Phenotypic Heterogeneity and Associations of Two Aldose Reductase Gene Polymorphisms With Nephropathy and Retinopathy in Type 2 Diabetes

YING WANG, MD, CHB
MAGGIE C.Y. NG, BSC, PHD
SHAO-CHIN LEE, PHD
WING-YEE SO, MBCHB, MRCP
PETER C.Y. TONG, MBCHB, MRCP
CLIVE S. COCKRAM, MD, FRCP
JULIAN A.J.H. CRITCHLEY, PHD, FRCP
JULIANA C.N. CHAN, MD, FRCP

OBJECTIVE — We investigated the phenotypic features of diabetic microvascular complications and their association with a $(CA)_n$ microsatellite and a *C*/*T* polymorphism at the 5′ region of the aldose reductase gene (*ALR2*) in a consecutive cohort of 738 Chinese type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — Of the entire patient cohort, 392 were free of diabetes complications, or uncomplicated, 159 had diabetic nephropathy, 66 had diabetic retinopathy, and 121 had both diabetic nephropathy and retinopathy. Nephropathy was defined as urinary albumin excretion rate (AER) ≥20 μg/min and albumin-to-creatinine ratio ≥3.5 mg/mmol in two urine collections. Retinopathy was defined by the presence of hemorrhages, exudates, laser marks, and fibrous proliferation or by a history of vitrectomy. $(CA)_n$ and *C*/*T* polymorphisms were examined by PCR followed by capillary electrophoresis and digestion with *Bfa*I, respectively.

RESULTS — In the whole cohort, patients with diabetic retinopathy (n = 187) had higher blood pressure and lower BMI, while those with diabetic nephropathy (n = 280) had higher blood pressure, waist-to-hip ratio, and lipid profile than those without the respective complications. The z+6 carriers of the $(CA)_n$ polymorphism were less common in patients with diabetic retinopathy than those without diabetic retinopathy (n = 551) (4.3 vs. 9.3%, P = 0.04). The CT/TT carriers had a higher AER than the CC carriers (30.2 ×/÷ 7.2 vs. 21.9 ×/÷ 6.9 μg/min, P = 0.03). Further subgroup analysis was performed after excluding uncomplicated patients with <5 years disease duration. The group with both diabetic nephropathy and retinopathy had higher frequencies of the z-2 allele (25.7 vs. 16.9%, P = 0.03) and T allele (26.4 vs. 18.5%, P = 0.04) and a lower frequency of the z+6 allele (1.7 vs. 5.5%, P = 0.054) than the uncomplicated group. Multiple logistic regression analysis confirmed that z-2 carrying (odds ratio 2.6, 95% CI 1.20–5.83, P = 0.02) and CT/TT genotypes (OR 2.5, 95% CI 1.19–5.19, P = 0.02) were independent predictors for both diabetic nephropathy and retinopathy.

CONCLUSIONS — Chinese type 2 diabetic patients exhibited phenotypic differences in terms of risk factors for both diabetic nephropathy and diabetic retinopathy. Both the z-2 allele of $(CA)_n$ polymorphism and T allele of *ALR2* were independently associated with severe diabetic microvascular complications.

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From the Divisions of Clinical Pharmacology and Endocrinology, Department of Medicine and Therapeutics, Prince of Wales Hospital, Chinese University of Hong Kong, Shatin, Hong Kong SAR.

Address correspondence and reprint requests to Professor Juliana C.N. Chan, Divisions of Clinical Pharmacology and Endocrinology, Department of Medicine and Therapeutics, Prince of Wales Hospital, Chinese University of Hong Kong, Shatin, N.T., Hong Kong SAR. E-mail: jchan@cuhk.edu.hk.

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Abbreviations: ACR, albumin-to-creatinine ratio; AER, urinary albumin excretion rate; ALR2, aldose reductase; FPG, fasting plasma glucose.

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

Glycemic control, blood pressure, and disease duration are known predictive factors for the development of diabetic microvascular complications, including nephropathy and retinopathy (1,2). However, not all diabetic patients develop microvascular complications. Family studies in Pima Indian and Caucasian populations suggest genetic influences in the development of these complications (3,4). There is also increasing evidence (5,6) showing that non-Caucasian (including Asian) diabetic patients have a higher risk of renal complications than Caucasian populations. In Hong Kong, up to 50% of type 2 diabetic patients attending hospital medical clinics have nephropathy and 30% have clinically evident retinopathy (7).

An excessive flux of glucose through the polyol pathway, in which aldose reductase (ALR2) is the first and rate-limiting enzyme, has been implicated in the pathogenesis of diabetic microvascular complications. ALR2 catalyzes NADPH-dependent reduction of glucose to sorbitol. Excessive accumulation of intracellular sorbitol has been associated with alteration in the intracellular milieu and signaling pathways (8,9). The *ALR2* gene, located on chromosome 7q35, has been reported (10) as a candidate locus for diabetic nephropathy in Pima Indians. The z-2 allele of the 5′-$(CA)_n$ polymorphism of *ALR2* was found to be associated with both nephropathy and retinopathy in Chinese type 2 diabetic patients (11–13). Recently, the T allele of the promoter *C*/*T* polymorphism was found to be associated with type 1 diabetic nephropathy in Caucasians (14). These findings support the notion that the *ALR2* genetic polymorphism may be implicated in the development of diabetes complications, albeit with marked variations in terms of risk associations in different patient populations. In this study, we examined the relationships of these two polymorphisms

Table 1—*Clinical and biochemical parameters of Chinese type 2 diabetic patients categorized according to their status of nephropathy and retinopathy.*

	No nephropathy	Nephropathy	*P* value	Adjusted *P* value (for age and duration of disease)	No retinopathy	Retinopathy	*P* value	Adjusted *P* value (for age and duration of disease)
n	458	280			551	187		
Sex (M/F)	178/280	129/151			229/322	78/109		
Age (years)	52.3 ± 12.6	60.8 ± 11.1	<0.001		53.4 ± 13.0	62.0 ± 9.6	<0.001	
Duration of diabetes (years)	4.2 ± 4.7	8.1 ± 6.3	<0.001		4.4 ± 4.6	9.4 ± 6.9	<0.001	
BMI (kg/m^2)	24.5 ± 4.1	25.2 ± 3.9	0.459	0.079	24.8 ± 4.1	23.9 ± 3.7	0.007	0.04
Waist-to-hip ratio	0.87 ± 0.1	0.89 ± 0.08	0.004	0.153	0.88 ± 0.08	0.89 ± 0.13	0.133	0.916
Systolic blood pressure (mmHg)	129 ± 21	145 ± 22	<0.001	<0.001	131 ± 21	147 ± 25	<0.001	<0.001
Diastolic blood pressure (mmHg)	79 ± 11	82 ± 13	<0.001	<0.001	80 ± 11	82 ± 14	0.01	0.029
HbA_{1c} (%)	7.7 ± 1.9	8.4 ± 2.2	<0.001	0.002	7.7 ± 1.9	8.8 ± 2.2	<0.001	<0.001
FPG (mmol/l)	8.5 ± 3.5	9.5 ± 4.4	<0.001	0.006	8.4 ± 3.4	10.2 ± 4.9	<0.001	<0.001
Total cholesterol (mmol/l)	5.4 ± 1.2	5.8 ± 1.4	<0.001	<0.001	5.5 ± 1.2	5.7 ± 1.4	0.019	0.106
HDL cholesterol (mmol/l)	1.31 ± 0.36	1.18 ± 0.37	<0.001	<0.001	1.27 ± 0.37	1.24 ± 0.37	0.337	0.638
LDL cholesterol (mmol/l)	3.28 ± 1.14	3.29 ± 1.56	0.963	0.643	3.24 ± 1.27	3.40 ± 1.43	0.169	0.331
Triglyceride (mmol/l)*	1.29 ×/÷ 1.82	1.82 ×/÷ 1.91	<0.001	<0.001	1.41 ×/÷ 1.91	1.55 ×/÷ 1.91	0.185	0.823
Plasma creatinine (μmol/l)	68 ± 16	116 ± 84	<0.001	<0.001	77 ± 36	114 ± 92	<0.001	<0.001
Spot urinary albumin-to-creatinine ratio (mg/mmol)	1.5 ×/÷ 25.	26.9 ×/÷ 4.7	<0.001	<0.001	2.7 ×/÷ 4.7	12.3 ×/÷ 7.6	<0.001	<0.001
4-h AER (μg/min)*	6.9 ×/÷ 1.8	213.8 ×/÷ 4.8	<0.001	<0.001	17.0 ×/÷ 5.5	74.1 ×/÷ 9.6	<0.001	<0.001
Nephropathy (%)	0	100.0			28.9	64.7	<0.001	<0.001
Retinopathy (%)	14.4	43.2	<0.001	<0.001	0	100.0		
Cardiovascular events (%)	3.1	11.1	<0.001	0.017	3.3	14.4	<0.001	0.001

Data are expressed as mean ± SD. *Geometric mean ×/÷ anti-log SD. Nephropathy was defined by one timed (4- or 24-h) AER ≥ 20 μg/min and a spot ACR ≥3.5 mg/mmol after excluding urinary tract infection. Cardiovascular events were defined as the presence of ischemic heart disease, stroke, heart failure, or peripheral vascular disease.

of the *ALR2* and other phenotyptic risk factors with diabetic nephropathy and retinopathy in a large cohort of Hong Kong Chinese type 2 diabetic patients.

RESEARCH DESIGN AND METHODS

— In Hong Kong, the majority of inhabitants are of Han Chinese origin, without any known ancestors of other ethnic origin. They are a typical socioeconomic representation of first- or second-generation migrants from Southern China who now live a westernized lifestyle. In this cross-sectional study, 738 Chinese patients (aged 55.5 ± 13 years, known disease duration 5.7 ± 5.7 years, [means ± SD]) with type 2 diabetes (15) were consecutively recruited between 1994 and 1999 from the Diabetes Center of the Prince of Wales Hospital. Patients taking ACE inhibitors, which may attenuate albuminuria, were excluded. Patients with hypertension were treated with calcium channel blockers, α-blockers, β-blockers, methyldopa, or diuretics. In addition, patients with microscopic hematuria or known history of non–diabetes-related renal diseases, such as obstructive uropathy, were excluded. Of 738 patients, 21.5% had diabetic nephropathy, 8.9% had diabetic retinopathy, 16.4% had both diabetic nephropathy and retinopathy, and 53.1% were free from complications, or uncomplicated. Informed consent was obtained from each participant. The study was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong.

Clinical examination and biochemical assays

Procedures for physical examination and methods for biochemical assays have been described previously (16). After at least 8 h of fasting, physical examination, and anthropometric measurements were performed for all patients. Blood samples were taken for routine biochemical and DNA assays. All patients provided two separate urine samples, one random spot and one timed (4- or 24-h) sample. Urinary albumin concentration was measured by immunoturbidimetry with intra- and interassay coefficient of variance of 3.3 and 6.7%, respectively. The lowest limit of detection was 2.5 mg/l (17). Nephropathy was defined as an albumin excretion rate (AER) ≥20 μg/min in a timed urine collection and albumin-to-creatinine ratio (ACR) ≥3.5 mg/mmol in

a random spot urine sample after excluding urinary tract infection. Retinopathy was defined by the presence of characteristic changes, including hemorrhages, exudates, laser marks, and fibrous proliferation, detected by direct ophthalmoscopy through dilated pupils by a diabetologist or ophthalmologist, or a history of vitrectomy.

Genotyping

Genomic DNA was extracted from whole blood by the phenol-chloroform method (18). Genotyping for the 5′-$(CA)_n$ microsatellite polymorphism was performed as described by Ko et al. (11) with a LI-COR DNA Analyzer (LI-COR, Lincoln, NE). All alleles were sized by comparing with a plasmid DNA containing a 23 (CA) repeats of *ALR2*, which was kindly donated by Dr. Shiro Maeda, Shiga University of Medical Science, Japan. Genotyping for the promoter (C/T) polymorphism was performed as described by Kao et al. (19). The presence of the C allele was indicated by a 206-bp fragment and T allele was indicated by 147- and 59-bp fragments after overnight digestion with *Bfa*I at 37°C.

Statistical analysis

The Statistical Package for Social Sciences (version 9.0; SPSS, Chicago, IL) was used. Skewed data, including triglyceride, AER, and ACR, were logarithmically transformed. Continuous variables are expressed as means ± SD or geometric means ×/÷ anti–log SD where appropriate, and analyzed using independent-sample *t* test and ANCOVA. The χ^2 test was used for analyzing allele and genotype frequencies and expressed as odds ratio (OR) with 95% CIs where appropriate. Multiple logistic regression analysis was used to identify independent predictive factors for microvascular complications. Linkage disequilibrium between the two polymorphisms was estimated by the GOLD program (20). Haplotype frequencies were estimated with the EH program (21) and compared between patients with no complications and those with both diabetic nephropathy and retinopathy. EH was run three times for both groups separately and then combined. The relevant statistic test was T = log likelihood (both diabetic nephropathy and retinopathy) + log likelihood (uncomplicated) − log likelihood (both diabetic nephropathy and retinopathy + uncomplicated). Twice this value was calculated as χ^2 distribution with a number of df to the number of haplotypes estimated. A P value <0.05 (two-tailed) was considered significant.

Table 2—*Genotype and allele frequencies of the 5′-$(CA)_n$ and promoter C/T polymorphisms of the aldose reductase gene in Chinese type 2 diabetic patients classified according to the presence or absence of nephropathy or retinopathy*

	No nephropathy	Nephropathy	No retinopathy	Retinopathy
n	458	280	551	187
Genotype frequency (%)				
x/x, x/z+6, z+6/z+6	91.5/8.1/0.4	93.2/6.8/0	90.9/8.9/0.2	95.7/3.7/0.5*
x/x, x/z+4, z+2/z+4	86.9/12.4/0.7	88.2/11.4/0.4	86.2/13.1/0.7	90.9/9.1/0
x/x, x/z+2, z+2/z+2	48.9/41.9/9.2	49.6/41.4/8.9	50.5/41.4/8.2	45.5/42.8/11.8
x/x, x/z, z/z	52.0/41.9/6.1	50.0/40.4/9.6	49.7/43.0/7.3	55.6/36.4/8.0
x/x, x/z−2, z−2/z−2	59.8/33.8/6.3	59.3/34.6/6.1	59.9/34.3/5.8	58.8/33.7/7.5
x/x, x/z−4, z−4/z−4	89.1/10.9/0	88.2/11.8/0	89.8/10.2/0	85.6/14.4/0
CC, CT, TT	65.9/28.4/5.7	60.4/34.3/5.4	65.3/29.6/5.1	59.4/33.7/7.0
Allele frequency (%)				
z+6	4.5	3.4	4.7	2.5
z+4	6.9	6.1	7.3	4.6
z+2	30.2	29.7	28.8	33.1
Z	27.1	30.0	28.8	26.2
z−2	23.3	23.4	23.0	24.4
z−4	5.5	5.9	5.1	7.2
X	2.5	1.5	2.3	2.0
C	80.1	77.6	80.1	76.3
T	19.9	22.5	19.9	23.8

*Statistically significant ($P < 0.05$) when the combined genotype (x/z+6 or z+6/z+6) was compared between patients with retinopathy (4.3%) and without retinopathy (9.1%). x = any other $(CA)_n$ allele.

RESULTS — After adjusting for age and duration of diabetes, patients with nephropathy had higher blood pressure, HbA_{1c}, fasting plasma glucose (FPG), total cholesterol, triglyceride, and creatinine, but lower HDL cholesterol than those without nephropathy. They also had a higher frequency of cardiovascular events and retinopathy. Patients with retinopathy had lower BMI but higher blood pressure, HbA_{1c}, FPG, and frequencies of cardiovascular events and nephropathy than those without retinopathy. Patients with nephropathy also had higher waist-to-hip ratio before adjustment (Table 1).

Thirteen alleles of 5′-$(CA)_n$ microsatellites were identified, including z+12, z+10, z+8, z+6, z+4, z+2, z, z-2, z-4, z-6, z-12 z-14, and z-16, where z corresponded to 24 (CA) repeats (11). Z+2, z, and z-2 were the major alleles, accounting for 81.4% of total alleles. The genotypic frequencies observed in this population were in Hardy-Weinberg equilibrium. In the whole cohort, the frequency of the z+6 carrying genotypes was significantly lower in those with than those without retinopathy (OR 0.45, 95% CI 4.3 vs. 9.1, 0.19–1.0, $P = 0.04$) (Table 2). The CT/TT genotype carriers ($n = 267$) had a higher urinary AER than the CC genotype carriers ($n = 471$) (30.2 ×/÷ 7.2 vs. 21.9 ×/÷ 6.9 μg/min, $P = 0.03$). This difference remained significant ($P = 0.04$) after adjustment for confounding variables, including age, duration of diabetes, blood pressure, and HbA_{1c}.

In this cohort analysis, many patients without complications had a relatively short duration of diabetes and were susceptible to the future development of complications. Thus, patients with <5 years diabetes duration ($n = 300$) were excluded in the subsequent analysis. The remaining patients ($n = 438$) were divided into four subgroups: 159 (36.3%) patients with diabetic nephropathy, 66 (15.1%) patients with diabetic retinopathy, 121 (27.6%) patients with both diabetic nephropathy and retinopathy, and 92 (21%) uncomplicated patients. No significant differences in the frequencies of z+6, z-2, and T allele were observed among the four groups. However, univaraiate analysis revealed higher frequencies of the z-2 allele (25.7 vs. 16.9%, OR

Table 3—*Genotype and allele frequencies of the 5′-(CA)$_n$ and promoter C/T polymorphisms in Chinese type 2 diabetic patients classified according to the presence or absence of nephropathy or retinopathy in subgroups.*

	Uncomplicated	Diabetic nephropathy	Diabetic retinopathy	Both diabetic nephropathy and retinopathy
n	92	159	66	121
Genotype frequency (%)				
x/x, x/z+6, z+6/z+6	89.1/10.9/0	90.6/9.4/0	93.9/4.5/1.5	96.7/3.3/0
x/x, x/z−2, z−2/z−2	68.5/29.3/2.2	61.0/34.6/4.4	62.1/31.8/6.1	57.0/34.7/8.3
CC, CT, TT	69.6/23.9/6.5	64.2/32.7/3.1	66.7/28.8/4.5	55.4/36.4/8.3
Allele frequency (%)				
z+6	5.5	4.7	3.9	1.7
z−2	16.9	21.7	22.0	25.7*
C	81.5	80.5	81.1	73.6
T	18.5	19.5	18.9	26.4*

*$P < 0.05$ compared with UC group. x = any other (CA)$_n$ allele. Uncomplicated: patients free of microvascular complications and with ≥5 years disease duration.

1.7, 95% CI 1.0–2.8, $P = 0.03$) and the T allele (26.4 vs. 18.5%, 1.6, 1.0–2.7, $P = 0.04$) as well as a trend of decreased frequency of the z+6 allele (1.7 vs. 5.5%, $P = 0.054$) (Table 3) in both diabetic nephropathy and retinopathy compared with the uncomplicated group.

Using age, sex, duration of disease, blood pressure, metabolic indices, and the three *ALR2* genotypes (z+6 carrying, z-2 carrying, and CT/TT) as independent variables, as well as the uncomplicated group as control (code = 0), the z-2 carrying (OR 2.64, 95% CI 1.02–5.83) and CT/TT genotypes (2.48, 1.19–5.19), age (1.06, 1.02–1.10), blood pressure (1.04, 1.02–1.06), HbA_{1c} (1.23, 1.03–1.46), log triglyceride (20.1, 3.73–107.7), and male sex (2.25, 1.10–4.61) were independent risk factors for prediction of both diabetic nephropathy and retinopathy with a correct rate of 76.9%. Since the duration of diabetes was comparable between the uncomplicated and the both diabetic nephropathy and retinopathy groups after excluding uncomplicated patients with <5 years disease duration, this variable was not put into the regression model.

As the 5′-(CA)$_n$ polymorphism and promoter C/T polymorphism are in close proximity, the degree of linkage disequilibrium and the association of haplotype with both diabetic nephropathy and retinopathy were investigated. The risk alleles (z-2 and T) were in weak linkage disequilibrium (D' = 0.34). The four haplotype frequencies differed significantly between the diabetic nephropathy and retinopathy group and the uncomplicated group ($\chi^2 = 11.48$, $P < 0.01$). Compared with x/C haplotype carriers, where x represented alleles other than z-2, the ORs for having severe diabetes complications for the x/T, z-2/C, and z-2/T haplotype carriers were 2.0 (95% CI 1.2–3.2), 2.1 (1.3–3.5), and 1.5 (0.3–9.3), respectively (Table 4).

CONCLUSIONS

Association between 5′-(CA)$_n$ polymorphism of *ALR2* gene and microvascular complications

Currently, the literature suggests that ALR2 may be associated with diabetic microvascular complications. An in vivo study (22) has demonstrated increased ALR2 levels in peripheral mononuclear cells in type 2 diabetic patients with neuropathy and retinopathy, but less so in patients with nephropathy. A Japanese study (23) has demonstrated that different alleles of the *ALR2* (CA)$_n$ polymorphism were associated with different levels of ALR2 in erythrocytes. However, the associations of ALR2 genetic polymorphisms with microvascular complications in humans remain conflicting. In the present study, we found that the z-2 carrying genotype was associated with increased risk of developing both diabetic nephropathy and retinopathy in a subgroup analysis. Our findings are in agreement with other reports (11–13,24,25) in China, Japan, and Chile that show associations between the z-2 allele and nephropathy and/or retinopathy in type 2 diabetic patients. However, two recent studies (26,27) reported a lack of association between the z-2 allele and microvascular complications in both type 1 and type 2 diabetic patients of Caucasian origin. This may be due to differences in ethnicity, study design, sample size, patient selection, and recruitment criteria. Nevertheless, our findings and those of other investigators suggest that z-2 allele of *ALR2* may be implicated in the development of diabetic microvascular complications in at least Chinese and Japanese type 2 diabetic patients.

In addition to the z-2 allele, we found that the z+6 carrying genotype was protective against retinopathy in the whole cohort and had a trend of protective effects in patients with coexisting nephropathy and retinopathy in the subgroup analysis. Despite the latter difference not reaching statistical significance, which might be due to the rarity of this allele (4.1%), our finding was consistent with a similar study (12) involving Chinese type 2 diabetic patients where this allele was protective against coexisting nephropathy and retinopathy. However, given the rarity of this allele, the relatively small

Table 4—*Haplotype-frequency of the (CA)$_n$ and C/T markers of ALR2 between Chinese type 2 diabetic patients without complications with ≥5 years of disease (uncomplicated) and those with both nephropathy and retinopathy*

Haplotype frequency (%)	Uncomplicated	Both diabetic nephropathy and retinopathy	OR (95% CI)
n	184	242	
z−2/T	0.9	1.0	1.5 (0.3–9.3)
x/T	17.5	25.4	2.0 (1.2–3.2)
z−2/C	15.9	24.6	2.1 (1.3–3.5)
x/C	65.6	49.0	1.0

x = non–z-2 allele; OR for each group was estimated by comparing with the group with x/C haplotype.

sample size, and subtle differences in definition of patient cohorts, these findings must be interpreted with caution.

Other $(CA)_n$ alleles have also been reported to be associated with either diabetic nephropathy or diabetic retinopathy. In a preliminary study, we had previously reported (28) an association between the z-4 allele and retinopathy in the late-onset diabetic patients ($n = 384$) included in the present cohort. The present extended study aimed to validate the previous finding, but we were unable to find this association in either early- or late-onset patients. In addition, a report on Chinese type 2 diabetes revealed a protective effect of the z+2 allele on nephropathy (13). Although our patients had similar frequencies of the z+2 allele (30.0 vs. 29.1%) and z-2 allele (23.3 vs. 22.1%), as reported in the previous study (13), we were unable to confirm this finding.

Association between promoter C/T polymorphism and microvascular complications

In the present study, although we were unable to show an association in the whole group between the T allele of the promoter C/T polymorphism and either nephropathy or retinopathy alone, the CT/TT genotype carriers had higher urinary AER than the CC carriers, even after adjustment for other conventional risk factors. This association was further strengthened by the association between the T allele and coexistence of nephropathy and retinopathy. Despite some inconsistencies (14,19), our results provide corroborative evidence to another study (14) in Caucasian type 1 diabetic patients in whom the T allele was associated with nephropathy. A recent study (29) also suggested that the C/T polymorphism might be associated with increased expression of ALR2 in Chinese population, although this has not been confirmed.

Phenotypic heterogeneity of diabetic microvascular complications

It is noteworthy that patients with retinopathy or nephropathy in our study had different clinical characteristics. Although there were close associations between retinopathy, nephropathy, and disease duration, a recent kidney biopsy study (30) in our population showed that 41% of type 2 diabetic patients with diabetic glomerulosclerosis did not have retinopathy. In the present study, we found that patients with nephropathy had higher waist-to-hip ratio and lipid profiles than those without nephropathy, while patients with retinopathy had a lower BMI but similar lipid profiles compared with those without retinopathy. On the other hand, logistic regression revealed that triglyceride, HbA_{1c}, systolic blood pressure, and sex were independent risk factors for patients with both nephropathy and retinopathy. The inclusion of *ALR2* genotype increased the predictive value of the model from 69 to 77%. Hence, our findings suggest that variable modifiable risk factors may have different effects on the development of diabetes complications and that genetic factors may be of particular importance in those with severe complications.

Compared with patients carrying the low-risk haplotype of x/C, those with the haplotypes of z-2 or T allele alone had twofold increased risk for both diabetic nephropathy and retinopathy. This was further confirmed in the logistic regression model, where both of these alleles were independent risk factors. However, these two risk alleles were only in weak linkage disequilibrium, and only 1% of the patients carried the z-2/T haplotype. Despite these associations, it remains plausible that there may be an undefined regulatory element of *ALR2* or another gene near *ALR2* that contribute to the development of diabetes complications. With the completion of the Human Genome Project, it is now apparent that up to 30% of the single nucleotide polymorphisms showed variations among different racial groups (31). Additionally, there is now evidence suggesting that haplotypes of variants within a gene may be more informative than a single gene variant in terms of disease prediction and that some of these haplotypes may be important in the regulation of gene function (32). Hence, all these factors may contribute to the apparently inconsistent findings regarding the clinical significance of the *ALR2* genetic polymorphisms in different populations. Although chance or undetected population stratification might explain some of these associations, given the relatively homogenous nature of our study population and the large sample size, these factors are not likely to affect our results, which are largely consistent with the current literature. Although prospective and family-based studies (33) are necessary to elucidate the nature of these associations, our studies have demonstrated phenotypic heterogeneity and the effects of *ALR2* genetic polymorphisms on the risk associations for diabetes complications. These markers can help provide better prevention for high-risk diabetic patients.

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Professor Julian A.J.H. Critchley, one of the senior investigators, unfortunately died after a tragic road traffic accident on 13 July 2001, at the age of 50 years. During his 12 years of study in Hong Kong, he provided significant contributions to our understanding of diabetes and metabolic syndrome.

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