Diabetologia (2012) 55:2402–2406
DOI 10.1007/s00125-012-2602-5

ARTICLE

KCNQ1 SNPS and susceptibility to diabetic nephropathy in East Asians with type 2 diabetes

X. L. Lim · S. Nurbaya · A. Salim · E. S. Tai · S. Maeda · Y. Nakamura · D. P. K. Ng

Received: 14 February 2012 / Accepted: 4 May 2012 / Published online: 14 June 2012

Abstract
Aims/hypothesis A Japanese study had earlier reported that *KCNQ1* single-nucleotide polymorphisms (SNPs) may be associated with diabetic nephropathy. To further investigate this finding, we analysed three SNPs, rs2237895, rs2237897 and rs2283228, within the *KCNQ1* locus for association with albuminuria among Chinese type 2 diabetic patients residing in Singapore. Albuminuria was analysed as both categorical (micro- and macroalbuminuria) and continuous traits ($\log_e$ albumin/creatinine ratio [ACR]).
Methods A total of 752 Chinese patients with type 2 diabetes were included in the study. Albuminuria was determined by ACR using spot urine samples, and renal function was approximated using estimated GFR. Genotyping was performed using invader and Taqman assays as appropriate. Multivariate regression analyses were used to analyse the associations between SNPs and renal traits.
Results Significant associations were detected between rs2283228 and macroalbuminuria ($p<0.001$, corrected $p<0.01$), as well as $\log_e$ ACR ($p=0.004$, corrected $p=0.036$) after multiple hypothesis testing and adjustment for potential confounding. A trend of increasing OR was observed with increasing severity of diabetic nephropathy (low and high microalbuminuria, macroalbuminuria). rs2237897, previously implicated in the earlier Japanese study, was also associated with macroalbuminuria, but this finding did not remain significant after correction for multiple testing. Meta-analyses of the Chinese and Japanese studies revealed both SNPs to be significantly associated with macroalbuminuria.
Conclusions/interpretation Together with the previous Japanese study, our findings support the hypothesis that, in addition to *KCNQ1* being an established type 2 diabetes gene, genetic variation in this gene may contribute to susceptibility to diabetic nephropathy in East Asians.

Keywords ACR · Chinese · Estimated GFR · Genetics · *KCNQ1* · Meta-analysis · Type 2 diabetes

Abbreviations

ACR	Albumin/creatinine ratio
eGFR	Estimated GFR
GWAS	Genome-wide association studies
IQR	Interquartile range
KCNQ1	Potassium voltage-gated channel KQT-like subfamily, member 1
SNP	Single-nucleotide polymorphism

Electronic supplementary material The online version of this article (doi:10.1007/s00125-012-2602-5) contains per reviewed but unedited supplementary material, which is available to authorised users.

X. L. Lim · S. Nurbaya · A. Salim · D. P. K. Ng (✉)
Department of Epidemiology and Public Health, Yong Loo Lin School of Medicine, National University of Singapore,
16 Medical Drive MD3,
117597, Singapore, Republic of Singapore
e-mail: danielpkng@gmail.com

E. S. Tai
Department of Medicine, National University of Singapore, Singapore, Republic of Singapore

S. Maeda
Laboratory for Endocrinology and Metabolism, RIKEN Center for Genomic Medicine, Yokohama, Japan

Y. Nakamura
Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan

Introduction

The gene *KCNQ1* encodes the pore-forming α subunit of a voltage-gated potassium channel and was initially identified as a type 2 diabetes susceptibility gene in genome-wide association studies (GWAS) [1, 2]. Three single-nucleotide polymorphisms (SNPs)—rs2237895, rs2237897 and rs2283228—within *KCNQ1* have been found to be strongly associated with type 2 diabetes in both East Asian and European populations. More recently, a study of Japanese patients with type 2 diabetes had suggested that, interestingly, *KCNQ1* may also confer susceptibility to diabetic nephropathy, as evidenced by the presence of macroalbuminuria [3]. In that report, results of the initial study showed an association with proteinuria, while replication studies showed a similar trend but individually failed to reach statistical significance. In view of this initial report by Ohshige et al [3], we have attempted to replicate this association with diabetic nephropathy in another East Asian population by studying 752 Chinese patients with type 2 diabetes. In addition, we also investigated whether these SNPs modulated albuminuria as a continuous trait ($\log_e$ albumin/creatinine ratio [ACR]).

Methods

Chinese patient population A total of 752 Chinese patients with type 2 diabetes from the Singapore Diabetes Cohort Study were included in this study. Briefly, the Singapore Diabetes Cohort Study was made up of consenting patients who had been previously diagnosed with type 2 diabetes at primary care facilities of the National Healthcare Group Polyclinics in Singapore. Information on demographics, lifestyle factors and medical family history were obtained by questionnaire. Patients' physical measurements and blood and spot urine specimens were taken, and their medical records were reviewed to obtain information on their metabolic control and the presence of comorbidities and complications. The research protocol was approved by the National Healthcare Group Domain-Specific Review Board.

Renal traits Mid-stream random spot urine samples were collected and transported chilled to the laboratory, centrifuged and stored at −80°C before analysis. Albuminuria was determined on the basis of the ACR (mg/mmol) using commercially available kits for albumin and creatinine measurements (Exocell, Philadelphia, PA, USA). Patients with ACR <3.4 mg/mmol were considered normoalbuminuric, while those with 3.4≤ACR<33.9 mg/mmol and ACR ≥33.9 mg/mmol were microalbuminuric and macroalbuminuric, respectively. Renal function (expressed as estimated GFR [eGFR]) was estimated using the simplified Modification of Diet in Renal Disease equation where eGFR (ml min^{-1} 1.73 m^{-2})=186.3×(plasma creatinine in mg/dl)$^{-1.154}$×(age in years)$^{-0.203}$×(0.742 for women)×(1.21 if participant is black) [4].

Japanese replication study For the purpose of replication and meta-analyses, we studied type 2 diabetic patients from the initial case–control study reported by Ohshige et al [3]. Cases (n=754) comprised patients with diabetic retinopathy and a urinary albumin excretion rate ≥200 μg/min or an ACR ≥33.9 mg/mmol, while controls (n=558) had retinopathy with a urinary albumin excretion rate <20 μg/min or ACR <3.4 mg/mmol.

Genotyping Three SNPs in intron 15 of *KCNQ1*, rs2237895 (A>C), rs2237897 (C>T) and rs2283228 (A>C), were genotyped using a multiplex PCR invader assay [5] and TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA). All SNPs had a more than 90% genotyping success rate.

Statistical analysis All statistical analyses were carried out using Stata V.10 (http://www.stata.com), assuming a two-sided test with a 5% level of significance. Genotype and allele frequencies were tested for Hardy–Weinberg equilibrium proportions using the χ^2 test. Differences in characteristics between sexes were compared using the χ^2 test for categorical variables. For continuous variables that were normally distributed, ANOVA was used to compare differences in mean between sex. Otherwise, the Kruskal–Wallis test was implemented and the medians were compared. Multivariate analyses on the association of individual *KCNQ1* SNPs with $\log_e$ ACR were performed using linear regression. Multivariate logistic regression was used to determine the associations between *KCNQ1* SNPs and the presence of micro- and macroalbuminuria assuming dominant, recessive and additive genetic models. Correction for multiple hypothesis testing was not straightforward, particularly as the renal traits or genetic models were not entirely independent. Nevertheless, to minimise type 1 error, Bonferroni correction was performed based on albuminuria analysed as categorical (micro- and macroalbuminuria) and continuous ($\log_e$ ACR) traits under three genetic models (3×3=9 comparisons). An uncorrected p value <0.006 would therefore be required to declare significance.

Meta-analysis was performed for each SNP under all three genetic models. Log_e OR was used as the measure of effect size, and the standard error of the $\log_e$ OR as a measure of the study's precision. The I^2 index was used to measure the extent of between-study heterogeneity. Since the I^2 index, like other heterogeneity indices, has little power in detecting heterogeneity with a small number of studies, a liberal type I error of 10% was used for detecting heterogeneity. If the studies were found to be heterogeneous, a random-effect meta-analysis method was performed. Otherwise, fixed-effect meta-analysis was performed.

Results

The clinical characteristics of the 752 type 2 diabetic patients analysed in this study are presented according to sex (electronic supplementary material [ESM] Table 1) and nephropathy status (ESM Table 2). Intersex differences were observed for WHR, diastolic blood pressure, and total and HDL-cholesterol. Diabetes duration, HbA_{1c}, blood pressure and eGFR were associated with nephropathy status. The genotype distributions of all three *KCNQ1* SNPs were in Hardy–Weinberg equilibrium regardless of whether the patients were analysed as a whole or categorised by sex or nephropathy status (ESM Tables 1 and 2). Moderate linkage disequilibrium was observed between the three SNPs in our Chinese patients, and this pattern was similar to that observed in the Japanese study based on pilot data from the 1000 Genomes Project (ESM Table 3).

SNP rs2283228 was strongly associated with macroalbuminuria in multivariate analyses under the recessive (uncorrected $p=1.492\times10^{-5}$, corrected $p=1.343\times10^{-4}$) and additive (uncorrected $p=8.167\times10^{-5}$, corrected $p=7.350\times10^{-4}$) models (Table 1). The dominant model revealed associations of only borderline significance, which did not withstand correction for multiple hypothesis testing (Table 1). SNP rs2237897, which was associated with macroalbuminuria in the earlier Japanese study [3], was also associated in our study, but the significance was borderline (uncorrected $p=0.025$ under the additive model, corrected p = NS).

None of the SNPs were associated with microalbuminuria (ACR=3.4 to <33.9 mg/mmol) regardless of the genetic model used (ESM Table 4). A trend of increasing OR was, however, observed when patients with low (ACR=3.4 to <7.5 mg/mmol) or high (ACR=7.5 to <33.9 mg/mmol) microalbuminuria and finally macroalbuminuria (ACR ≥33.9 mg/mmol) were compared. For instance, the OR for rs2283228 increased from low microalbuminuria (OR 1.19, 95% CI 0.57, 2.46) to high microalbuminuria (OR 1.88, 95% CI 0.98, 3.61) to macroalbuminuria (OR 6.00, 95% CI 2.68, 13.41) under the recessive model (Table 1 and ESM Table 4). This increasing trend was also observed for rs2283228 under the additive and dominant models.

With respect to the analysis of albuminuria as a continuous trait, SNP rs2283228 was significantly associated with $\log_e$ ACR under the recessive model ($p=0.004$, corrected $p=0.036$) even after adjustment for eGFR (Table 2). The other significant covariates included diabetes duration and HbA_{1c}. This SNP also showed borderline association with $\log_e$ ACR under the additive model ($p=0.028$, corrected p = NS) (Table 2). Patients who were 'CC' recessive had the highest ACR reading (median=2.0 mg/mmol, interquartile range [IQR]=0.6–14.7 mg/mmol) compared with 'AA' (1.6 mg/mmol, IQR=0.7–4.2 mg/mmol) and 'AC' (1.5 mg/g, IQR=0.7–4.9 mg/mmol) genotypes ($p=0.0331$).

To investigate whether rs2283228 could be replicated in another East Asian population, this SNP was genotyped in a case–control study of type 2 diabetic Japanese patients

Table 1 Association between *KCNQ1* SNPs and macroalbuminuria in multivariate analyses based on recessive, dominant and additive models

Model type	rs2237895 (A>C)		rs2237897 (C>T)		rs2283228(A>C)	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Recessive						
KCNQ1 SNP	0.47 (0.13, 1.67)	0.244	2.15 (0.85, 5.40)	0.104	6.00 (2.68, 13.41)	1.492×10^{-5}
Covariate						
HbA_{1c}	1.67 (1.24, 2.25)	0.001	1.82 (1.34, 2.48)	1.334×10^{-4}	1.81 (1.33, 2.47)	1.744×10^{-4}
eGFR	0.98 (0.96, 0.99)	0.010	0.98 (0.96, 1.00)	0.028	0.97 (0.95, 0.99)	0.002
Dominant						
KCNQ1 SNP	1.00 (0.52, 1.94)	0.993	2.10 (1.00, 4.40)	0.049	2.50 (1.21, 5.16)	0.013
Covariate						
HbA_{1c}	1.67 (1.25, 2.24)	0.001	1.80 (1.33, 2.45)	1.827×10^{-4}	1.75 (1.28, 2.38)	4.191×10^{-4}
eGFR	0.98 (0.96, 0.99)	0.008	0.98 (0.96, 1.00)	0.032	0.98 (0.96, 0.99)	0.011
Additive						
KCNQ1 SNP	0.87 (0.53, 1.42)	0.575	1.77 (1.07, 2.93)	0.025	2.76 (1.67, 4.57)	8.167×10^{-5}
Covariate						
HbA_{1c}	1.67 (1.24, 2.24)	0.001	1.83 (1.34, 2.49)	1.444×10^{-4}	1.83 (1.33, 2.52)	2.119×10^{-4}
eGFR	0.98 (0.96, 0.99)	0.007	0.98 (0.96, 1.00)	0.030	0.97 (0.96, 0.99)	0.005

Only covariates (HbA_{1c} and eGFR) that were statistically significant in the final model are shown. Other covariates considered in the adjustment that were not significant included sex, age, type 2 diabetes duration, BMI, WHR, systolic and diastolic blood pressure, mean arterial pressure, triacylglycerol, cholesterol, LDL-cholesterol, HDL-cholesterol, treatment with antihypertensive and lipid-lowering drugs, and diabetes treatment

Table 2 Association of $\log_e$ ACR with *KCNQ1* SNPs in Chinese patients with type 2 diabetes under recessive, dominant and additive models

Model type	rs2237895 (A>C)		rs2237897 (C>T)		rs2283228(A>C)	
	Coefficient (95% CI)	*p* value	Coefficient (95% CI)	*p* value	Coefficient (95% CI)	*p* value
Recessive						
KCNQ1 SNP	−0.091 (−0.420, 0.238)	0.586	0.153 (−0.239, 0.544)	0.445	0.497 (0.161, 0.834)	0.004
Covariate						
T2DM duration	0.015 (−0.0001, 0.030)	0.052	0.011 (−0.004, 0.026)	0.143	0.015 (0.0003, 0.030)	0.045
HbA_{1c}	0.302 (0.179, 0.426)	1.932×10^{-6}	0.331 (0.206, 0.455)	2.507×10^{-7}	0.295 (0.173, 0.417)	2.452×10^{-6}
eGFR	−0.007 (−0.013, −0.001)	0.016	−0.006 (−0.011, 0.0001)	0.056	−0.007 (−0.013, −0.002)	0.012
Dominant						
KCNQ1 SNP	0.103 (−0.134, 0.340)	0.395	0.130 (−0.105, 0.365)	0.277	0.127 (−0.103, 0.357)	0.277
Covariate						
T2DM duration	0.014 (−0.001, 0.029)	0.058	0.011 (−0.004, 0.027)	0.135	0.015 (−0.0003, 0.029)	0.055
HbA_{1c}	0.302 (0.178, 0.425)	1.932×10^{-6}	0.327 (0.203, 0.451)	3.244×10^{-7}	0.297 (0.174, 0.419)	2.337×10^{-6}
eGFR	−0.007 (−0.013, −0.001)	0.018	−0.005 (−0.011, 0.0003)	0.064	−0.006 (−0.012, −0.001)	0.027
Additive						
KCNQ1 SNP	0.028 (−0.140, 0.196)	0.746	0.107 (−0.071, 0.286)	0.239	0.186 (0.020, 0.351)	0.028
Covariate						
T2DM duration	0.015 (−0.0005, 0.030)	0.057	0.012 (−0.004, 0.027)	0.136	0.015 (−0.00005, 0.030)	0.049
HbA_{1c}	0.301 (0.178, 0.425)	2.028×10^{-6}	0.329 (0.204, 0.453)	2.780×10^{-7}	0.296 (0.174, 0.418)	2.337×10^{-6}
eGFR	−0.007 (−0.013, −0.001)	0.016	−0.006 (−0.011, 0.0003)	0.062	−0.007 (−0.012, −0.001)	0.021

Only covariates (T2DM duration, HbA_{1c} and eGFR) that were statistically significant in the final model were shown. Other covariates considered in the adjustment that were not significant included sex, age, BMI, WHR, systolic and diastolic blood pressure, mean arterial pressure, triacylglycerol, cholesterol, LDL-cholesterol, HDL-cholesterol, treatment with antihypertensive and lipid-lowering drugs, and diabetes treatment

In this analysis, ACR was expressed in mg/g which can be converted to mg/mmol after dividing by 8.84

T2DM, type 2 diabetes mellitus

previously included in the initial study by Ohshige et al [3] (see the Methods section). In multivariate analyses, rs2283228 was significantly associated with macroalbuminuria (ESM Table 5). In meta-analyses combining data from Chinese and Japanese patients, macroalbuminuria was significantly associated with rs2283228 under the dominant model with a pooled OR of 1.50 (95%CI 1.15, 41.96) (meta-analysis *p* value=0.003) (ESM Fig. 1). A similar but non-significant trend was observed for this SNP under the recessive and additive models (ESM Fig. 1). Macroalbuminuria was significantly associated with rs2237897 under the dominant (meta-analysis *p* value=0.002), recessive (meta-analysis *p* value=0.031) and additive (meta-analysis *p* value=0.001) models (ESM Fig. 2).

Discussion

Our study has provided evidence to support the hypothesis that genetic variation in *KCNQ1* can significantly affect albuminuria in Chinese patients with type 2 diabetes. This result is consistent with the earlier report by Ohshige et al [3], which showed that *KCNQ1* SNPs were associated with proteinuria in Japanese patients with type 2 diabetes. In the Japanese population, rs2237897 was the SNP most strongly associated with proteinuria. While we did find some evidence of replication of this SNP in our Chinese patients, our study also revealed a novel positive association with rs2283228, which we subsequently replicated in the Japanese population. Meta-analyses of the Chinese and Japanese datasets revealed both SNPs to be positively associated with macroalbuminuria. As with the original GWAS findings linking *KCNQ1* to type 2 diabetes, the effect of these genetic variants would seem to be more relevant in East Asian populations as opposed to certain other human populations (e.g. those of European descent), as these *KCNQ1* SNPs may be substantially less polymorphic.

A major limitation of our study is the modest number of Chinese patients with macroalbuminuria. Thus, although the associations were significant even after correction for multiple hypothesis testing, one might wish to be cautious in drawing conclusions. Encouragingly, however, we were able to successfully follow up on our positive findings in an independent collection of Japanese patients. It was also notable that, as the severity of diabetic nephropathy increased—namely, as it progressed from low to high

microalbuminuria and finally macroalbuminuria—a rise in the magnitude of association (OR) was observed. This, in our opinion, is at least consistent with the notion that the association with macroalbuminuria was not by chance. Another limitation is that the ACR readings were based on a single urine sample collected at entry into the Chinese study. This could potentially affect the precision of the disease classification and inadvertently made it more difficult to detect minor genetic effects. On the other hand, this ACR reading correlated highly with multiple urinary dipstick measurements obtained in visits to the clinic preceding their entry into our patient population. Any disease misclassification that might have inadvertently occurred did not appear to have a large negative effect on our study, as highly significant associations were readily detected.

It is worth reiterating that *KCNQ1* was initially identified as a gene predisposing to type 2 diabetes. Yet, its implication in renal traits is biologically plausible. For instance, KCNQ1 (potassium voltage-gated channel, KQT-like subfamily, member 1) molecularly assembles with KCNE1 (potassium voltage-gated channel, Isk-related family, member 1) to form a potassium ion channel that has been localised to the brush border of the proximal tubule. This potassium channel facilitates the maintenance of an ion potential gradient, which serves to drive Na^+ secretion in the nephron [6]. Given that the SNPs are intronic, it is not clear how they would act to affect the molecular biology of KCNQ1. Potentially, this may affect its gene expression, but a survey of the current literature did not shed light on the potential functional roles of these *KCNQ1* SNPs. Unfortunately, this lack of functional information is commonplace for many of the GWAS associated SNPs. Future studies aimed at delineating this biological aspect will certainly aid our understanding of the role of these disease genes.

Funding This study was supported by a grant from the Singapore National Medical Research Council (NMRC/1115/2007) awarded to D. P.K. Ng.

Contribution statement XLL wrote the paper and performed the statistical analyses; AS, SN, EST, SM and YN contributed to data acquisition and statistical analyses and drafted the paper; DPKN conceived the study, contributed to the laboratory and statistical analyses and wrote the paper. All authors approved the final manuscript.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

References

1. Yasuda K, Miyake K, Horikawa Y et al (2008) Variants in KCNQ1 are associated with susceptibility to type 2 diabetes mellitus. Nat Genet 40:1092–1097
2. Unoki H, Takahashi A, Kawaguchi T et al (2008) SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. Nat Genet 40:1098–1102
3. Ohshige T, Tanaka Y, Araki S et al (2010) A single nucleotide polymorphism in KCNQ1 is associated with susceptibility to diabetic nephropathy in Japanese subjects with type 2 diabetes. Diabetes Care 33:842–846
4. National Kidney Foundation (2002) K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 39:S1–S266
5. Ohnishi Y, Tabaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y (2001) A high-throughput SNP typing system for genome-wide association studies. J Hum Genet 46:471–477
6. Lang F, Rehwald W (1992) Potassium channels in renal epithelial transport regulation. Physiol Rev 72:1–32