The Review of DIABETIC STUDIES

ORIGINAL DATA

ENPP1 K121Q Polymorphism is not Related to Type 2 Diabetes Mellitus, Features of Metabolic Syndrome, and Diabetic Cardiovascular Complications in a Chinese Population

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■ Abstract

BACKGROUND: Ectoenzyme nucleotide pyrophosphate phosphodiesterase 1 (ENPP1) is known to influence insulin sensitivity by inhibiting insulin receptor signaling. A DNA polymorphism in the *ENPP1* gene at exon 4 (K121Q) was demonstrated to be associated with insulin resistance, type 2 diabetes mellitus (T2DM), and a risk of early myocardial infarction, albeit with controversy. Our aim was to investigate any association of *ENPP1* K121Q alleles with T2DM, features of the metabolic syndrome, and diabetic cardiovascular complications in a Chinese population of Han origin. METHODS: The *ENPP1* K121Q polymorphism was determined by a restriction fragment-length polymorphism-polymerase chain reaction in 1,862 patients with T2DM and 844 non-diabetic subjects. RESULTS: The genotype distributions or Q-allele frequency were not statistically different between the diabetic and non-diabetic groups. The anthropometric parameters, systolic and diastolic blood pressures, lipid profiles, and serum creatinine levels of subjects with different *ENPP1* K121Q polymorphisms were not statistically different in the two groups or even in the pooled data. When sub-group analyses of diabetic subjects were stratified according to BMI levels (greater or less than 27), gender, age of diabetes onset (older or younger than 60 years), and the presence or absence of a diabetic family history; this polymorphism was still not associated with T2DM. Nor was the *ENPP1* K121Q polymorphism associated with the prevalence of coronary artery disease and ischemic cerebrovascular disease in patients with T2DM. CONCLUSION: The *ENPP1* K121Q polymorphism is not related to T2DM, features of the metabolic syndrome, or diabetic macrovascular complications in a Chinese population.

Keywords: ENPP1 · T2DM · metabolic syndrome · coronary artery disease · cerebrovascular disease

Introduction

The ectoenzyme nucleotide pyrophosphate phosphodiesterase 1 (ENPP1, also know as plasma cell membrane glycoprotein 1, or PC-1) is a class II membrane glycoprotein that adversely influences insulin sensitivity by inhibiting insulin receptor signaling [1, 2]. In addition to plasma cells, ENPP1 is widely expressed in the muscle, liver, renal tubules, salivary duct epithelium, epididymis, adipose tissue, chondrocytes, pancreas, capillary endothelium in the brain, and the kidneys [3]. The physiologic functions of ENPP1 in these tissues are still relatively unknown. However, it has been found that over-expression of ENPP1 inhibits insulin receptor tyrosine kinase activity and subsequent cellular signaling in various cells [4, 5]. The ENPP1/PC-1 content is greater in cultured skin fibroblasts, skeletal muscle, and fat cells from insulin-

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resistant subjects [6-8]; moreover, the amount of ENPP1 over-expression directly correlates with the level of insulin resistance [6, 7]. A functional missense DNA polymorphism in exon 4 that causes an amino acid change from lysine to glutamine at codon 121 (K121Q) has been described [9]. Studies in vitro have shown that the Q variant of *ENPP1* has a stronger interaction with the insulin receptor than with the K variant and reduces insulin receptor autophosphorylation [10]. It is therefore a stronger inhibitor of insulin signaling and has been associated with insulin resistance in Sicilians [9], South Asians [11], and Finns and Swedes [12, 13]. Furthermore, Q variant carriers are associated with obesity in Caucasians and African-Americans [14, 15], and early onset of coronary artery disease in Caucasians [16, 17]. However, the role of the *ENPP1* 121Q allele on the pathogenesis of insulin resistance in other ethnic groups remains controversial [18-20].

Conflicting results have also been reported about the effect of 121Q variance on the risk for T2DM [9, 12, 15, 17, 18, 21-24]. Since there is still no data associating *ENPP1* K121Q polymorphism with the risk of T2DM and diabetic cardiovascular diseases among ethnic Chinese, this present study was designed to investigate whether a genetic variation of the *ENPP1* gene could be associated with components of metabolic syndrome, T2DM, and diabetic coronary artery disease and ischemic cerebrovascular disease in Chinese subjects living in Taiwan.

Subjects and Methods

Subjects

We studied 1,862 patients with T2DM, who entered a disease management program at the Diabetic Clinic of Pingtung Christian Hospital from January 2002 to June 2004; the program was being administrated by Taiwan's Bureau of National Health Insurance [25, 26]. Diagnosis of T2DM was based upon WHO criteria [27]. Patients presenting symptoms suggestive of type 1 diabetes – defined as diabetic ketoacidosis, acute presentation with heavy ketonuria (3+), or a continuous requirement of insulin within 1 year of diagnosis – were excluded [28].

We recruited 844 non-diabetic subjects without clinical evidence of major diseases from an unselected population. The subjects underwent routine medical check-ups and were enrolled as the control group. The selection criteria for a non-diabetic were subjects older than 50 years who had fasting plasma glucose levels lower than 100 mg/dl and no family history of T2DM. This case-control study was approved by the human research ethics committee of our hospital, and informed consent was obtained from each patient.

All of the study subjects were of Han Chinese origin and all lived in the same region at the time of the study. All of the patients underwent complete physical examinations and routine biochemical analyses of blood and urine, as well as an assessment of the presence and extent of macrovascular or microvascular complications. Anthropometric parameters that were measured included the body mass index (BMI) and the waist-to-hip ratio (WHR). Blood pressures were taken by a trained nurse with a digital automatic blood pressure monitor (Omron, model HEM-907, Omron, Japan) in a seated position after the subjects had rested for 5 minutes.

Plasma biochemical parameters were also measured after overnight fasting including triglycerides, total cholesterol, low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), uric acid, creatinine, and glucose. These were measured using standard commercial methods on a parallel, multichannel analyzer (Hitachi 7170A, Tokyo, Japan) as done in previous reports [26].

Ischemic cerebrovascular disease (CVD) was defined as acute or sudden focal neurological defects lasting more than 24 hours, and positive brain lesion images by computed tomography, magnetic resonance imaging, or magnetic resonance angiography examination. Patients with CVD were all enrolled in an outpatient disease management program and all were clinically stable and had no manifestations of acute illness [29]. The diagnosis of coronary artery disease (CAD) was based on the documented history of significant coronary artery stenosis (>75%) in one or more major coronary arteries, myocardial infarction, coronary angioplasty, or a coronary artery bypass operation [30].

ENPP1 gene K121Q polymorphism

Genomic DNA was prepared from peripheral blood using standard techniques. The K121Q polymorphism of the *ENPP1* gene in individuals from Italy was determined as previously described [9]. Exon 4 amplimers were obtained using the following primer pair: 4-forward, 5-GCAATTCTGTGTTCACTTT GGA-3; and 4-reverse, 5-GAGCACCTGACCTTGA CACA-3. The PCR was carried out in a final volume of 25 µl, containing 100 ng genomic DNA, 1.5 mmol/l $MgCl_2$, 0.2 mmol/l of each deoxynucleotide triphosphate, 0.5 pmol of each primer, and 0.05 units of *Taq* polymerase (Takara Tag™, Takara Shuzo Co., Ltd, Otsu Shiga, Japan).

Table 1. *ENPP1* gene K121Q genotype distributions in control subjects and type 2 diabetic patients

Genotype	T2DM		Controls		WFH	WOFH#	BMI≥27	BMI<27	Male	Female	ADO≥60	ADO<60
	n	%	n	%	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
KK	681	80.7	1515	81.4	82.6	79.9	81.8	81.2	80.8	81.8	81.9	81.2
KQ	155	18.4	333	17.9	17.0	19.1	17.8	17.9	18.6	17.3	17.5	18.0
QQ	8	0.9	14	0.8	0.4	1.0	0.4	0.9	0.6	0.9	0.6	0.8
KQ+QQ	163	19.3	347	18.6	17.4	20.1	18.2	18.8	19.2	18.2	18.1	18.8
Total (n)	844		1862		997	726	564	1298	838	1024	477	1385
OR (95% CI)*	0.96 (0.78-1.18)				0.88 (0.69-1.11)	1.05 (0.82-1.35)	0.98 (0.61-1.57)	0.96 (0.76-1.22)	0.88 (0.65-1.19)	1.03 (0.77-1.37)	0.92 (0.69-1.24)	0.96 (0.78-1.20)

Legend: n: number of subjects in the study group. WFH: with family history. WOFH: without family history. ADO: age of diabetes onset. BMI: body mass index. OR: odds ratio. CI: confidence interval. * KK vs. KQ+QQ. # 139 individuals with unknown family history of diabetes were excluded from calculation.

After an initial denaturation of 2 min at 94°C, the samples underwent 30 cycles at 94°C for 1 min, 55°C for 40 seconds, and 72°C for 40 seconds, with a final extension of 10 mins at 72°C in a thermal cycler (Gene Amp PCR System 9700, Perkin-Elmer, Foster City, CA). The 208-bp product was restricted with *Ava*II for 2 hours at 37°C. The unrestricted 208-bp product represents the K allele, while the Q allele was cut into 53- and 155-bp fragments. The three genotypes were scored after running on a 2.0% agarose gel and staining with ethidium bromide. To assure that the genotyping was of sufficient quality, we performed random duplicates in over 10% of the samples, and carried out controls from carriers and non-carriers in each genotyping assay. A technician who was blinded to the phenotype performed the assays. No genotype errors were detected in the random duplicates.

Statistical analysis

The data are shown as the mean ± SD. The normal distribution and homogeneity of variance were tested before further statistical analyses. The χ^2 test was used to assess the deviations from the Hardy-Weinberg equilibrium for genotype frequencies, and it was also used for comparison of the allele and genotype frequencies between different study groups. The odds ratios (OR) and 95% confidence intervals (CI) were calculated by logistic regression analysis.

Since there are only a small number of individuals with the QQ genotype, we tested whether variable means differed significantly between subjects with and without the Q variant (KK vs. KQ and QQ). Separate analyses were performed in subgroups of diabetic patients defined by their gender, BMI (BMI < 27 or ≥ 27), age at diagnosis of diabetes (<60 or ≥60 years old), and family history of T2DM (yes or no). A comparison of variables between the groups of genotypes was performed using ANOVA or two-tailed Student's *t*-test.

Before statistical testing, fasting plasma glucose and serum triglycerides were logarithmically transformed to achieve a normal distribution. Differences were considered statistically significant with a p-value < 0.05. All statistical analyses were performed using the Statistical Package for Social Science program (SPSS for Windows, version 10.0, SPSS Inc, Chicago, IL). A meta-analysis of case-control studies published on PubMed before February 2006 on the frequency of *ENPP1* X121Q (K121Q+Q121Q) sought to estimate the difference in risk for patients with diabetes vs. those without diabetes. The studies were combined using inverse variance weights, and the Q statistic was used to test for heterogeneity. These computations were made using Comprehensive Meta-Analysis version 2.0 (Biostat, Englewood, NJ).

Results

Tables A1A, A1B and A1C compares the clinical characteristics of control and diabetic subjects based on the genotype. Because we enrolled subjects older than 50 years as non-diabetic controls to ensure this group is less prone to diabetes and relative insulin sensitivity, the mean ages for control subjects (64.5 ± 9.5 yr) are older than for the diabetic subjects (62.2 ± 12.1 yr, p < 0.001) as expected. Diabetic subjects had significantly higher BMI, serum creatinine levels, and variables for metabolic syndrome, such as WHR, fasting glucose, systolic and diastolic blood pressures, and serum triglyceride levels than the controls. They exhibited lower total cholesterol, and HDL- and LDL-cholesterol levels than the controls. The anthropometric parameters, systolic and diastolic blood pressures, lipid profiles, and serum creatinine levels among the

subjects with different *ENPP1* K121Q polymorphisms were not statistically different among control and diabetic subjects and even in the pooled data (all $p > 0.05$, Tables A1A, A1B and A1C).

In both groups we studied, the genotype distributions of this polymorphism were in Hardy-Weinberg equilibrium. The *ENPP1* K121Q genotype distributions or allele frequency were not significantly different between the diabetic and control groups (Table 1). To identify possible relationships pertaining only to specific subgroups, we performed a series of sub-analyses in diabetic patients based on BMI levels (greater or less than 27), gender, age at diabetic onset (older or younger than 60 years) and the presence or absence of a diabetic family history. *ENPP1* K121Q polymorphism was still not associated with T2DM (Table 1).

not statistically different among diabetic patients with or without macrovascular complications. In our subgroup analysis based on smoking status, *ENPP1* K121Q polymorphism was still not associated with a higher risk of CAD or CVD in smokers or non-smokers with T2DM (data not shown).

We performed a MEDLINE search of publications before February 2006 for ENPP1 or PC-1 K121Q polymorphism and type 2 diabetes. The search was limited to articles published in English. There were 9 case-control studies published in the literature [9, 12, 15, 17, 18, 21-24], and only one article [24], which did not show detailed genotype distribution, was excluded from our meta-analysis. We summarized the results of the published studies (including this present one) on the role of polymorphism in risk for T2DM in Table 2.

Table 2. Studies on the association between ENPP1 K121Q polymorphism and type 2 diabetes

Authors	Population	T2DM (KK/KQ+QQ)*	Controls (KK/KQ+QQ)*	OR	95% CI	Q allele carrier (%)
Pizzuti, *et al.*	Caucasian, Sicilian	132 (81/51)	121 (80/41)	1.23	0.73 – 2.05	36.4
Gu, *et al.*	Caucasian, Finnish, Swedish	392 (304/88)	147 (110/37)	0.86	0.55 – 1.34	23.2
Rasmussen, *et al.*	Caucasian, Danish	404 (299/105)	593 (428/165)	0.91	0.68 – 1.21	24.0
Kubaszek, *et al.*	Caucasian, Finnish	94 (66/28)	394 (305/89)	1.45	0.88 – 2.40	81.2
Hamaguchi, *et al.*	Dominican	358 (66/292)	397 (97/300)	1.43	1.01 – 2.03	78.4
Abate, *et al.*	Caucasian, American	141 (86/55)	717 (530/187)	1.81	1.24 – 2.64	28.2
Abate, *et al.*	South Asian Indian	223 (148/75)	456 (344/112)	1.56	1.10 – 2.21	27.5
Abate, *et al.*	South Asian Indian	121 (67/54)	962 (646/316)	1.65	1.12 – 2.42	34.2
Bacci, *et al.*	Caucasian, Italian	969 (671/298)	638 (463/175)	1.18	0.94 – 1.47	29.4
Matsuoka, *et al.*	Caucasian	40 (31/9)	629 (402/227)	0.51	0.24 – 1.10	35.3
Matsuoka, *et al.*	African-American	30 (1/29)	312 (112/200)	16.24	2.18 – 120.34	67.0
Current study	Chinese	1862 (1515/347)	844 (681/163)	0.94	0.75 – 1.16	18.8
All studies		4766(3335/1431)	6210(4198/2012)	1.18	1.06 – 1.30	

Legend: OR: odds ratio. CI: confidence interval. * number of subjects in the study group (number of subjects carrying alleles KK in relation to the number of subjects carrying alleles KQ and QQ).

Tables A2A, A2B and A2C show diabetic subjects stratified according to the presence or absence of coronary artery and ischemic cerebrovascular disease. For our disease-free control group, we recruited 378 patients known to have had diabetes for over 10 years, who were free of cardiovascular disease and had a similar distribution of age, gender, and known diabetic durations. *ENPP1* K121Q genotype distributions were

A total of 4,766 type 2 diabetic subjects and 6,210 control subjects were collected. The pooled OR of *ENPP1* K121Q/Q121Q was 1.18 with a 95% CI ranging from 1.06 to 1.30 ($p = 0.002$) associated with T2DM across various populations. The test for heterogeneity was significant ($Q = 32.36$, $df = 11$, $p < 0.001$) as evidence of differences among the various published studies.

Discussion

Our results show that the genotype distributions of *ENPP1* K121Q polymorphism were not associated with T2DM or any features of metabolic syndrome in a Chinese population. Subgroup analysis of diabetic patients according to their gender, age at diabetes onset, obesity status, or presence or absence of diabetic family history, did not disclose any association between *ENPP1* K121Q polymorphism and T2DM. Nor was *ENPP1* K121Q polymorphism associated with diabetic macrovascular complications in this large case-controlled study.

The *ENPP1* gene K121Q polymorphism is a functional missense [9], and the 121Q variant binds insulin receptors more strongly than the 121K variant [10]. There is, therefore, an association of this polymorphism with insulin resistance and related metabolic abnormalities have been reported [9, 11-17]. Yet, not all reports confirm the association of *ENPP1* K121Q polymorphism with insulin resistance [18-20], and, indeed, conflicting results have been reported about the effect of the *ENPP1* 121Q variant on the risk of developing T2DM [9, 12, 15, 17, 18, 21-24]. Although the meta-analysis of the association between *ENPP1* K121Q polymorphism and the risk for T2DM revealed significant positive results (Table 2), only three studies [15, 22, 23] demonstrated significant associations, including the test of heterogeneity ($p < 0.001$), which indicated the differences among the various published studies.

The frequency of the 121Q allele carriers in the ethnic Chinese of our study was 18.8%. This was lower than among Caucasians (23.2% - 36.4%), South Asian Indians (27.5% - 34.2%), African-Americans (67.0%), and Dominicans (78.4%) (Table 2). The average BMI of our diabetic subjects was 25.0 ± 3.4 in males and 25.8 ± 4.0 in females and was lower than in Caucasians. Clearly, there must be other genetic and/or environmental factors beyond ENPP1 that account for the ethnic differences in susceptibility to T2DM. However, whether genetic epistasis does in fact modulate human insulin sensitivity is not known. From a single gene perspective, the effects of a genetic variation on insulin resistance on clinical overt hyperglycemia appear to be very small [31].

In the study of Abate and colleagues, significant association between *ENPP1* K121Q polymorphism and genetic susceptibility to T2DM was observed in South Asian Indian and Caucasian populations. However, the control subjects used in this study were too young (42-46 vs. 53-59 years old for controls and diabetics respectively) to provide convincingly strong evidence to draw a conclusion.

The frequency of the Q allele carrier in Dominicans (78.4%) and African-Americans (67.0%) were markedly higher than in other ethnic groups reported [15, 23]. Whether the high prevalence of Q allele in this population could explain the high prevalence of insulin resistance and T2DM in these populations [32] is an interesting topic that requires further study. Our results implying the absence of an association between *ENPP1* 121Q variants and genetic susceptibility of T2DM in an ethnic Chinese population are consistent with most existing reports, indicating that such associations can be context-dependent and population-specific [33, 34].

Our results also failed to show any association between coronary artery disease and ischemic cerebrovascular disease in type 2 diabetic subjects with the Q variant. In contrast, there are reports showing that K121Q polymorphism of the *ENPP1* gene is associated with an earlier onset of myocardial infarction in diabetic and non-diabetic subjects [16, 17] in a Caucasian population. The study of racial and ethnic differences in disease and the detection of risk factor levels must be based on solid hypotheses that can evaluate the interaction of lifestyle and possible genetic attributes. Differences in constitutional factors, diet, physical activity, age, gender, nutrition status, and personal habits all influence the prevalence and susceptibility of both insulin resistance and atherosclerotic diseases [35, 36]. Our findings indicate that a single genetic polymorphism of an insulin signaling gene is not enough to induce the development of complex diseases like atherosclerosis.

Limitations of our study include the cross-sectional design of this study, which prohibited us from concluding any causal relationship between the *ENPP1* gene K121Q polymorphism and T2DM or in diabetic complications. Further investigations by large-scale and cohort studies may be needed to confirm the role of *ENPP1* in the pathogenesis of T2DM and its complications.

In conclusion, our data indicates that the *ENPP1* gene K121Q polymorphism is not related to T2DM and diabetic macrovascular complications in an ethnic Chinese population.

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■ Appendix

Table A1A. Clinical characteristics of patients with type 2 diabetes

Parameter	Genotype			p
	KK	KQ + QQ	Total	
n	1515	347 (333+14)	1862	
Age (yr)	62.0 ± 12.3	62.8 ± 11.1	62.2 ± 12.1	0.268
Gender (M/F)	677/838	161/186	838/1024	0.563
BMI (M)	24.9 ± 3.4	25.2 ± 3.2	25.0 ± 3.4	0.407
BMI (F)	25.9 ± 4.0	25.3 ± 3.8	25.8 ± 4.0	0.058
WHR (M)	0.95 ± 0.06	0.95 ± 0.07	0.94 ± 0.06	0.421
WHR (F)	0.94 ± 0.09	0.95 ± 0.09	0.94 ± 0.09	0.233
Fasting glucose (mmol/l)	9.5 ± 3.5	9.8 ± 3.5	9.5 ± 3.5	0.148
HbA1c (%)	8.5 ± 2.2	8.6 ± 2.2	8.5 ± 2.2	0.458
Systolic BP (mmHg)	143.8 ± 22.9	143.7 ± 23.0	143.8 ± 23.0	0.898
Diastolic BP (mmHg)	85.1 ± 13.1	84.6 ± 12.9	85.0 ± 13.1	0.512
T-cholesterol (mmol/l)	5.2 ± 1.2	5.2 ± 1.1	5.2 ± 1.2	0.332
Triglyceride (mmol/l)	1.9 ± 2.8	1.8 ± 1.7	1.9 ± 2.6	0.679
HDL-cholesterol (mmol/l)	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	0.661
LDL-cholesterol (mmol/l)	3.2 ± 0.9	3.2 ± 0.9	3.2 ± 0.9	0.453
Creatinine (μmol/l)	88.4 ± 53.0	97.2 ± 88.4	88.4 ± 61.9	0.509

Legend: Data are mean ± SD. n: number of subjects in the study group. p: KK vs. KQ/QQ subjects by unpaired Student's *t*-test. M: male. F: female. WHR: waist to hip ratio. BMI: body mass index. BP: blood pressure.

Table A1B. Clinical characteristics of control subjects

Parameter	Genotype			p1	p2
	KK	KQ + QQ	Total		
n	681	163 (155+8)	844		
Age (yr)	64.5 ± 9.5	64.5 ± 9.4	64.5 ± 9.5	0.948	< 0.001
Gender (M/F)	308/373	82/81	390/454	0.243	0.705
BMI (M)	23.7 ± 3.1	24.3 ± 3.5	23.8 ± 3.2	0.194	< 0.001
BMI (F)	24.2 ± 3.4	24.3 ± 3.4	24.2 ± 3.4	0.930	< 0.001
WHR (M)	0.91 ± 0.08	0.91 ± 0.09	0.91 ± 0.09	0.601	< 0.001
WHR (F)	0.97 ± 0.08	0.86 ± 0.08	0.87 ± 0.08	0.376	< 0.001
Fasting glucose (mmol/l)	5.2 ± 0.3	5.2 ± 0.3	5.2 ± 0.3	0.630	< 0.001
HbA1c (%)	nd	nd	nd		
Systolic BP (mmHg)	131.5 ± 20.3	128.4 ± 20.3	130.9 ± 20.3	0.078	< 0.001
Diastolic BP (mmHg)	76.9 ± 12.3	79.3 ± 12.2	76.8 ± 12.2	0.578	< 0.001
T-cholesterol (mmol/l)	5.4 ± 1.0	5.3 ± 1.1	5.4 ± 1.0	0.426	< 0.001
Triglyceride (mmol/l)	1.2 ± 0.8	1.2 ± 0.7	1.2 ± 0.7	0.867	< 0.001
HDL-cholesterol (mmol/l)	1.3 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	0.274	< 0.001
LDL-cholesterol (mmol/l)	3.4 ± 0.9	3.4 ± 1.0	3.4 ± 0.9	0.968	< 0.001
Creatinine (μmol/l)	86.6 ± 42.4	84.9 ± 23.9	86.6 ± 39.8	0.500	0.015

Legend: Data are mean ± SD. n: number of subjects in the study group. p1: KK vs. KQ/QQ subjects by unpaired Student's *t*-test. p2: comparisons of variables between diabetic and non-diabetic groups adjusted by gender, age, and BMI. M: male. F: female. WHR: waist to hip ratio. BMI: body mass index. BP: blood pressure. nd: not done.

Table A1C. Clinical characteristics of control subjects and patients with type 2 diabetes

Parameter	Genotype			p
	KK	KQ + QQ	Total	
n	2196	510	2706	
Age (yr)	63.6 ± 17.3	64.0 ± 10.7	63.7 ± 16.3	0.654
Gender (M/F)	985/1211	243/267	1228/1478	0.237
BMI (M)	24.6 ± 3.4	24.9 ± 3.3	24.6 ± 3.4	0.217
BMI (F)	25.4 ± 3.9	25.0 ± 3.7	25.3 ± 3.9	0.129
WHR (M)	0.94 ± 0.06	0.94 ± 0.07	0.94 ± 0.06	0.903
WHR (F)	0.93 ± 0.09	0.93 ± 0.10	0.93 ± 0.09	0.766
Fasting glucose (mmol/l)	8.1 ± 3.5	8.3 ± 3.6	8.2 ± 3.5	0.317
HbA1c (%)				
Systolic BP (mmHg)	140.0 ± 22.9	138.8 ± 23.2	139.8 ± 22.9	0.290
Diastolic BP (mmHg)	82.6 ± 13.4	82.0 ± 13.3	82.5 ± 13.4	0.362
T-cholesterol (mmol/l)	5.3 ± 1.2	5.2 ± 1.1	5.3 ± 1.1	0.229
Triglyceride (mmol/l)	1.7 ± 2.4	1.6 ± 1.5	1.7 ± 2.2	0.679
HDL-cholesterol (mmol/l)	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	0.826
LDL-cholesterol (mmol/l)	3.3 ± 0.9	3.2 ± 0.9	3.3 ± 0.9	0.571
Creatinine (µmol/l)	88.4 ± 53.0	88.4 ± 70.7	88.4 ± 53.0	0.733

Legend: Data are mean ± SD. n: number of subjects in the study group. p: KK vs. KQ/QQ subjects by unpaired Student's *t*-test. M: male. F: female. WHR: waist to hip ratio. BMI: body mass index. BP: blood pressure.

Table A2A. Clinical characteristics of patients with type 2 diabetes and with coronary artery disease

Parameter	Coronary artery disease			p
	KK	KQ + QQ	Total	
n	104	22	126	
Age (yr)	69.7 ± 9.4	71.5 ± 8.2	70.0 ± 9.2	0.431
Gender (M/F)	55/49	12/10	67/59	0.887
Diabetic duration (yr)	14.1 ± 7.6	14.6 ± 9.5	14.2 ± 7.9	0.799
BMI	25.4 ± 3.0	24.7 ± 3.4	25.3 ± 3.1	0.320
WHR	0.96 ± 0.08	0.99 ± 0.08	0.97 ± 0.08	0.133
Fasting glucose (mmol/l)	9.6 ± 3.5	9.8 ± 3.1	9.7 ± 3.4	0.813
HbA1c (%)	8.4 ± 1.9	8.7 ± 2.6	8.5 ± 2.1	0.514
Systolic BP (mmHg)	152.8 ± 21.0	150.6 ± 23.1	152.4 ± 21.3	0.659
Diastolic BP (mmHg)	87.6 ± 12.8	84.9 ± 15.5	87.1 ± 13.3	0.384
T-cholesterol (mmol/l)	5.3 ± 1.2	5.6 ± 1.5	5.4 ± 1.3	0.298
Triglyceride (mmol/l)	2.1 ± 1.5	2.0 ± 1.3	2.0 ± 1.4	0.734
HDL-cholesterol (mmol/l)	1.1 ± 0.3	1.2 ± 0.4	1.1 ± 0.3	0.457
LDL-cholesterol (mmol/l)	3.2 ± 0.9	3.6 ± 1.3	3.3 ± 1.0	0.206
Genotype (n, %)*	104 (82.5)	22 (17.5)	126 (100)	

Legend: Data are mean ± SD. n: number of subjects in the study group. p: KK vs. KQ/QQ subjects by unpaired Student's *t*-test. WHR: waist to hip ratio. BMI: body mass index. BP: blood pressure. * KK vs. KQ/QQ.

Table A2B. Clinical characteristics of patients with type 2 diabetes and with cerebrovascular disease

Parameter	Cerebrovascular disease			p
	KK	KQ + QQ	Total	
n	102	17	119	
Age (yr)	69.3 ± 9.5	69.7 ± 11.9	69.4 ± 9.8	0.895
Gender (M/F)	42/60	6/11	48/71	0.647
Diabetic duration (yr)	14.4 ± 8.5	17.3 ± 8.9	14.8 ± 8.6	0.201
BMI	24.5 ± 2.7	24.2 ± 2.7	24.4 ± 2.6	0.780
WHR	0.96 ± 0.06	0.96 ± 0.04	0.96 ± 0.05	0.889
Fasting glucose (mmol/l)	9.8 ± 3.9	9.5 ± 3.4	9.7 ± 3.8	0.812
HbA1c (%)	8.4 ± 1.9	8.6 ± 1.5	8.4 ± 1.8	0.728
Systolic BP (mmHg)	151.0 ± 22.8	160.5 ± 21.2	152.3 ± 22.8	0.109
Diastolic BP (mmHg)	86.1 ± 14.3	93.5 ± 15.8	87.1 ± 14.7	0.054
T-cholesterol (mmol/l)	5.5 ± 1.4	5.5 ± 1.5	5.5 ± 1.4	0.967
Triglyceride (mmol/l)	2.1 ± 1.4	2.3 ± 1.7	2.1 ± 1.4	0.638
HDL-cholesterol (mmol/l)	1.1 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	0.785
LDL-cholesterol (mmol/l)	3.3 ± 1.1	3.4 ± 1.1	3.3 ± 1.1	0.720
Genotype (n, %)*	102 (85.7)	17 (14.3)	119 (100)	

Legend: Data are mean ± SD. n: number of subjects in the study group. p: KK vs. KQ/QQ subjects by unpaired Student's *t*-test. WHR: waist to hip ratio. BMI: body mass index. BP: blood pressure. * KK vs. KQ/QQ.

Table A2C. Clinical characteristics of patients with type 2 diabetes and without macrovascular diseases

Age (yr)	Non-macrovascular disease			p1	p2	p3
	KK	KQ + QQ	Total			
n	299	79	378			
Age (yr)	69.7 ± 6.9	69.7 ± 6.5	69.7 ± 6.8	0.943	0.678	0.648
Gender	135/164	33/46	168/210	0.591	0.089	0.430
Diabetic duration (yr)	14.8 ± 3.9	15.2 ± 3.6	14.9 ± 3.8	0.506	0.154	0.865
BMI	24.8 ± 3.3	25.0 ± 3.4	24.9 ± 3.4	0.658	0.207	0.158
WHR	0.96 ± 0.07	0.97 ± 0.08	0.96 ± 0.08	0.503	0.382	0.505
Fasting glucose (mmol/l)	10.0 ± 3.1	10.1 ± 3.8	10.0 ± 3.3	0.740	0.296	0.412
HbA1c (%)	8.8 ± 2.2	8.6 ± 2.1	8.8 ± 2.2	0.440	0.194	0.112
Systolic BP (mmHg)	148.6 ± 22.7	147.3 ± 19.5	148.3 ± 22.1	0.643	0.007	0.088
Diastolic BP (mmHg)	85.0 ± 12.9	85.1 ± 11.8	85.0 ± 12.7	0.917	0.110	0.123
T-cholesterol (mmol/l)	5.1 ± 1.0	5.1 ± 1.3	5.2 ± 1.1	0.698	0.066	0.010
Triglyceride (mmol/l)	1.8 ± 1.3	1.8 ± 1.2	1.8 ± 1.3	0.999	0.068	0.014
HDL-cholesterol (mmol/l)	1.2 ± 0.3	1.2 ± 0.1	1.2 ± 0.3	0.591	0.146	0.206
LDL-cholesterol (mmol/l)	3.2 ± 0.8	3.1 ± 1.0	3.2 ± 0.9	0.191	0.168	0.119
Genotype (n, %)*	299 (79.1)	79 (20.9)	378 (100)		0.404	0.111

Legend: Data are mean ± SD. n: number of subjects in the study group. p1: KK vs. KQ/QQ subjects by unpaired Student's *t*-test. p2: clinical characteristics of between CAD and non-macrovascular complication by unpaired Student's *t*-test. p3: Clinical characteristics of between CVA and non-macrovascular complications by unpaired Student's *t*-test. WHR: waist to hip ratio. BMI: body mass index. BP: blood pressure. * KK vs. KQ/QQ.

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