Original Research

DISTRIBUTION OF *KCNJ11* RS5219 POLYMORPHISM IN VIETNAMESE POPULATION AND ITS ASSOCIATION WITH HYPERGLYCEMIA

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ABSTRACT

Aims: *KCNJ11* gene, encoding ATP-sensitive channel subunits, involves in insulin secretion. The study aimed at investigating the distribution of the *KCNJ11* E23K (rs5219) polymorphism and its association with prediabetes and type 2 diabetes (T2D) in Vietnamese population.

Methods: A cross-sectional study randomly recruited 2.676 participants aged 40-64 years from a general population in Ha Nam province, Vietnam. Glycemic status of the subjects was classified based on fasting plasma glucose and oral glucose tolerance test. PCR-restriction fragment length polymorphism method was applied to detect the *KCNJ11*-rs5219 polymorphism. Genotype frequencies were compared to find the distribution difference among normoglycemic, prediabetes, and T2D groups. Generalized linear models and multinomial logistic regression analysis were used to determine the associations of the *KCNJ11*-rs5219 polymorphism with prediabetes and T2D.

Results: The frequencies of minor K allele in normoglycemic, prediabetes and T2D groups were 33.2, 32.6 and 35.4%, respectively. Genotype distribution of the E23K polymorphism was in Hardy-Weinberg equilibrium and not significantly different among the three glucose groups ($p > 0.05$). Fasting plasma glucose and 2-h glucose levels were not significantly different among three genotypes EE, EK, and KK ($p > 0.05$). After adjusted for social-economic status, lifestyle and clinical patterns, no significant association was found between the *KCNJ11*-rs5219 polymorphism and hyperglycemia with ORs from 0.80 to 1.29.

Conclusions: The minor K allele was predominant and genotype frequency in the population was remained constant among generations. The study suggests no significant association between the *KCNJ11*-rs5219 polymorphism and hyperglycemia in the Vietnamese population.

Keywords: *prediabetes, type 2 diabetes, rs5219, KCNJ11, Vietnamese*

I. INTRODUCTION

Hyperglycemia including prediabetes and type 2 diabetes (T2D) occurs when there is too much sugar in the blood because of defection in insulin secretion, insulin action, or both. The estimated prevalence of diabetes in adults aged 20 – 79 years has more than tripled, from an estimated 151 million (4.6%) in 2000 to 537 million (10.5%) in 2021. By estimate, 643 million people will have diabetes by 2030 (11.3%) and 783 million (12.2%) by 2045 [1]. In addition, three quarters (75%) of those with diabetes were living in low-and middle-income countries with lacking of primary health care [2]. The prediabetes

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prevalence is often two to three times higher than the diabetes prevalence and varies among populations, ethnic groups.

In Vietnam, the age–adjusted comparative diabetes prevalence was 6.1% [1]. There were still 73% of diabetic subjects without knowing the condition [3].

In general, T2D which is associated to age, family history of obesity, lifestyle and genetics results from the deficiency of insulin secretion and insulin activity [3, 4]. It is not surprising that most T2D risk alleles appear to be associated with pancreatic β-cell dysfunction [5].

The pancreatic islet adenosine triphosphate-sensitive potassium ion channel complex (KATP) plays a critical role in glucose-stimulated insulin secretion. KATP channel is a hetero-octameric complex which consisted of four sulfonylurea receptor (SUR1) subunits and four Kir6.2 subunits [6]. Potassium inwardly-rectifying channel, subfamily J, member 11 (*KCNJ11*), which encodes the subunit protein of KATP (Kir6.2), is highly expressed in the pancreas. *KCNJ11* gene locates on chromosome 11, encoding a protein with 390 amino acids. Mutations in the *KCNJ11* gene can reduce the ability of ATP to inhibit the activity of the KATP channel and induce enhanced ability of Mg-ATP to simultaneously stimulate the function of KATP channel, leading to defective insulin secretion [7]. Especially, the *KCNJ11* E23K (rs5219) polymorphism is caused by a switch of guanine (G) to adenine (A) at codon 23, resulting in a glutamic acid (E) to lysine amino acid (K) in the *KCNJ11* gene. The polymorphism has been associated with T2D in European, Asian and Arab populations [8, 9]. Mutations in the *KCNJ11* E23K gene could set the stage for the descending sensitivity of the ion channel to ATP, which makes the channel consume more ATP until it is closed. As a result, insulin release is damaged and the risk of T2D is increased [10]. Moreover, the E23K variant was reported independently to increase the risk of developing prediabetes in the prospective study, supporting its role in glycaemic progression in Southern Chinese, with the effect of being more evident at the stage when normal glucose tolerance subjects progressed to prediabetes [11].

While previous studies have shown that the *KCNJ11* E23K polymorphism plays a critical role in the development of T2D, most of these studies did not focus on prediabetes. In addition, the genetic background of *KCNJ11* gene for T2D and prediabetes has not been investigated in Vietnam. Therefore, it is necessary to evaluate the distribution of the E23K polymorphism and the association of this genetic variation with prediabetes and type 2 diabetes susceptibility in Vietnamese population.

II. METHODS

2.1. Subjects and data collection

The cross-sectional study consisted of 2.676 participants aged 40-64 years (2171 normoglycemic, 409 prediabetes, and 96 T2D cases). They were randomly recruited from a general population in Ha Nam province located in the Red River Delta region, Vietnam. The details of the study were reported previously [3]. In summary, all participants were interviewed by trained surveyors to collect data on social-economic status (age, sex, residence, marital status, occupation, education, income level,

family history of diabetes) and lifestyle patterns (smoking and drinking comsumptions, leisure time spent sitting, siesta, and watching television). Clinical parameters were measured including body fat percentage, systolic blood pressures (SBP), glycemic status, and lipid profile.

Blood samples were collected and centrifuged immediately in the morning after a participant had fasted for 8h–16h prior to the clinic visit. The glycemic status of subjects was determined using both fasting plasma glucose level (FPG) and oral glucose tolerance test (OGTT) with 75 g glucose after 2h [12]. Glucose and lipid profile were analyzed using a semi-autoanalyzer (Screen Master Lab; Hospitex Diagnostics LIHD112, Italy) with commercial kit (Chema. Diagnostica, Italy). Plasma glucose was measured by glucose oxidase method. Lipid profile including triglycerides, high-density lipoprotein cholesterol (HDL-C) were measured by enzymatic methods.

2.2. Hyperglycemic diagnosis

Both FPG and OGTT were used to identify prediabetes and diabetes status [12]. Participants were classified as having diabetes if they had FPG $\geq$ 7.0 mmol/L or OGTT $\geq$ 11.1 mmol/L or previous diagnosis of diabetes and current use of hypoglycemic drugs. Prediabetes was classified if FPG was between 5.6 and 6.9 mmol/L, and/or OGTT was between 7.8 and 11.0 mmol/L. Normoglycemic level was classified when FPG < 5.6 mmol/L and OGTT < 7.8 mmol/L.

2.3. Genotyping

The *KCNJ11* E23K polymorphism genotyping method was reported previously [13]. Briefly, peripheral blood samples were obtained from each participant and genomic DNA was extracted from 300 µl blood samples, using Wizard® Genomic DNA Purification Kit (Promega Corporation, USA). The purity and concentration of DNA was measured by NanoDrop. The forward and reverse primers in PCR reaction to genotype the *KCNJ11* E23K polymorphism were 5'-GACTCTGCAGTGAGGCCCTA-3' and 5'-ACGTTGCAGTTGCCTTTCTT-3', respectively. The *Ban*II restriction enzyme (New England Biolabs, Beverly, MA) was selected to incubate 210bp PCR products and distinguish the C or T allele after electrophoresis in 3% agarose gel or 12% polyacrylamide gel [9, 14]. Distribution patterns of the rs5219 polymorphism were KK (2 bands: 178 bp and 32 bp), EK (4 bands: 178 bp, 150 bp, 32 bp and 28 bp), and EE (3 bands: 150 bp, 32 bp and 28 bp).

2.4. Statistical analysis

Allele and genotype frequencies were compared among 3 glycemic groups (normoglycemic, prediabetes, and T2D) and checked for Hardy-Weinberg equilibrium by χ^2 test.

The values of FPG and OGTT in the group with EE genotype were compared with either EK or KK genotype groups, using generalized linear models adjusted for the covariates including social-economic status (age, sex, residence, marital status, occupation, education, and income level), lifestyle (smoking and alcohol comsumptions, leisure time spent sitting, siesta, and watching television), and clinical patterns (body fat percentage, SBP, HDL-C, triglycerides, and family history of diabetes).

Association of the *KCNJ11* E23K polymorphism with prediabetes and T2D was analysed using multinomial logistic

regression adjusted for the covariates. Odd ratios (ORs) with 95% confidence interval (95% CI) were reported for the risk allele. The level of significance was set to 0.05 for all analyses. The above statistical procedures were performed using SPSS version 20.0 (SPSS, Chicago, USA).

III. RESULTS

3.1. Distribution of *KCNJ11* E23K polymorphism in the population

Table 1 shows the frequencies of genotypes and alleles of the *KCNJ11* E23K polymorphism in the total sample and each of normoglycemic, prediabetes and diabetes groups. We found that genotypic distribution of the E23K polymorphism followed Hardy-Weinberg equilibrium in total sample and in normoglycemic group (p = 0.832), prediabetes group (p=0.924), and diabetes group (p=0.382). The frequency of minor allele (K) in normoglycemic, prediabetes, diabetes, and the total groups were 33.2, 32.6, 35.4%, and 33.6%, respectively. No significant difference in genotype and allele frequencies was observed among normoglycemic, prediabetes and diabetes groups (p > 0.05).

Table 1. *Genotype and allele frequencies of KCNJ11 E23K polymorphism among groups*

	Normoglycemic group (n = 2171)	Prediabetes group (n = 409)	Type 2 diabetes group (n= 96)	Total (n = 2676)	p-value
Genotype					
EE	958 (44.1)	186 (45.5)	42 (43.8)	1186 (44.3)	0.859
EK	965 (44.4)	179 (43.8)	40 (41.7)	1184 (44.2)	
KK	248 (11.4)	44 (10.8)	14 (14.6)	306 (11.4)	
Allele					
E	2935 (66.8)	551 (67.4)	124 (64.6)	3556 (66.4)	0.685
K	1461 (33.2)	267 (32.6)	68 (35.4)	1796 (33.6)	
P_{HWE}	0.832	0.924	0.382	0.687	

The data are presented as n (%). HWE: Hardy-Weinberg equilibrium. p-values were from χ^2 test.

3.2. Association of the *KCNJ11* E23K polymorphism with hyperglycemia

Table 2 shows the concentration of FPG and 2-h glucose in people aged 40-64 years in Ha Nam province. The results couldn't show the difference of FPG and OGTT levels among three genotypes EE, EK, and KK (p > 0.05).

Table 3 shows that no significant association was found between E23K polymorphism in *KCNJ11* gene and prediabetes or T2D in univariate and multinomial logistic regression after adjusted for social-economic status, lifestyle, and clinical patterns (p > 0.05).

Table 2. *Comparison of fasting plasma glucose and 2-h glucose levels among three KCNJ11 E23K genotypes*

KCNJ11 E23K	Fasting plasma glucose	*p*-value	2-h glucose tolerance	*p*-value
Univariate				
EE	4.81 (4.76 – 4.87)	Reference	5.78 (5.67 – 5.88)	Reference
EK	4.77 (4.72 – 5.83)	0.321	5.73 (5.63 – 5.83)	0.542
KK	4.80 (4.69 – 4.91)	0.865	5.76 (5.55 – 5.96)	0.974
After adiustment				
EE	5.05 (4.68 – 5.45)	Reference	6.23 (5.51 – 6.96)	Reference
EK	5.02 (4.64 – 5.40)	0.271*	6.18 (5.45 – 6.90)	0.488*
KK	5.03 (4.63 – 5.42)	0.857*	6.15 (5.40 – 6.90)	0.664*

*Data are means (95%CI). *p-value was received from generalized linear models adjusted for social-economic status, lifestyle, and clinical patterns.*

Table 3. *Association of KCNJ11 E23K polymorphism with prediabetes and type 2 diabetes*

KCNJ11 E23K	Prediabetes		Type 2 diabetes	
	OR (95% CI)	*p*- value	OR (95% CI)	*p*- value
Univariate				
EE	1.00		1.00	
EK	0.91 (0.64 – 1.21)	0.621	1.29 (0.69 – 2.40)	0.425
KK	0.96 (0.76 – 1.19)	0.689	0.95 (0.61 – 1.47)	0.804
After adjustment				
EE	1.00		1.00	
EK	0.80 (0.53 – 1.19)	0.268*	1.08 (0,52 – 2.23)	0.844*
KK	0.97 (0.76 – 1.24)	0.804*	1.11 (0,67 – 1.84)	0.688*

** p-value was received from multinomial logistic regression adjusted for social-economic status, lifestyle, and clinical patterns.*

IV. DISCUSSION

In the present study, we reported that the frequency of minor allele (K) in normoglycemic, prediabetic and T2D groups were 33.2%, 32.6% and 35.4%, respectively. No significant difference in genotype and allele frequencies was observed among normoglycemic, prediabetic and T2D groups ($p > 0.05$). The frequency of K allele was commonly seen in different populations, exept for African population (2.34%) [15]. The frequency of K allele of total sample was (33.6%) lower than that in Chinese Han population (41%) [16], in Danish population (39%) [9], in a UK population (37%) [17], in Iranian population (36%) [18], in a Japanese population (36%) [8], but higher than that in an Arabic population (30.5%) [19].

Prediabetes and T2D are complex disorders that involve the interaction

between multiple genes and environmental factors in their pathogenesis. The association of the *KCNJ11* E23K polymorphism with prediabetes and T2D has been reported to be inconsistent. A 12-year study in a Sounthern Chinese population showed that the *KCNJ11* E23K polymorphism could predict glycemic progression with its effect in the early T2D stage (HR = 1.25, 95%CI= 1.09-1.44), but not with the progression to T2D [11]. Accoding five genetic models, a research on an Indian population showed that the dominant (EE *vs.* EK+KK, p = 0.022) and additive (EK *vs.* EE+KK, p = 0.021) models, but not the recessive model (KK *vs.* EE+EK, p = 0.727) of the *KCNJ11* E23K polymorphism were linked to diabetes [20]. The E23K polymorphism can affect the insulin secretion pathway. The K allele of this locus impairs insulin secretion by reducing ATP sensitivity of the KATP channel, hence resulting in over-activity of the channel and subsequent suppression of insulin secretion [21]. This effect on insulin secretion is more significant in carriers of the KK genotype compared with carriers of the EE genotype.

The *KCNJ11* polymorphism is associated with T2D and hypertension in the Korean population [22]. However, many studies couldn't find the association between the E23K polymorphism with neither prediabetes nor T2D, in line with our finding. A study in the Central Bohemian population of the Czech Republic could not confirm the Kir6.2 E23K as a genetic marker for T2D although they found a correlation between E23K of the *KCNJ11* gene and C-peptide levels, which may be considered a measure of pancreatic β-cell activity [23]. This association was not observed in cohort studies. Two Finnish prospective studies showed that the E23K polymorphism had no effect on the development of diabetes [24]. KK genotype was not found in the Mauritanians of black African descent and E23K variant was not associated with T2D (OR = 0.69, 95% CI = 0.04–11.32, p = 0.793) [25]. The *KCNJ11* E23K polymorphism was not associated with T2D in the Iranian population after adjusting for the confounding effects of age, gender and body mass index. However, it may play a role in disease progression in the presence of obesity when following subgroup analysis of individuals with and without diabetes based on BMI in the recessive model (p = 0.03) [18].

The study had limitations. This is a cross-sectional nature which can not give a causal conclusion on the *KCNJ11* E23K polymorphism. Moreover, the study focused only on one polymorphism and did not evaluate the interaction between genetic factors and environmental factors in the pathogenesis of hyperglycemia.

V. CONCLUSION

The *KCNJ11* E23K polymorphism is found to be highly frequency in the Vienamese population. The study suggested that the *KCNJ11* E23K variant had no significant association on genetic architecture of hyperglycemia in the Vietnamese population. Further large-scale investigations and cohort studies should be conducted to identify the effect of the E23K variants in the pathogenesis of prediabetes and type 2 diabetes.

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