Original Article

KCNQ1 Variants Associate with Type 2 Diabetes in Malaysian Malay Subjects

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Abstract

Introduction: Type 2 diabetes (T2D) candidate gene: potassium voltage-gated channel, KQT-like subfamily, member 1 (KCNQ1) was suggested by conducting a genome wide association study (GWAS) in Japanese population. Association studies have been replicated among East Asian populations; however, the association between this gene and T2D in Southeast Asian populations still needs to be studied. This study aimed to investigate the association of KCNQ1 common variants with type 2 diabetes in Malaysian Malay subjects. Materials and Methods: The KCNQ1 single nucleotide polymorphisms (SNPs): rs2237892, rs2283228, and rs2237895 were genotyped in 234 T2D and 177 normal Malay subjects. Results: The risk allele of the rs2283228 (A) was strongly associated with T2D (OR = 1.7, *P* = 0.0006) while the rs2237892 (C) was moderately associated with T2D (OR = 1.45, P = 0.017). The recessive genetic models showed that rs2283228 was strongly associated with T2D (OR = 2.35, *P* = 0.00005) whereas rs2237892 showed a moderate association with T2D (OR = 1.69, *P* = 0.01). The haplotype block (TCA), which contained the protective allele, correlated with a protection from T2D (OR = 0.5, *P* = 0.003). Furthermore, the diplotype (CAA-TCA) that contained the protective haplotype was protected against T2D (OR = 0.46, *P* = 0.006). Conclusion: The KCNQ1 SNPs, haplotypes and diplotypes are associated with T2D in the Malaysian Malay subjects.

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Key words: Diplotypes, Haplotypes, KCNQ1, SNPs, Type 2 diabetes

Introduction

Diabetes is the most common metabolic disease that affects 246 million people worldwide. The International Diabetes Federation (IDF) predicts that the total number of people living with diabetes will increase to 380 million within the next 20 years.[1] Diabetes, mostly type 2 diabetes mellitus (T2D), now affects 5.9% of the world's adult population, with almost 80% of patients originating from developing countries.[2] More than 1.3 million Malaysians have diabetes and the IDF has predicted that this number would be doubled by 2025.

A new approach known as genome-wide association (GWAS) study has been applied to complex diseases including T2D and has resulted in the identification of a growing number of trait susceptibility loci for T2D.[3] Two independent GWAS studies have identified a novel T2D susceptibility gene: potassium voltage-gated channel, KQT-like subfamily, member 1 (KCNQ1) in East Asian subjects.[4,5] Very recently, 2 GWAS studies on Chinese Han and European populations confirmed KCNQ1 as T2D susceptibility gene.[6,7] The association of T2D with KCNQ1 variants was replicated in studies among Chinese,[8-10] Singaporeans,[11,12] Indians[13] and in some Euro-Caucasians.[4,14,15]

The KCNQ1 gene contains 16 exons and spans more than 404 kb on chromosome 11p15.5, and encodes the pore-forming alpha subunit of the voltage-gated K+ channel (KvLQT1) which plays an important role in controlling the repolarisation process of the ventricles.[16,17] *KCNQ1* is ubiquitously expressed in epithelial cells, including the endocrine and exocrine pancreas.[18] *KCNQ1* was reported

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to be expressed in insulin-secreting cells, and inhibition of this potassium channel significantly increased insulin secretion.[19]

This research focused on the association of single nucleotide polymorphisms (SNPs) and haplotypes of the KCNQ1 gene with type 2 diabetes mellitus in Malaysian Malays subjects.

Materials and Methods

Subjects and Data Collection

T2D Malay subjects aged between 30 and 70 years who attended the University Malaya Medical Centre (UMMC), Kuala Lumpur for treatment, were approached and asked to participate in this study. For the control group, physically normal Malay subjects who attended the UMMC for medical checkups were recruited. Venous blood samples (10 ml) were collected from each subject after obtaining an informed consent form.

Biochemical Analyses

Glucose, triglyceride, total cholesterol and high-density lipoprotein cholesterol (HDLc) were measured by an automated analyzer Dimension® RxL Max® Integrated Chemistry System, and insulin was measured by an ADVIA Centaur Assay XP Immunoassay System (Siemens Healthcare Diagnostics Inc. Deerfield, IL USA) Insulin resistance was calculated using the Homeostasis Model Assessment (HOMA2) Calculator v2.2, which is available from the Oxford Center for Diabetes, Endocrinology and Metabolism. This program used fasting insulin and blood glucose measurements to calculate the insulin resistance.

Genetic Analyses

KCNQ1 SNPs: rs2237892, rs2283228, and rs2237895 were selected for genotypic analysis based research by Unoki et al and Yasuda et al who found that these SNPs were associated with T2D in Asian populations.[4,5] The SNP sequences were downloaded from the US National Library of Medicine website, and specific primers were designed for each SNP using FastPCR programme. DNA extraction was achieved through salt precipitation adapted from the Puregene method. All SNPs were amplified by thermocycler using a 96 microwell plate (Real-Time PCR Systems, StepOnePlus Applied Biosystems Inc, Foster City, CA, USA). The KCNQ1 SNPs: rs2237895, rs2283228, and rs2237892 were genotyped by SmaI, BstNI and BsoBI restriction enzymes respectively. Polyacrylamide gel electrophoresis (7%) was used for detection of these digested PCR products.

Statistical Analysis

HelixTree 7.0 SNP and Variation Suite for Genetic Statistics (SVS) was used to study the deviation of SNPs from the Hardy-Weinberg equilibrium and to study the linkage disequilibrium (LD) between SNPs and construct haplotypes and diplotypes of related SNPs. The association of SNP alleles with T2D was performed with the De Finetti program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). The other statistical analyses were carried out using SPSS version 11.5. The missing data were listwise deleted (when any of the variables were missing, the entire observation was omitted from the analysis). The associations of the KCNQ1 SNPs, recessive, dominant and additive genetic models and the association of haplotypes and diplotypes with T2D were evaluated by hierarchical logistic regression controlled for age and gender.

Results

Two hundred and thirty-four T2D and 177 normal Malay subjects signed the consent forms and donated blood. The demography and biochemical parameters of the subjects are depicted in Table 1. The T2D subjects had a higher age, they had a higher BMI, larger waist and waist to

Table 1. Demography and Biochemical Parameters

Parameters	Normal n = 177	Type 2 diabetes n = 234	*P* value
Gender (Female)	96	128	
(Male)	81	106	
Age (years)	44.9 ± 10.69	48.5 ± 7.51	0.0001
Body mass index BMI (kg/m^2)	27.9 ± 6.66	29.6 ± 5.38	0.004
Waist (cm)	91.6 ± 17.3	97.4 ± 12.4	0.0002
Hip (cm)	106 ± 12.3	106 ± 11.2	0.83
Waist/Hip ratio	0.865 ± 0.096	0.920 ± 0.074	1.1E-9
Systolic blood pressure (mmHg)	134 ± 19.0	140 ± 18.6	0.018
Diastolic blood pressure (mmHg)	83 ± 10.3	84 ± 10.0	0.35
Fasting insulin (pmol/L)	80.7 ± 59.7	132 ± 101	5.04E-10
Fasting glucose (mmo/L)	5.14 ± 0.61	8.85 ± 3.6	2.8E-38
Triglyceride (mmol/L)	1.46 ± 0.79	2.02 ± 1.23	4.9E-8
Total cholesterol (mmol/L)	5.38 ± 0.99	5.05 ± 1.31	0.004
HDL cholesterol (mmol/L)	1.38 ± 0.355	1.16 ± 0.26	1.8E-11
Insulin resistance (IR)	1.50 ± 1.08	2.67 ± 1.89	3.1E-14

hip ratio, higher systolic blood pressure, blood glucose, insulin, insulin resistance, triglyceride and lower HDLc than normal subjects, all of which were consequences of T2D. The total cholesterol was lower in T2D patients than in normal subjects, whereas no differences between control and T2D subjects were found regarding hip measurements and diastolic blood pressure.

The 3 SNPs included in this study did not deviate from the Hardy-Weinberg equilibrium (HWE) in normal Malay subjects, HWE $P > 0.05$. The risk allele frequencies of rs2237892, rs2283228 and rs2237895 in normal subjects were 0.67, 0.65 and 0.22 versus 0.75, 0.76 and 0.28 in diabetic patients respectively (Table 2). The risk allele of the rs2283228 (A) was strongly associated with T2D (OR = 1.7, $P = 0.0006$) while the rs2237892 (C) was moderately associated with T2D (OR = 1.45, $P = 0.017$). The association of T2D with the risk allele of rs2237895(C) was borderline significant (OR = 1.38, $P = 0.052$). The results showed that the recessive genetic models of rs2283228 and rs2237892 are risk factors for T2D in the Malaysian Malay subjects (OR = 2.35, $P = 0.00005$; OR = 1.67, $P = 0.01$) respectively. The additive genetic models of these SNPs also showed a risk for T2D (OR = 1.7, $P = 0.001$; OR = 1.4, $P = 0.018$) respectively (Table 2).

Three-SNP haplotype and diplotype block was identified with significant LD. This block was made up of 2237892, rs2283228 and rs2237895 (Fig. 1). The possible haplotypes for each individual were adjusted to more than 0.5 resulting in 8 haplotypes and 21 diplotypes. Furthermore, 5 haplotypes and 6 diplotypes (with frequencies in the total sample of more than 0.02) were analysed further for their association with T2D. The most common haplotype was CAC, which is composed of the risk allele of rs2237892 (CC), rs2283228 (AA), and rs2237895 (CC) was borderline associated with a higher risk for T2D (OR = 1.4, $P = 0.092$). In contrast, the haplotype TCA that contains the protective homozygous of rs2237892 (TT) and rs2283228 (CC) showed a strong protection from T2D (OR = 0.5, $P = 0.0033$) (Table 3). The diplotype CAA-CAC that contains the risk haplotypes block was associated with a higher risk for T2D (OR = 1.9, $P =$

Table 2. Association of the Recessive, Dominant and Additive Genetic Models with Type 2 Diabetes in Malaysian Malay Subjects

SNP	Group	Risk allele (frequency)	OR (95% CI)	*P* value	Genotype n(distribution %)			#Receive model		#Dominant model	
								OR (95% CI)	***P*** value	OR (95% CI)	***P*** value
		C			TT	CT	CC				
rs2237892	normal	(0.67)	1.45	0.017	21(11.9)	75(42.4)	81(45.7)	1.69	0.01	1.41	0.31
	diabetic	(0.75)	(1.07-1.96)		20(8.5)	79(33.8)	135(57.7)	(1.13-2.5)		(0.73-2.73)	
		A			CC	AC	AA				
rs2283228	normal	(0.65)	1.7	0.00062	21(11.9)	83(46.9)	73(41.2)	2.35	0.00005	1.36	0.35
	diabetic	(0.76)	(1.25-2.30)		21(9.0)	72(30.7)	141(60.3)	(1.56-3.54)		(0.71-2.61)	
		C			CC	AC	AA				
rs2237895	normal	(0.22)	1.38	0.052	8(4.5)	62(35.0)	107(60.5)	1.85	0.16	1.36	0.14
	diabetic	(0.28)	(0.99-1.90)		20(8.5)	91(38.9)	123(52.6)	(0.79-4.34)		(0.91-2.03)	

In the additive model shown in Table 2 (Con't), genotype of homozygote for the non-risk allele (0/0), heterozygote (1/0) and homozygote for the risk allele (1/1 were coded as 0, 1 and 2 respectively). The recessive model was defined as 1/1 vs1/0+0/0 and dominant model as 1/1+1/0 vs 0/0.
#; Controlled for age and gender.

Table 2. (Con't)

SNP	Group	#Additive model	
		OR (95% CI)	***P*** **value**
rs2237892	normal	1.4	0.018
	diabetic	(1.07-1.94)	
rs2283228	normal	1.7	0.001
	diabetic	(1.26-2.31)	
rs2237895	normal	1.3	0.08
	diabetic	(0.97-1.86)	

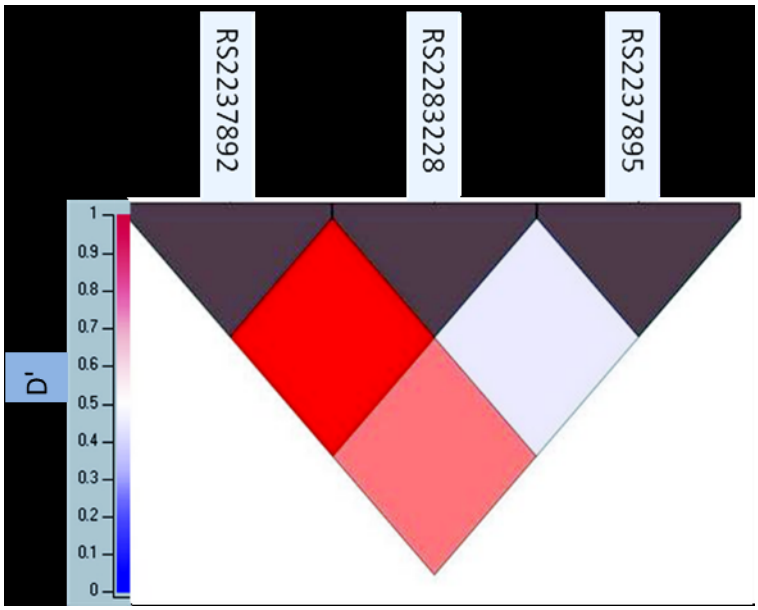

Fig. 1. Pairwise linkage disequilibrium among KCNQ1 SNPs in Malaysian Malays. Values in the upper represent KCNQ1 SNPs while values in the left represent D' value.

0.019). However, the diplotype CAA-TCA containing the risk haplotype (CAA) and the protective haplotype (TCA) was associated with protection from T2D (OR = 0.46, *P* = 0.0058) (Table 3).

Discussion

The rate of occurrence of the *KCNQ1* SNPs in the Asian populations was higher compared to the European populations. Our results showed that the frequencies of rs2237892, rs2283228 and rs2237895 among Malaysian Malays were similar to the Singaporean Chinese and the Malays.[11] This study found that rs2237892 was associated with T2D in Malaysian Malays, a finding that has been reported from previous studies on Japanese, Korean and Chinese subjects,[5] and this association was replicated on Chinese subjects.[8-10] However, the results reported from Singaporean studies were conflicting. Tan et al[11] reported the association between T2D and this SNP in Singaporean Chinese but not in Malays; however, the same group later reported that this SNP was associated with T2D in Malays but not in Chinese.[12] These contradictory results might be due to the small sample size of diabetic Malays in the first study compared to the second study (100 vs 1076). Rs2283228 was reported to be associated with diabetes

Table 3. Association of Common Haplotypes and Diplotypes with Type 2 Diabetes in Malaysian Malay Subjects

Haplotypes rs2237892, rs2283228, rs2237895	Frequency Normal n = 177	Frequency Type 2 diabetes n = 234	Odds ratio	95% confidence interval	*P* Value
Haplotypes					
CAC	0.35	0.43	1.43	0.94-2.15	0.09
TCA	0.32	0.20	0.5	0.31-0.79	0.003
CAA	0.19	0.24	1.36	0.83-2.23	0.22
CCA	0.06	0.04	0.73	0.29-1.88	0.52
TAA	0.04	0.05	1.48	0.56-3.89	0.43
Diplotypes					
CAA-CAA	0.19	0.224	1.36	0.83-2.23	0.22
CAA-CAC	0.14	0.22	1.91	1.11-3.27	0.02
CAA-TCA	0.22	0.13	0.46	0.27-0.80	0.006
TCA-CAC	0.13	0.12	0.87	0.47-1.59	0.65
TCA-TCA	0.07	0.05	0.74	0.31-1.73	0.48
CAC-CAC	0.03	0.07	2.34	0.83-6.60	0.11

Controlled for age and gender.

in Japanese and Danish,[4] but not in Singaporean Chinese subjects.[4,11] However, our study showed that this SNP was strongly associated with T2D in Malaysian, differing from the finding of Tan et al[11] in Singaporean Malays. These conflicting results might be due to the fact that the sample size of T2D in our study was double that of Tan's study.[11]

However, the association of T2D with haplotypes and diplotypes is incomparable in magnitude with that observed between T2D and individual SNPs; it confirms the association of T2D with the SNPs and conveys a higher risk for T2D than the one attributed to the single SNP in the Malaysian Malays.

The increased risk for T2D linked to the KCNQ1 gene is likely to be caused by a reduction in insulin secretion.[8-11] The pore-forming alpha subunit of the voltage-gated K+ channel (KvLQT1) (encoded by *KCNQ1*) and the regulatory beta subunit ISK (encoded by potassium channel, voltage-gated, ISK-related subfamily, member 1; KCNE1 gene) coassemble to form the I(KS) potassium channel in the pancreas.[20] Intrinsically, there is a possibility that KCNQ1 polymorphisms alter the role of the I(KS) potassium channel, causing decreased insulin secretion, leading in time to T2D.[11] However, homozygous Kcnq1-null mice have been reported not to show hyperglycaemia, or glucose intolerance, and the contribution of the KCNQ1 encoded protein to the molecular pathogenesis of type 2 diabetes remains unclear.[4] Recently, Boini et al[21] found that both blood glucose and insulin levels were lower in kcnq1–/– than in kcnq1+/+ mice. In addition, the uptake of glucose into skeletal muscle, liver, kidney and lung tissue was significantly higher in kcnq1–/– than in kcnq1+/+ mice, thus leading to a suggestion that *KCNQ1* is a novel molecule affecting insulin sensitivity of the glucose metabolism. In conclusion, our study replicates the association of *KCNQ1* common variants with T2D in Malaysian Malay subjects. In addition, *KCNQ1* haplotypes and diplotypes proved to be associated with T2D.

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