



Evaluation of associations between single nucleotide polymorphisms in the *FRMD3* and *CARS* genes and diabetic nephropathy in a Kuwaiti population

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ABSTRACT. Diabetic nephropathy is the leading cause of end-stage kidney disease in the world. Many single nucleotide polymorphisms (SNPs) have been associated with diabetic nephropathy. SNPs at the 4.1 protein ezrin, radixin, moesin domain 3 (*FRMD3*) and cysteinyl t-RNA synthetase (*CARS*) genes have a well-established relationship with diabetic nephropathy. However, this association has not been evaluated in a Kuwaiti population. DNA was extracted from blood samples obtained from patients with diabetic nephropathy (N = 38); the genes of interest were amplified, and the SNPs were genotyped. Diabetics without nephropathy (N = 64) were used as controls. The risk (G and C) and non-risk (C and T) allele frequencies of the SNPs at the rs1888747 and rs739401 loci of *FRMD3* and *CARS*, respectively, did not differ significantly between the diabetics with (case)

and without (control) nephropathy ($P > 0.05$). These findings suggest that the molecular mechanisms involved in diabetic nephropathy may be different in a Kuwaiti population, compared to other populations (such as Japanese and Caucasian Europeans). The discrepancies observed in our study could also be attributed to the smaller sample size analyzed in this study. Therefore, further analyses with larger samples are required to identify the susceptibility genes in a Middle-Eastern population.

Key words: SNP; *FERM3*; *CARS*; Diabetic nephropathy

INTRODUCTION

Diabetic nephropathy is a progressive kidney disease caused by longstanding diabetes, which is the leading cause of end-stage renal disease in the western world (Thomas et al., 2012). Although diabetes deleteriously affects many organ systems, its impact on the kidney is the most significant. Some degree of renal dysfunction is observed in at least half of the patients with type 2 diabetes (Parving et al., 2006; Thomas et al., 2006) and approximately one in three patients with type 1 diabetes (Rossing et al., 1995; Krolewski et al., 1996; Hovind et al., 2003; Pambianco et al., 2006). However, not all patients with diabetes-mediated hyperglycemia develop this disease (Pambianco et al., 2006; Parving et al., 2006; Thomas et al., 2006). Diabetic nephropathy is a complex disease since both environmental and genetic factors appear to play a role in its development.

Family aggregation studies have provided strong evidence indicating that susceptibility to diabetic nephropathy is an inherited trait, with some families showing an increased risk of renal disease compared to others despite a shared predisposition for diabetes (Seaquist et al., 1989; Harjutsalo et al., 2004; Freedman et al., 2007). Certain ethnic groups are believed to be at a greater risk of developing nephropathy (Cooke et al., 2012; Palmer et al., 2012). This led to research analyzing a possible correlation between gene expression and the development of diabetes-associated end stage renal disease. Several genes have been proposed to cause or influence susceptibility to diabetic nephropathy. Products of a wide range of genes might mediate a sequence of pathological changes to renal tissues causing end-stage renal disease (Harjutsalo et al., 2004). Polymorphisms in the genes coding for 4.1 protein ezrin, radixin, moesin (FERM) domain containing-3 (*FRMD3*) and cysteinyl-tRNA synthetase (*CARS*) are associated with diabetic nephropathy in both type 1 and 2 diabetes (Pezzolesi et al., 2009; Pezzolesi et al., 2010; Maeda et al., 2010; Mooyaart et al., 2011).

The *FRMD3* gene is located at 9q21.32 on chromosome 9 (Pezzolesi et al., 2011). The protein encoded by this gene is a single-pass membrane protein primarily found in ovaries. A similar protein in erythrocytes helps determine the shape of red blood cells; however, the function of the encoded protein has not been determined. Some studies have suggested that this gene has a tumor suppressive role, and there is evidence linking defects in this gene to susceptibility to diabetic nephropathy in type 1 diabetes (Pezzolesi et al., 2011). This gene has several transcript variants encoding different isoforms. Two of these single nucleotide polymorphisms (SNPs) have been associated with diabetic nephropathy in genome-wide association studies in patients with type I diabetes: The G allele of SNP rs1888747 and the A allele of SNP rs10868025 (Pezzolesi et al., 2009; Maeda et al., 2010; Pezzolesi et al., 2010; Mooyaart et al., 2011).

The *CARS* gene, located on chromosome 11 at position 11p15.5, encodes a class-1 aminoacyl-tRNA synthetase (Cruzen et al., 1993). This gene is one of several located near the imprinted gene domain on chromosome 11p15.5, an important tumor-suppressor gene region. Alterations in this region have been associated with Beckwith-Wiedemann syndrome, Wilms tumor, rhabdomyosarcoma, adrenocortical carcinoma, and lung, ovarian, and breast cancers (Karnik et al., 1998). In addition, 2 SNPs in this gene, C allele of SNP rs739401 and the A allele of SNP rs451041, have been associated with diabetic nephropathy in genome wide association studies performed in patients with type 1 diabetes (Pezzolesi et al., 2009; Maeda et al., 2010; Pezzolesi et al., 2010; Mooyaart et al., 2011).

The aim of this study was to identify and analyze the profile of the *FRMD3* and *CARS* genes in diabetic nephropathy patients in Kuwait. Identification of the molecular characteristics of genes involved in the development of diabetic nephropathy could help increase our understanding of the pathogenesis of the disease, as well as determine the diagnostic tools for the identification of susceptible patients. The allele frequency of biallelic polymorphisms in the DNA of patients with diabetic nephropathy was determined using SNPs. Type 2 diabetic patients without nephropathy were used as controls.

MATERIAL AND METHODS

The *FRMD3* and *CARS* genes in 38 patients with diabetic nephropathy were genotyped and compared against the respective genes in 64 diabetic subjects exhibiting no evidence of diabetic renal dysfunction, as described in the literature (>300 mg albumin in urine every 24 h). The latter subjects (without nephropathy) represented the control group.

The SNPs in the two genes were genotyped using DNA extracted from the samples provided by the patients and controls. Informed consent was obtained from all subjects (or their guardians). The study protocol was approved by a local human Ethics Committee.

The genes of interest were amplified by real-time polymerase chain reaction (PCR), prior to allelic discrimination using the TaqMan® GTXpress™ Master Mix (Applied Biosystems, Foster City, CA, USA) according to the manufacturer protocol.

Genotyping frequencies of the risk and non-risk alleles were determined by direct counting. The data was statistically analyzed using the chi-squared test, performed on 2 x 2 contingency tables using the Vassar Stats online calculator. Odds ratios with 95% confidence intervals and person probability values were also calculated using this program. P values < 0.05 were considered statistically significant.

The G risk allele at position rs1888747 of the *FRMD3* gene and the C risk allele at position rs739401 of the *CARS* gene, and their respective non-risk alleles (C and T) were analyzed in this study.

RESULTS

SNPs in the *FRMD3* and *CARS* genes were not significantly associated with diabetic nephropathy (P = 0.43) Table 1). The frequency of occurrence of the risk allele G in the *FRMD3* gene was higher in patients with and without diabetic nephropathy than that of the non-risk allele C (P = 0.66 and 0.72, respectively). However, the risk allele frequency of the *FRMD3* gene was not significantly associated with any of the tested groups (P = 0.43). The genotyping of SNPs in the *CARS* genes yielded identical results in the study and control groups. The risk (C) and non-risk allele (T) frequencies of the *CARS* gene were 0.54 and 0.46 in both groups, respectively (P = 1.00).

Table 1. Association between SNPs in the *FRMD3* and *CARS* genes and diabetic nephropathy.

Gene	SNP	Chromosome		Risk allele frequency	Non-risk allele frequency	P	OR (95%CI)
<i>FRMD3</i>	rs1888747	9	Nephropathy (N = 76)	G 50 (0.66)	C 26 (0.34)	0.43	1.33 (0.72-2.45)
			No nephropathy (N = 128)	92 (0.72)	36 (0.28)		
<i>CARS</i>	rs739401	11	Nephropathy (N = 76)	C 41 (0.54)	T 35 (0.46)	1.00	(0.57-11.00 (0.57-0.76))
			No nephropathy (N = 128)	69 (0.54)	59 (0.46)		

DISCUSSION

The principal goal of our study was to compare the risk allele frequencies of the *FRMD3* and *CARS* genes in diabetic patients with and without diabetic nephropathy in Kuwait. The expression of the risk allele did not differ significantly between the diabetic patients with and without nephropathy (study and control groups, respectively); therefore, these results showed no association between SNPs in these genes and diabetic nephropathy.

Pezzolesi et al. (2009) reported a strong association between the SNP rs1888747 in *FRMD3* and diabetic nephropathy. However, this study was exclusively conducted in patients with type 1 diabetes. Here, the investigators genotyped 360,000 SNPs in 820 cases (284 with proteinuria and 536 with end-stage renal disease) and 885 control subjects with type 1 diabetes.

Maeda et al. (2010) also obtained a similar association between SNPs in *FRMD3* and diabetic nephropathy in patients with both type 1 and 2 diabetes. This study tried to replicate the previous study by Pezolesi et al. (2009) in over 1500 ethnic Japanese patients with and without diabetic nephropathy (each). An additional genome-wide association study performed by Pezolesi et al. (2010) in GoKinD samples obtained from 943 patients with diabetic nephropathy and a similar number of diabetic controls (without nephropathy) revealed a significant and strong association between SNP rs1888747 in *FRMD3* and diabetic nephropathy.

We also studied an SNP at locus rs739401 in *CARS*. Pezolesi et al. (2009) and Maeda et al. (2010) reported a strong association between this SNP and diabetic nephropathy.

In our study, we observed no significant differences between the risk and non-risk allele frequencies in *FRMD3* and *CARS*. The expression of the risk allele of *FRMD3* gene was high in diabetics with and without nephropathy ($P = 0.66$ and 0.72 , respectively). Similarly, the risk allele of *CARS* was highly expressed in diabetics with and without nephropathy ($P = 0.54$ in both groups). Therefore, despite the high expression of risk alleles, SNP rs1888747 in *FRMD3* and SNP rs739401 in *CARS* were not associated with diabetic nephropathy in Kuwaiti diabetics.

CONCLUSIONS

The role of SNPs in diabetic nephropathy has been previously analyzed in Arab countries (Ezzidi et al., 2009; Nemr et al., 2010). However, we are the first to report the association between these SNPs and diabetic nephropathy in the Middle-Eastern Arab region. The number of patients included in our study was considerably lower than that in previous studies, which could be responsible for the conflicting results obtained. A more probable explanation could be that the genetic predisposition of the Kuwaiti, and by the extension, the Middle-Eastern/Arab, population to diabetes differs considerably to that in populations extensively studied and reported previously (e.g., Japanese, Caucasian Europeans, etc.).

We intend to conduct further studies of the correlation between these SNPs (and others

in other genes) and diabetic nephropathy with a larger Kuwaiti/Arab sample size. This could help determine the predisposition of Middle Eastern patients to diabetes, and allow for the subsequent analyses of biochemical pathways related to these genes.

Conflicts of interest

The authors declare no conflict of interest.

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