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

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**A B S T R A C T**

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 kcnq1 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.

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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) and diabetic nephropathy (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 (Ohshima 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 insulin expression and decreased insulin exocytosis and secretion (Rosengren et al., 2012; Tan et al., 2008). 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 (Slats 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 gene 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; Smilinich 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):

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eGFR (mL/min./1.73 m^2) = 186 \times \frac{\text{[plasma creatinine (mg/dl)]}}{\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; 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’CTTGTGCCCCGTGACCCAC and 5’GGCTTCGACGCTCAACCTG 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’CTTATACGTCGGCCCATGCTG and 5’TAGTAGGTCTGCTGTTAATGGCTTGG, and the PCR fragments were digested with Tail 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 fractioning through electrophoresis in 3% agarose gels (suppl. Figs. 1 and 2). We used the following primers: Intron 10 (21 bp indel; variant rs143094382) and 5’GGGAAAGGATATCTCAATTAGGCT and 5’GAAAGGGCCCTGTGGTGTG; Intron 11 (13 bp indel; rs144613775) and 5’GCA GGGGCTGTATCTGAGGC and 5’ GCCCTCCACCTGGGACCAA; Intron 12 (35 bp indel; variant rs374600880) and 5’AAATCTAGAACGGAGAGATCAAGGC and 5’AAATGAGGAGATCAAGGCTGATT.

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™ Designer; Life Technologies ) to amplify the coding region of the KCNQ1 gene (16 exons; transcript sequence ENST00000302509; www.ensembl.org) including 5 bases of