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ORIGINAL ARTICLE

−607 C/A polymorphism in the promoter of IL-18 gene is associated with 2 h post-loading plasma glucose level in Chinese

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Abstract The purpose of this study is to find out whether the −607 C/A polymorphism in IL-18 gene promoter will affect serum IL-18 concentrations and glucose metabolism in Chinese subjects. In 232 patients with impaired glucose regulation (IGR) or type 2 diabetes and 212 subjects of normal glucose regulation (NGR), −607 C/A polymorphism was analyzed by polymerase chain reaction with sequence specific primers. Serum IL-18 concentrations were determined by ELISA in 200 subjects. Compared with subjects with NGR, those with type 2 diabetes and IGR had significant higher IL-18 concentrations [114.4 (92.9–149.2) and 102.1 (67.5–138.2) vs. 77.3 (53.8–107.8) pg/ml, $P = 0.0026$ and $P < 0.0001$, respectively]. A significantly higher proportion of A/A genotype of −607 C/A polymorphism presented in patients with type 2 diabetes compared with subjects of NGR (23.8% vs. 10.9%, $P = 0.005$). Subjects with an A/A genotype also had higher 2 h post-loading plasma glucose (2 h-PPG) compared with C/A and C/C genotypes even after adjustments of age, sex and BMI [8.94 (7.55–12.3) vs. 7.80 (6.00–9.87) and 7.23 (5.66–8.99) mmol/l, P both < 0.05]. Multiple linear regression revealed that BMI ($P < 0.0001$) and 2 h-PPG ($P = 0.019$) were independently associated with IL-18 concentrations. In conclusion, subjects with IGR or type 2 diabetes had significantly higher concentrations of IL-18 than those with NGR. Genotype A/A of IL-18 gene promoter −607 C/A polymorphism was associated with higher prevalence of type 2 diabetes and 2 h post-loading plasma glucose level.

Keywords Interleukin-18 · Single nucleotide polymorphism · Type 2 diabetes · 2 h post-loading plasma glucose

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Introduction

Recent studies have suggested that activated innate immunity and chronic inflammation implicated in the pathogenesis of type 2 diabetes [1, 2]. The pro-inflammatory cytokine interleukin-18 (IL-18) is now recognized as a central regulator of innate and acquired immune responses [3]. IL-18 is mainly secreted by macrophages and dendritic cell. However, adipocytes have been found to produce IL-18 as well [4]. Studies have shown that IL-18 plays an early role in the inflammatory cascade, inducing the production of tumor necrosis factor-α (TNF-α) [5] and interleukin-6 (IL-6) [6] which in turn regulates the synthesis of C-reactive protein (CRP) in the liver [7]. Epidemiological studies have found that IL-18 is associated with BMI, blood pressure, triglycerides, high-density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), and fasting insulin level [8]. High IL-18 concentration has been observed in patients with type 2 diabetes and metabolic syndrome [9, 10]. Prospective studies also provided evidence that IL-18 was a potent predictor of the development of type 2 diabetes [11].

Giedraitis et al. [12] found single nucleotide polymorphism (−607 C/A) in promoter region might influence IL-18 expression. The SNP was found to associate with various diseases, including type 1 diabetes [13, 14], chronic hepatitis B virus infection [15], allergic rhinitis [16], and

atopic asthma [17]. Moreover, Laurence et al. [18] argued that variations within IL-18 gene influenced serum IL-18 concentrations and clinical outcome in patients with CAD. Although the correlation between IL-18 and type 2 diabetes and effects that the polymorphism may exert on the promoter activity have been explored, data about the relationship between the polymorphism of IL-18 and type 2 diabetes was scarce.

Therefore, our study was aimed at investigating the associations of −607 C/A polymorphism of IL-18 gene and IL-18 concentrations, type 2 diabetes in a Chinese population.

Results

Clinical characteristics

Clinical characteristics of the study subjects are shown in Table 1. Compared with subjects in normal glucose regulation (NGR) group, those with diabetes (DM) were more frequently men, had older age, remarkably higher waist circumference (WC), waist to hip ratio (WHR), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose (FPG), 2 h post-loading plasma glucose (2 h-PPG), total cholesterol (TC), triglyceride (TG), homeostasis model assessment for insulin resistance index (HOMA-IR). Meanwhile, in comparison with subjects in impaired glucose regulation (IGR) group, those with DM also had significantly higher WHR, SBP, DBP, FPG, 2 h-PPG, and HOMA-IR.

IL-18 concentrations and glucose tolerance status

As compared with subjects in NGR group, those with DM and IGR had significantly higher concentrations of IL-18 [114.4 (92.9–149.2) and 102.1 (67.5–138.2) vs. 77.3 (53.8–107.8) pg/ml, $P < 0.0001$ and $P = 0.0026$, respectively] (Fig. 1), even after adjustments for age, sex, and BMI, differences still persisted ($P = 0.0004$ and $P = 0.039$, respectively). Whereas, no difference was found between IGR and diabetic patients ($P = 0.20$).

IL-18 concentrations and metabolic traits

We further analyzed the association between metabolic risk factors and IL-18 concentration. Results from Pearson's correlation analysis revealed that age, BMI, SBP, Lg 2 h-PPG, Lg HOMA-IR, and Lg TG were significantly related to IL-18 concentration (Table 2). After performing multiple linear regression, BMI and 2 h-PPG were the only two variables which were independently associated with IL-18 concentration. Table 2 also displayed that BMI explained 18% ($P < 0.0001$) variability of IL-18 concentration, while 2 h-PPG explained 2.9% ($P = 0.019$).

Table 1 Clinical characteristics of study subjects

	NGR ($n = 212$)	IGR ($n = 152$)	DM ($n = 80$)	P value
Age (years)	32.2 ± 11.9	39.0 ± 16.6*	45.5 ± 14.4*#	<0.0001
Sex (male/female)	60/152	39/113*	40/40*#	0.0003
BMI (kg/m^2)	25.9 ± 6.0	28.1 ± 4.9*	26.7 ± 4.2	0.0005
WC (cm)	82.9 ± 14.2	89.8 ± 11.4	90.3 ± 0.7	<0.0001
WHR	0.85 ± 0.08	0.89 ± 0.09*	0.92 ± 0.06*#	<0.0001
SBP (mmHg)	116 ± 15	121 ± 14*	127 ± 18*#	<0.0001
DBP (mmHg)	75 ± 9	78 ± 10*	83 ± 10*#	<0.0001
FPG (mmol/l)	5.00 (4.69–5.34)	5.70 (5.13–6.33)*	7.80 (7.00–10.03)*#	<0.0001
2 hPPG (mmol/l)	5.93 (4.36–6.70)	8.70 (8.10–9.64)*	14.20 (12.28–18.20)*#	<0.0001
HOMA-IR	2.40 (1.48–4.23)	3.07 (1.76–4.20)	3.82 (2.08–6.84) *#	<0.0001
TC (mmol/l)	4.67 ± 0.85	5.14 ± 1.04*	5.12 ± 1.19*	<0.0001
TG (mmol/l)	1.26 (0.93–1.95)	1.80 (1.28–2.54)*	1.80 (1.43–2.70) *	<0.0001
HDL-c (mmol/l)	1.35 ± 0.36	1.30 ± 0.37	1.24 ± 0.28	0.088
LDL-c (mmol/l)	2.87 ± 0.78	3.03 ± 0.80	3.12 ± 0.72	0.048

Data are expressed as means ± SD or median (interquartile range). *NGR* normal glucose regulation, *IGR* impaired glucose regulation, *DM* type 2 diabetes, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FPG* fasting plasma glucose, *2 hPPG* 2 h post-loading plasma glucose, *TC* total cholesterol, *TG* triglyceride, *HDL-c* high-density lipoprotein-cholesterol, *LDL-c* low-density lipoprotein-cholesterol

* $P < 0.05$, IGR and DM compared with NGT subjects by Bonferroni test

# $P < 0.05$, DM compared with IGR subjects by Bonferroni test

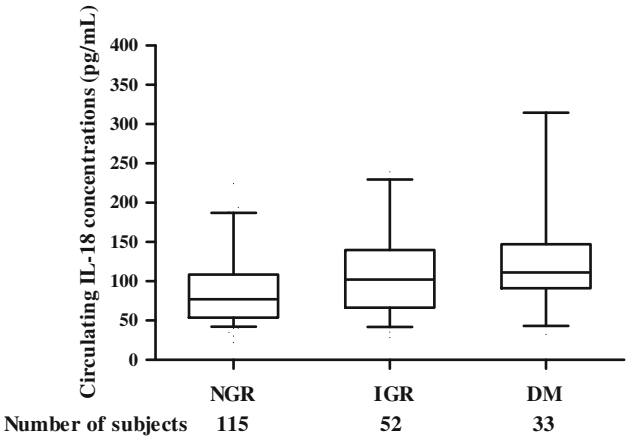

Fig. 1 Circulating IL-18 concentrations in different glucose regulation status. *Whiskers* represent 5–95 percentiles

Genotype frequency

The genotype distributions in accordance with Hardy–Weinberg equilibrium (HWE) in total population ($\chi^2 = 1.45$, $P = 0.48$) and in subgroups (NGR: $\chi^2 = 2.38$, $P = 0.30$; IGR: $\chi^2 = 0.053$, $P = 0.97$; DM: $\chi^2 = 0.10$, $P = 0.95$). The proportion of −607 A/A genotype was significantly higher in IGR and DM patients compared with NGR subjects ($P = 0.022$ and $P = 0.0053$, respectively; Table 3). After the adjustments for age, sex, and BMI, the significant difference still remained ($P = 0.035$ and $P = 0.016$, respectively).

IL-18 genotypes and metabolic traits

In comparison with those with C/A or C/C genotypes, subjects with A/A genotypes had higher FPG [5.80 (5.20–6.95) vs. 5.35 (4.87–6.51) or 5.30 (4.90–6.20) mmol/l, P both < 0.05], and 2 h-PPG [8.94 (7.55–12.3) vs. 7.80 (6.00–9.87) or 7.23 (5.66–8.99) mmol/l, P both < 0.05, Table 4]. Significantly higher HOMA-IR was also detected in subjects with A/A genotypes compared with those with C/A genotypes [3.43 (2.17–5.45) vs. 2.68 (1.63–4.26), $P < 0.05$]. However, BMI, blood pressure, lipids profile did not differ between three genotypes. After further adjustments for age, sex, and BMI, the relationship between genotype and 2 h-PPG still persisted ($P = 0.002$), while the relationship of genotype with FPG and HOMA-IR dramatically attenuated ($P = 0.053$ and $P = 0.083$, respectively).

We also observed the IL-18 concentrations in different genotypes in NGR group. Subjects with AA genotype had significantly higher concentration of IL-18 than those with CA and CC genotypes [117.6 (88.5–156.4) vs. 78.9 (71.0–87.8) and 69.6 (59.0–82.1) pg/ml, $P = 0.023$ and $P = 0.011$, respectively]. However, after adjustments for age, sex and BMI, the differences disappeared ($P = 0.24$ and $P = 0.089$, respectively).

Table 3 Distribution of the −607 C/A genotypes in NGR, IGR, and T2DM

	CC *n* (%)	CA *n* (%)	AA *n* (%)	*P*1 value	*P*2 value
NGR ($n = 212$)	74 (34.9)	115 (54.3)	23 (10.9)		
IGR ($n = 152$)	43 (28.3)	78 (51.3)	31 (20.4)	0.022	0.035
DM ($n = 80$)	19 (24.8)	42 (52.5)	19 (23.8)	0.0053	0.016

*P*1 values were unadjusted

*P*2 values were adjusted for age and sex

Table 2 Correlations between age, sex and metabolic traits with serum interleukin-18

Log 10 (IL-18)	Pearson correlation coefficients	*P* value	Standardized regression coefficients	Partial R^2	*P* value
Age	−0.14	0.044	–	–	–
Sex	−0.028	0.70	–	–	–
BMI	0.48	<0.0001	0.39	0.18	<0.0001
SBP	0.17	0.016	–	–	–
DBP	0.093	0.20	–	–	–
Log FPG	0.13	0.067	–	–	–
Log 2 hPPG	0.31	<0.0001	0.16	0.029	0.019
Log HOMA-IR	0.41	<0.0001	–	–	–
LogTG	0.27	0.0004	–	–	–
TC	0.051	0.50	–	–	–
HDL	−0.088	0.26	–	–	–
LDL	−0.0091	0.96	–	–	–
−607 C/A genotype	0.16	0.024	–	–	–

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FPG* fasting plasma glucose, *2 hPPG* 2 h post-loading plasma glucose, *TC* total cholesterol, *TG* triglyceride, *HDL-c* high-density lipoprotein-cholesterol, *LDL-c* low-density lipoprotein-cholesterol

Table 4 Clinical characteristics of study subjects according to the genotypes of IL-18 SNP−607

	Genotypes			*P* value	
	CC ($n = 136$)	CA ($n = 235$)	AA ($n = 73$)	*P*1	*P*2
Age (years)	35.5 ± 14.6	37.8 ± 15.1	38.2 ± 15.5	0.30	–
Sex (male/female)	44/92	73/162	24/49	0.94	–
BMI (kg/m^2)	26.6 ± 5.1	26.7 ± 5.6	27.7 ± 5.0	0.35	–
SBP (mmHg)	119 ± 17	121 ± 15	121 ± 16	0.48	0.96
DBP (mmHg)	77 ± 10	78 ± 10	79 ± 10	0.35	0.85
FPG (mmol/l)	5.30 (4.90–6.20)	5.35 (4.87–6.51)	5.80 (5.20–6.95)	**0.024**	0.053
2 hPPG (mmol/l)	7.23 (5.66–8.99)	7.80 (6.00–9.87)	8.94 (7.55–12.30)	**0.0003**	**0.002**
HOMA-IR	2.71 (1.60–4.58)	2.68 (1.63–4.26)	3.43 (2.17–5.45)	**0.044**	0.083
TC (mmol/l)	4.89 ± 1.12	4.92 ± 1.00	4.96 ± 0.84	0.90	0.99
TG (mmol/l)	1.51 (1.02–2.20)	1.57 (1.08–2.25)	1.74 (1.29–2.60)	0.36	0.73
HDL-c (mmol/l)	1.35 ± 0.42	1.31 ± 0.33	1.26 ± 0.29	0.31	0.46
LDL-c (mmol/l)	2.94 ± 0.82	3.01 ± 0.77	2.90 ± 0.73	0.55	0.53

Data are expressed as mean ± SD or or median (interquartile range). *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FPG* fasting plasma glucose, *2 hPPG* 2 h post-loading plasma glucose, *TC* total cholesterol, *TG* triglyceride, *HDL-c* high-density lipoprotein-cholesterol, *LDL-c* low-density lipoprotein-cholesterol

*P*1 values were unadjusted

*P*2 values were adjusted for age, sex, and BMI

Bold values demonstrate that differences between groups are statistically significant

Discussion

The present study showed that IL-18 concentrations were remarkably higher in IGR and DM patients compared with NGR subjects. Significantly higher proportions of −607 A/A genotype presented in IGR and DM patients in comparison with that in NGR subjects. Moreover, −607 A/A genotype was associated with higher 2 h-PPG levels.

Abundant epidemiological studies have indicated a close relationship between IL-18 and type 2 diabetes, metabolic syndrome and atherosclerosis [8–10, 18, 19]. Serum IL-18 concentration was found to be elevated in diabetic patient compared with NGR subjects and increased progressively with the escalating number of metabolic traits of metabolic syndrome [8–10]. Higher IL-18 concentration correlated with poorer prognosis in patients with established coronary artery disease (CAD) [18, 19]. In consistence with some previous studies [9, 10], our results showed higher IL-18 concentrations in diabetic patients compared with NGR subjects. Moreover, we also found that in IGR patients, IL-18 concentration was significantly higher than of NGR subjects, which implied that IL-18 might take part in the early phase of pathogenesis of type 2 diabetes.

Several studies have showed that serum IL-18 concentration was associated with nutritional states, such as hyperglycemia [20], and fat mass increase [21–23]. Esposito et al. [18] found that IL-18 concentration was elevated by acute hyperglycemia in humans through an oxidative mechanism. We also observed a positive correlation of serum IL-18 concentration with BMI, PPG, and serum TG. BMI contributed most to the variation of serum Il-18 concentration. In particular, adipocytes from obese individuals secreted 3-fold more IL-18 than those from lean ones [24]. In small samples of obese premenopausal women, IL-18 concentration was elevated in human obesity and that weight loss was associated with proportional reductions in IL-18 levels [21]. Conversely, an animal study demonstrated that IL-18 deficiency resulted in obesity and insulin resistance in mice and the phenotype could be rescued by exogenous administration of IL-18 [25]. Recently, another animal study also indicated an essential role of IL-18 in regulating fat distribution and the deficiency of IL-18 was correlated with decreased energy expenditure [26]. These seemingly conflicting results suggested that IL-18 probably act as a feedback signal to oppose obesity, hyperglycemia, and positive energy balance [26]. Alternatively, it might be a consequence of sensitivity in those subjects to the effect of IL-18.

Giedraitis et al. [12] stated that −607 SNP in the promoter of IL-18 gene was associated with decreased IL-18 expression. Several studies demonstrated that the A allele might be involved in pathogenesis of chronic hepatitis B virus infection [15], allergic rhinitis [16], and atopic asthma [17]. Results regarding the association of −607 SNP and type 1 diabetes were not consistent. Kretowski et al. [13] reported a decreased proportion of −607 A/A genotype in type 1 diabetic patients relative to control subjects. However, Szeszko et al. [14] pointed out that the

genotype distributions of Kretowski's control subjects were not in HWE which may due to ethnic diversity in control subjects. But unfortunately, in Szeszko's study, although higher proportion of −607 A/A genotype was detected in type 1 diabetic patients compared with control group, the difference did not reach statistical significance. In contrast to previous studies, in our study, a significantly higher proportion of −607 A/A genotype was detected in patients with type 2 diabetes compared with NGR subjects. This contradiction may be because of different pathogenesis between type 1 and type 2 diabetes which is greatly influenced by environmental factors. In addition, different genetic influence among races should take into consideration. For instance, A allele at position of −607 was a protective allele from type 1 diabetes in a Polish population [13], while in a population from UK, the significant association was not found [14].

In conclusion, the results of this study showed that subjects with IGR or type 2 diabetes had significantly higher concentrations of IL-18 than those with NGR. Genotype A/A of IL-18 gene promoter −607 C/A polymorphism was associated with higher prevalence of type 2 diabetes and 2 h-PPG level.

Materials and methods

Study population

A total of 468 subjects were recruited from the specialized outpatient clinic for obesity in Ruijin Hospital affiliated to Shanghai Jiao-tong University School of Medicine and a community in Shanghai, China, and underwent a 75-g oral glucose tolerance test (OGTT). All subjects were unrelated Han Chinese living in the Shanghai region, and given informed consent. The Institutional Review Board of the Ruijin Hospital approved the study protocol. None of the study participants was taking any medication or ever instructed in lifestyle modification. Glycemic abnormalities were characterized as IGR ($6.1 \leq \text{FPG} < 7.0$ mmol/l or/and $7.8 \ll 11.1$ mmol/l), and type 2 diabetes mellitus (FPG $\geq$ 7.0 mmol/l or 2 h-PPG $\geq$ 11.1 mmol/l) according to the 1999 World Health Organization criteria [27]. A FPG level less than 6.1 mmol/l and a level below 7.8 mmol/l was considered to represent NGR.

Blood pressure was measured in the seated position after a 10-min rest, in the non-dominant arm to the nearest 2 mmHg using mercury sphygmomanometer. Two measurements were taken in 1 min apart and the mean of the two was used in analysis. Weight and height were measured with light clothes and without shoes. Body mass index (BMI) was calculated using the formula of weight/height^2 (kg/m^2). Waist circumference (WC) and hip circumference (HC) were measured at umbilical level and at the level of maximum extension of buttock, respectively.

Overnight fasting blood specimens were obtained for the measurements of plasma glucose, insulin, serum lipids, and IL-18 levels. Serum lipids were measured using an automated biochemical instrument (Hitachi, 7600 2020, Japan). Serum insulin was measured by radioimmunoassay (Sangon Company, Shanghai, China). Insulin resistance was estimated using HOMA-IR, according to the formula HOMA-IR = fasting insulin (mU/l) × FPG (mmol/l)/22.5. Serum IL-18 was measured using ELISA kits (R&D Systems, Inc., USA).The intra- and inter-assay coefficients of variation were less than 10 and 15%, respectively.

Twenty-four subjects were excluded from the study due to unsuccessful genotyping. A total of 444 subjects were eventually analyzed [80 type 2 diabetic patients, 152 subjects of impaired glucose regulation (IGR, IFG, and/or IGT) and 212 of NGR]. IL-18 levels were determined in 200 (33 diabetic patients, 52 subjects of IGR and 115 of NGR) age- and sex-matched subjects from all the participants according to 607 C/A genotype.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes. The sequence-specific polymerase chain reaction (SSP-PCR) was used to detect the single nucleotide polymorphisms (SNPs) at position −607 in the promoter region of IL-18 gene [12]. A common reverse primer 5′-TA ACCTCATTCAGGACTTCC-3′ and two sequence-specific forward primers 5′-GTTGCAGAAAGTGTAAAAATT ATTAC-3′ and 5′-GTTGCAGAAAGTGTAAAAATTAT TAA-3′ were adopted. A forward primer 5′-CTTTGCT ATCATTCCAGGAA-3′ was used to amplify a 301-bp fragment covering the polymorphic site as an internal positive control. Amplification product of 196 bp was detected in the case of −607 C/A variation.

Statistical analyses

Statistical analyses were performed using SAS (Version 8.0) statistical software package. Continuous variables were presented as means ± SD or median and interquartile ranges for variables with skewed distribution (fasting plasma glucose, 2 h post-loading plasma glucose, serum triglyceride, serum fasting insulin, HOMA-IR, and serum IL-18 levels). Means of continuous variables among different groups were compared using the ANOVA, and post hoc comparisons were performed with the Bonferroni test. χ^2 test was adopted to compare categorical variables. Coefficients of correlation were calculated using Pearson's method. Multivariate linear regression models were created to find out the independent variables that related to

circulating IL-18 concentrations. Two-tailed *P* value of less than 0.05 was considered statistically significant.

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Conflict of interest statement No conflicts of interest were declared.

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