

and treatment of diabetic nephropathy. *Lancet* 352:213–219, 1998
13. Nishida Y, Oda H, Yorioka N: Effect of lipoproteins on mesangial cell proliferation. *Kidney Int* (Suppl. 71):S51–S53, 1999
14. Botma GJ, Verhoeven AJ, Jansen H: Hepatic lipase promoter activity is reduced by the C-480T and G-216A substitutions present in the common LIPC gene variant, and is increased by upstream stimulatory factor. *Atherosclerosis* 154:625–632, 2001
15. Skoglund-Andersson C, Ehrenborg E, Fisher RM, Olivecrona G, Hamsten A, Karpe F: Influence of common variants in the CETP, LPL, HL and APO E genes on LDL heterogeneity in healthy, middle-aged men. *Atherosclerosis* 167:311–317, 2003
16. Tan KC, Shiu SW, Chu BY: Effects of gender, hepatic lipase gene polymorphism and type 2 diabetes mellitus on hepatic lipase activity in Chinese. *Atherosclerosis* 157:233–239, 2001
17. Ordovas JM, Corella D, Demissie S, Cupples LA, Couture P, Coltell O, Wilson PW, Schaefer EJ, Tucker KL: Dietary fat intake determines the effect of a common polymorphism in the hepatic lipase gene promoter on high-density lipoprotein metabolism: evidence of a strong dose effect in this gene-nutrient interaction in the Framingham Study. *Circulation* 106: 2315–2321, 2002
18. Tai ES, Corella D, Deurenberg-Yap M, Cutter J, Chew SK, Tan CE, Ordovas JM, Singapore National Health Survey: Dietary fat interacts with the −514C→T polymorphism in the hepatic lipase gene promoter on plasma lipid profiles in a multiethnic Asian population: the 1998 Singapore National Health Survey. *J Nutr* 133:3399–3408, 2003
19. Todorova B, Kubaszek A, Pihlajamaki J, Lindstrom J, Eriksson J, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Tuomilehto J, Uusitupa M, Laakso M: The G-250A promoter polymorphism of the hepatic lipase gene predicts the conversion from impaired glucose tolerance to type 2 diabetes mellitus: the Finnish Diabetes Prevention Study. *J Clin Endocrinol Metab* 89:2019–2023, 2004
20. Hokanson JE, Cheng S, Snell-Bergeon JK, Fijal BA, Grow MA, Hung C, Erlich HA, Ehrlich J, Eckel RH, Rewers M: A common promoter polymorphism in the hepatic lipase gene (LIPC-480C→T) is associated with an increase in coronary calcification in type 1 diabetes. *Diabetes* 51:1208–1213, 2002
21. Fan YM, Salonen JT, Koivu TA, Tuomainen TP, Nyyssonen K, Lakka TA, Salonen R, Seppanen K, Nikkari ST, Tahvanainen E, Lehtimaki T: Hepatic lipase C-480T polymorphism modifies the effect of HDL cholesterol on the risk of acute myocardial infarction in men: a prospective population based study. *J Med Genet* 41:e28, 2004
22. Sheetz MJ, King GL: Molecular understanding of hyperglycemia's adverse effects for diabetic complications. *JAMA* 288:2579–2588, 2002
23. Singleton JR, Smith AG, Russell JW, Feldman EL: Microvascular complications of impaired glucose tolerance. *Diabetes* 52: 2867–2873, 2003
24. Haddy N, De BD, Chemaly MM, Maurice M, Ehnholm C, Evans A, Sans S, Do Carmo MM, De BG, Siest G, Visvikis S: The importance of plasma apolipoprotein E concentration in addition to its common polymorphism on inter-individual variation in lipid levels: results from Apo Europe. *Eur J Hum Genet* 10:841–850, 2002
25. Jansen H, Chu G, Ehnholm C, Dallongeville J, Nicaud V, Talmud PJ: The T allele of the hepatic lipase promoter variant C-480T is associated with increased fasting lipids and HDL and increased preprandial and postprandial LpCIII:B: European Atherosclerosis Research Study (EARS) II. *Arterioscler Thromb Vasc Biol* 19:303–308, 1999
26. Ashavaid TF, Shalia KK, Kondkar AA, Todur SP, Nair KG, Nair SR: Gene polymorphism and coronary risk factors in Indian population. *Clin Chem Lab Med* 40:975–985, 2002
27. Tu AY, Albers JJ: Glucose regulates the transcription of human genes relevant to HDL metabolism: responsive elements for peroxisome proliferator-activated receptor are involved in the regulation of phospholipid transfer protein. *Diabetes* 50: 1851–1856, 2001
28. Pirart J: [Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973]. *Diabetes Metab* 3:97–107, 1977 [article in French]
29. Chavers BM, Mauer SM, Ramsay RC, Steffes MW: Relationship between retinal and glomerular lesions in IDDM patients. *Diabetes* 43:441–446, 1994