



KCNQ1 gene variants in the risk for type 2 diabetes and impaired renal function in the Spanish Renastur cohort



Cristina Riobello^a, Juan Gómez^a, Helena Gil-Peña^b, Salvador Tranche^c, Julián R. Reguero^d, Jesús M. de la Hera^d, Elías Delgado^{e,g}, David Calvo^d, César Morís^{d,g}, Fernando Santos^{b,g}, Pablo Coto-Segura^{f,g}, Sara Iglesias^a, Belén Alonso^a, Victoria Alvarez^a, Eliecer Coto^{a,g,h,*}

^a Genética Molecular-Laboratorio Medicina, HUCA, Oviedo, Spain

^b Pediatría, HUCA, Oviedo, Spain

^c Centro de Salud ElCristo, Oviedo, Asturias, Spain

^d Cardiología-Fundación Asturcor, HUCA, Oviedo, Spain

^e Endocrinología, HUCA, Oviedo, Spain

^f Dermatología, HUCA, Oviedo, Spain

^g Universidad de Oviedo, Oviedo, Spain

^h Red investigación renal (REDINREN), Madrid, Spain

ARTICLE INFO

Article history:

Received 19 December 2015

Received in revised form

4 March 2016

Accepted 6 March 2016

Available online 9 March 2016

Keywords:

Potassium channels

KCNQ1 gene

Next generation sequencing

Estimated glomerular filtration rate

Type 2 diabetes

Gene polymorphisms

ABSTRACT

Several common *KCNQ1* gene polymorphisms have been associated with the risk of type 2 diabetes (T2DM) and diabetic nephropathy. This effect is explained by the role of the *knq1* protein as a potassium channel that in the pancreatic beta-cells drives an electrical signal that facilitates glucose-stimulated insulin secretion. The *KCNQ1* gene is also expressed in the kidney, and could thus be implicated in the risk of developing impaired renal function. To test this hypothesis, we genotyped six common *KCNQ1* gene variants (three single nucleotide polymorphisms, rs2237892, rs2237895, and rs231362, and three intronic indels) in 681 healthy elderly individuals (>65 years old) from the Spanish Renastur cohort. None of the six variants was associated with T2DM (180 diabetics vs. 581 non-diabetics). The intron 12 insertion allele was associated with a reduced estimated glomerular filtration rate (eGFR<60, n = 90 vs. eGFR≥60, n = 591; II vs ID + DD genotypes, p = 0.031, OR = 2.06, 95%CI = 1.12–4.14). We also performed a next generation sequencing search of variants in the coding regions of the *KCNQ1* gene in 100 individuals with the extreme eGFR values. We found two rare amino acid changes (p.K393N and p.P408A) and the 393 Asn variant was found only among diabetics (n = 4; p = 0.05). The two rare alleles were present in the two eGFR groups.

Our results suggest that a common *KCNQ1* intron 12 indel polymorphism is a risk factor for impaired renal function independent of T2DM. If this association is confirmed by others, further research to determine the mechanism that drives this association would be warranted.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Genome wide association studies (GWAS) have identified *KCNQ1* single nucleotide polymorphisms (SNPs) that are linked to the risk of type 2 diabetes (T2DM) in East Asian and European

* Corresponding author. Genética Molecular, Hospital Universitario Central de Asturias, 33011, Oviedo, Spain.

E-mail address: eliecer.coto@sespa.es (E. Coto).

populations (Unoki et al., 2008; Yasuda et al., 2008; Voight et al., 2010; Li et al., 2014; Qian et al., 2015). *KCNQ1* may also confer susceptibility to diabetic nephropathy (Ohshige et al., 2010; Liu et al., 2011; Lim et al., 2012; Chen et al., 2013; Hanson et al., 2013). The T2DM-risk alleles may also be associated with the risk of developing new onset diabetes among kidney transplanted patients treated with tacrolimus (Tavira et al., 2011).

The *KCNQ1* gene encodes for the potassium voltage-gated channel, KQT-like subfamily, member 1 (KV7.1) (Barhanin et al., 1996; Sanguinetti et al., 1996). This protein forms part of the pore

of a voltage-gated potassium channel in several tissues/organs, such as the heart, pancreas, and kidney (Zheng et al., 2007; Jespersen et al., 2005; Abbott, 2014). *KCNQ1* gene mutations are found in patients/families with long QT syndrome (LQTS), which is a Mendelian form of cardiac arrhythmia (Bokil et al., 2010). In the pancreas, the voltage-gated potassium channel drives an electrical signal in the beta-cells that facilitates glucose-stimulated insulin secretion (Ashcroft, 2005; Yamagata et al., 2011).

In mice, the deletion of the *Kcnq1* gene leads to decreased non-fasted plasma glucose and insulin concentrations (Boini et al., 2009). On the other hand, overexpression of *Kcnq1* in cultured cells results in increased density of the *Kcnq1* current and of total K^+ current, and impaired insulin secretion in the presence of glucose (Yamagata et al., 2011). These results suggest that increased *Kcnq1* gene expression might limit insulin secretion by pancreatic β -cells. The rs2237895 risk allele has been linked to decreased exocytosis and insulin secretion, suggesting that the risk alleles of this, and other *KCNQ1* intronic SNPs, may have a gain-of-function effect resulting in increased susceptibility to T2DM through increased protein expression and decreased insulin exocytosis and secretion (Rosengren et al., 2012; Tan et al., 2009). In contrast, LQTS patients with loss of function mutations in the *KCNQ1* gene exhibit increased insulin secretion due to delayed repolarization of the beta-cell (Torekov et al., 2014).

In the kidney, the *Kcnq1* protein is abundantly expressed in cells of the brush border membrane of the proximal tubule, where it mediates net K^+ secretion and facilitates Na^+ secretion in the nephron (Lang and Rehwald, 1992; Vallon et al., 2005). A recent study has also found that *Kcnq1* is expressed in the base of kidney primary cilia, pointing to a role in the pathophysiology of cystic kidney diseases (Slaats et al., 2015). Interestingly, Liu et al. (2011) identified several *KCNQ1* gene SNPs in association with eGFR in African Ancestry individuals, and show that knockdown of the *kcnq1* gene in the zebrafish results in abnormal kidney development and filtration.

The role of *Kcnq1* protein on beta-cell and kidney function in the mouse might explain the association of *KCNQ1* SNPs with T2DM and diabetic nephropathy. These nucleotide variants are intronic and a direct effect on protein function and/or expression is not evident. The *KCNQ1* intron variants might have a regulatory effect on several upstream genes that have been linked to the risk for T2DM. For instance, introns 10–11 encode for long non-coding RNA (lncRNA) designated as *KCNQ1OT1* that regulates the expression of the cyclin-dependent kinase inhibitor 1C (*Cdkn1c*), which is a cell-cycle inhibitor that has been linked to the risk for T2DM (Lee et al., 1997; Smilnich et al., 1999; Schultz et al., 2014). It is possible that intronic SNPs have an effect on the regulation of the lncRNA, or are in linkage disequilibrium with some *KCNQ1* functional variants thereby serving as surrogate markers for other functional variants.

Our main objective was to determine whether *KCNQ1* SNPs were linked to the risk for T2DM or reduced renal filtration in the RENASTUR population, which is a cohort of elderly Caucasians from Spain. In addition, we performed next generation sequencing (NGS) of the whole coding sequence of the *KCNQ1* gene in individuals with extreme values of eGFR to identify *KCNQ1* gene variants that are linked to impaired renal function.

2. Methods

2.1. Study subjects

A total of 681 individuals from the RENASTUR cohort were genotyped. All them were Caucasian, aged 65–85 years (45% male) and from the region of Asturias (Northern Spain, total population one million), and were recruited through Primary Health Care

Centers to evaluate renal function in healthy elderly people (Tavira et al., 2013; Coto et al., 2014). Individuals with a history of chronic renal disease were not eligible for the study. The main characteristics of the study cohort are summarized in Table 1. Age, sex, and smoking were self-reported, and the body mass index (BMI) was calculated by weight and height measured at the time of inclusion in the study. Individuals with a documented history of hypertension (HT) or who were receiving antihypertensive drugs were considered as hypertensives, and those with a history of type 2 diabetes (T2DM) or who were receiving antidiabetic drugs were considered as diabetics. The biochemical profile of all the participants were obtained from fasting blood samples collected by venipuncture.

The estimated glomerular filtration rate (eGFR) was calculated with the Modification of Diet in Renal Disease (MDRD) formulae (Levey et al., 1999):

$$eGFR (\text{ml/min./1.73 m}^2) = 186 \times [\text{plasma creatinine (mg/dl)}]^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if black}).$$

2.2. *KCNQ1* SNPs genotyping

SNP rs2237895 (A/C) was genotyped through real time PCR with a Taqman assay (assay id. [C_16171034_10](https://www.appliedbiosystems.com/); www.appliedbiosystems.com/) following the manufacturer's instructions (LifeTechnologies). In the case of SNPs rs2237892 and rs231362, we used restriction enzyme digestion of a polymerase chain reaction fragment (PCR-RFLP) (Tavira et al., 2011). Briefly, for rs2237892, the DNA was PCR amplified with primers 5'CTTGTGCCCTTGTCACCCAC and 5'GGCTCCAGCCTCCAAGCTG to give a 354 bp fragment. After digestion with the restriction enzyme *MspI* followed by electrophoresis in 3% agarose gels, the two alleles were visualized as bands of 354 bp (allele T) and 269 + 85 bp (allele C). For rs231362 A/G we used primers 5'CTTATC ACTGTCCGGCC-CATCTG and 5'TAGTAGGTGTCTGGTAAATGCTCTTGG, and the PCR fragments were digested with *TaqI* to visualize the two alleles (A = 261 bp, G = 161 + 100 bp) in 3% agarose gels.

2.3. *KCNQ1* intron indels genotyping

We sought to investigate indel polymorphisms in the region that overlaps *KCNQ1* and *KCNQ1OT1* (introns 10–12 of *KCNQ1*; sequence id ENSG00000053918; www.ensembl.org/). To facilitate the genotyping in agarose gels, we limited the search to >12 bp indels. We identified three indels that were genotyped by PCR-amplification followed by allele-size fractionation through electrophoresis in 3% agarose gels (suppl. Figs. 1 and 2). We used the following primers: Intron 10 (21 bp indel; variant rs143094382) 5'GGGAAGGATATCTCAATTGAAGCT and 5'CAAGGGGGCCTGGTGTGG; intron 11 (13 bp indel; rs144613775) 5'GCA GGGGCTGTATCTGAGGC and 5'GGCTCCATGGGACCAA; intron 12 (35 bp indel; variant rs374600880) 5'AATCTTACAGAAAGGAGGATCAAGGC and 5'AAGATGGGATGCTGAGCCTTGATT.

2.4. *KCNQ1* next generation sequencing

We searched for DNA variants in the *KCNQ1* gene through multiplex amplification of DNA pools and semiconductor next generation sequencing (NGS). We had previously demonstrated that this was a valid approach to identify rare variants that are diluted by the common allele (Gómez et al., 2014). Multiplex amplification (*Ampliseq*) was undertaken using an online tool (*IonAmpliseq*TM Designer; Life Technologies) to amplify the coding region of the *KCNQ1* gene (16 exons; transcript sequence ENST00000302509; www.ensembl.org) including 5 bases of

Download English Version:

<https://daneshyari.com/en/article/2195613>

Download Persian Version:

<https://daneshyari.com/article/2195613>

[Daneshyari.com](https://daneshyari.com)