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NOS3 RS1799983 AND RS2070744 POLYMORPHISMS AND THEIR ASSOCIATION WITH ADVANCED CHRONIC KIDNEY DISEASE AND CORONARY HEART DISEASE IN CANARIAN POPULATION WITH TYPE 2 DIABETES

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Abstract

Context. Different polymorphisms of the endothelial nitric oxide synthase gene (NOS3) have been related to diabetic kidney disease.

Objective. To evaluate the association between advanced diabetic chronic kidney disease (ACKD) and the rs1799983 and rs2070744 poymorphisms of NOS3 in a population from the Gran Canaria island.

Design. Cross-sectional case-control study.

Subjects and methods. Polymorphisms were genotyped in 152 subjects with ACKD secondary to type 2 diabetes [estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m^2], 110 subjects with type 2 diabetes for 20 or more years since diagnosis without ACKD (eGFR ≥45 mL/min/1.73m^2 and albumin/creatinine ratio <300 mg/g and/or 24-h urinary albumin excretion <300 mg) and 292 healthy controls. Association between both polymorphisms and established coronary heart disease (CHD) was also analyzed in both groups with diabetes.

Results. A greater proportion of homozygous individuals for the risk allele C of rs2070744 was found among subjects with ACKD. Association between ACKD and rs2070744 was observed in a recessive genetic model, both for comparison to subjects with diabetes but no ACKD [OR 2.17 (95% CI: 1.17-4.00), p=0.014] and for comparison to healthy controls [OR 1.61 (1.03-2.52), p=0.036]. The frequency of the C allele was significantly higher among subjects with CHD, but only in the group with ACKD. No associations were found for rs1799983.

Conclusions. NOS3 rs2070744 is associated with ACKD in population with type 2 diabetes from Gran Canaria. A link between this genetic variant and CHD in Canarian subjects with type 2 diabetes could be restricted to cases with ACKD.

Keywords: NOS3, endothelial nitric oxide synthase, type 2 diabetes, chronic kidney disease, diabetic nephropathy, coronary heart disease.

INTRODUCTION

Endothelial dysfunction is integrally involved in all stages of atherosclerosis and has been associated with an increased risk of onset and progression of cardiovascular disease (1). The main functional implication of endothelial dysfunction is the impairment of endothelial dependent vasodilatation, a process principally regulated by the production and release of nitric oxide in endothelial cells in response to shear stress. Nitric oxide diffuses into the vascular smooth muscle layer and stimulates cyclic GMP-mediated vasodilatation. In addition, it inhibits other key events in the development of atherosclerosis, such as the adhesion and migration of leukocyte to blood vessels, platelet aggregation and smooth muscle cell proliferation (2).

In the blood vessel wall, nitric oxide is generated from the metabolism of L-arginine by endothelial nitric oxide synthase (eNOS), one of the three isoforms of nitric oxide synthase (3). The gene encoding eNOS (NOS3) is located in chromosome 7q35-36 and consists of 26 exons with a total size of 21 kb (4). It has been extensively screened for variation and several polymorphisms have been found to influence plasmatic levels and endothelial production of nitric oxide (5). Three of them, in particular, have attracted much attention, as they have been consistently associated with cardiovascular disease (6, 7), namely the 4b/4a variant, a 27 bp-repeat VNTR in intron 4, and two single nucleotide polymorphisms (SNPs): G894T (rs1799983), which causes a substitution of 298Asp for 298Glu in exon 7, and T-786C (rs2070744) in the

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promoter region.

All three isoforms of nitric oxide synthase are expressed in the renal glomeruli, where nitric oxide plays an important role in sodium and water homeostasis and contributes to maintain normal vascular tone, regulating the normal pressure–natriuresis response and the tubuloglomerular feedback, inhibiting sodium tubular reabsorption and modulating the sympathetic nerve activity (8). As expected, NOS3 is mainly expressed in renal vascular endothelial cells, including the afferent and efferent arterioles (8), and genetic variants that reduce its transcription have been associated with hypertension (9), development and progression of different forms of nondiabetic renal disease (10-12), and a greater overall risk of end-stage chronic kidney disease (CKD) (13).

However, the relationship between NOS3 polymorphisms and CKD has been primarily studied in subjects with diabetes. According to the most recently published meta-analysis (14), based on a total of 49 case-control studies, the three main polymorphisms of NOS3 have been associated with increased susceptibility to diabetic nephropathy in subjects with type 2 diabetes, particularly the 4b/4a and G894T polymorphisms. However, according to this report (14), only six studies (15-20) have investigated the relationship between diabetic nephropathy and rs2070744, and none of them was performed on European population. Therefore, association studies between rs2070744 and diabetes-related CKD could be still of interest, particularly in less studied ethnic groups. The present investigation was aimed to evaluate the association between NOS3 rs1799983 and rs2070744 and the presence of advanced CKD among subjects with type 2 diabetes from the island of Gran Canaria, Spain. In addition, given both the known relationship between NOS3 variants and the development of atherosclerotic disease (6, 7) and the exceptionally high risk of coronary heart disease (CHD) in patients with diabetes and CKD (21), the association between both SNPs and the presence of CHD was also assessed on the same sample of subjects with type 2 diabetes, according to the presence or absence of CKD.

MATERIALS AND METHODS

Subjects

Study population was composed of participants from the Caracterización de la Enfermedad Renal Crónica Asociada a Diabetes (CERCA-Diabetes) Study, a survey directed to characterize type 2 diabetes-related advanced CKD in the Southern area of the Gran Canaria Island, Spain. Cases were all 152 subjects who attended a first consultation at the Advanced Chronic Kidney Disease Office of the Hospital Universitario Insular from February 2011 to February 2015, as long as they had an estimated glomerular filtration rate (eGFR) less than 30 mL/min/1.73 m^2 by MDRD-4 (Modification of Diet of Renal Disease) formula and the leading cause of CKD was determined to be type 2 diabetes. Controls were 110 patients with type 2 diabetes recruited from the Endocrinology Department of the same center with the following eligibility criteria: 20 or more years since diabetes diagnosis, eGFR Modification of Diet of Renal Disease-4 (MDRD) ≥ 45 mL/min/1.73m^2 and spot urine albumin/creatinine ratio < 300 mg/g and/or 24 h urinary albumin excretion < 300 mg. Additionally, NOS3 SNPs were also analyzed in samples of 292 healthy blood donors of 50 or more years of age from the same population, without diabetes or CKD.

Survey procedures

Blood, first morning void urine and 24 h urine samples were collected from subjects after nine hours fasting. Data collection sheet was recorded through a questionnaire covering sociodemographic information, lifestyle habits, personal and familial medical history and use of medication. Subjects were classified as smokers or non-smokers (including in the latter those who had quitted smoking). Physical activity was measured by asking about work-related and leisure time activities, using the questions designed for the 2006 Spanish National Health Survey. Subsequently, a clinical exam was performed. Blood pressure was taken twice, in a sitting position, using a manual sphygmomanometer and the mean of the two readings was used for statistical analysis. Diagnosis of diabetic retinopathy and cardiovascular disease was accepted when they were reported in the patient's medical record. Severe diabetic retinopathy was defined as proliferative retinopathy treated with panretinal photocoagulation or vitrectomy and/or clinically significant macular edema treated with focal laser photocoagulation or intravitreal angiogenesis inhibitors or glucocorticoids. CHD was defined by documented diagnosis of acute coronary syndrome and/or percutaneous or surgical coronary revascularization.

Biochemical analyses were performed at the Biochemistry Department of the Hospital Universitario Insular. Methodological details of the samples' management were published previously (22).

All participants signed an informed consent before their inclusion in the study. The study was

approved by the local Ethics Committee (case file CEIC-CHMI-491).

Genotyping

Genotyping services were provided by the Human Genotyping lab at Spanish National Cancer Research Centre. Genotyping was performed using OpenArray technology (Thermo Fisher Scientific, Massachusetts, USA) following manufacturer's instructions. Briefly, DNA samples were loaded into custom designed arrays including assays for rs1799983 and rs2070744, using OpenArray® AccuFill System (Thermo Fisher Scientific). QuantStudio™ 12K Flex system (Thermo Fisher Scientific) was used for samples amplification and fluorescent data collection. Hapmap samples with known genotype were included as internal control of the process. Genotypes were assigned using Taqman Genotyper Software (Thermo Fisher Scientific).

Statistical analyses

Categorical variables are expressed as frequencies (%) and continuous variables as mean and standard deviation (SD) when data followed a normal distribution, or as median and interquartile range (25^{th} – 75^{th} percentile) when distribution departed from normality. The percentages were compared, as appropriate, using the Chi-square ($\chi 2$) test or the exact Fisher test, the means by the t-test, and the medians by the Wilcoxon test for independent data. The Hardy-Weinberg equilibrium was assessed in the whole of the genotyped subjects, by the Chi-square test. The association between the studied SNPs (rs1799983 and rs2070744) and advanced CKD or CHD was evaluated by the codominant, dominant, recessive and additive inheritance patterns. In rs1799983, G was considered the normal allele and T the risk allele. In rs2070744, T was considered the normal allele and C the risk allele. Odds ratio (OR) and 95% confidence intervals (CI) were obtained for each of the allele inheritance models on each group by univariate logistic regression. Statistical significance was set at $p < 0.05$. Data were analyzed using the R package, version 3.3.1 (R Development Core Team, 2016) (23).

RESULTS

Main demographic and clinical characteristics of the study participants are summarized in Table 1. It was noteworthy that subjects with advanced CKD showed a more unfavorable cardiovascular risk profile (greater proportion of smokers, higher measures of blood pressure and waist circumference and worse levels of triglycerides and HDL cholesterol), and a greater prevalence of established CHD.

Genotype frequencies of the SNPs were in

Table 1. Demographic and clinical characteristics of the population with type 2 diabetes

	Advanced chronic kidney disease		
	Yes (N = 152)	No (N = 110)	P
Age (years)	69.6 ± 11.4	68.1 ± 8.3	0.228
Male sex (%)	57.2	36.4	< 0.001
Duration of diabetes (years)	18.8 ± 9.9	26.5 ± 6.0	< 0.001
Body Mass Index (kg/m^2)	32.1 ± 6.2	32.7 ± 6.2	0.430
Waist (cm)	111.2 ± 13.8	107.2 ± 13.4	0.021
Systolic blood pressure (mmHg)	148.7 ± 24.4	140.2 ± 22.1	0.004
Diastolic blood pressure (mmHg)	78.5 ± 11.3	72.8 ± 10.4	< 0.001
Hypertension (%)	99.3	88.2	< 0.001
eGFR* (mL/min/1.73 m^2)	22.3 (18.4 – 25.8)	72.9 (64.5 – 82.3)	< 0.001
Albumin/Creatinine (mg/g)	552 (138 – 2458)	0 (0 – 18)	< 0.001
Proteinuria (g/24 h)	1.4 (0.5 – 4.8)	0.1 (0.1 – 0.2)	< 0.001
HbA_{1c} (%)	7.3 (6.6 – 8.2)	7.5 (7.0 – 8.3)	0.032
Diabetic retinopathy (%)	58.5	61.5	0.636
Severe diabetic retinopathy (%)	48.7	47.3	0.824
Smoking (%)	16.9	3.9	0.005
Sedentary life style (%)	39.0	35.1	0.566
LDL cholesterol (mmol/L)	2.18 ± 0.91	2.01 ± 0.62	0.089
HDL cholesterol (mmol/L)	1.09 ± 0.34	1.20 ± 0.30	0.004
Triglycerides (mmol/L)	1.74 (1.33 – 2.45)	1.48 (1.08 – 1.77)	< 0.001
Metabolic syndrome (%)	94.0	84.9	0.032
Coronary heart disease (%)	35.1	19.3	0.005

*eGFR: estimated glomerular filtration rate.

agreement with the Hardy-Weinberg equilibrium (p = 0.461 for rs1799983 and 0.639 for rs2070744).

Table 2 shows allelic and genotypic frequencies of NOS3 SNPs in both groups of subjects with type 2 diabetes and in the control group of healthy blood donors. There were no significant differences for rs1799983. Nonetheless, for rs2070744, and compared to the two other groups, the proportion of homozygous subjects for the risk allele C was greater in the group with type 2 diabetes and advanced CKD (30.5% *vs.* 16.8% in the group with diabetes without advanced CKD and *vs.* 21.4% in healthy blood donors; p = 0.021 and p = 0.022, respectively). In both comparisons, the association between CKD and rs2070744 fit to a recessive inheritance model (Table 3).

As described above, the prevalence of established CHD was significantly higher among participants with advanced CKD. The demographic and clinical characteristics of subjects with or without CHD are provided separately for both groups of subjects with diabetes, as supplementary material. Tables 4 and 5 show allelic and genotypic frequencies of rs1799983 and rs2070744, respectively, for subjects with advanced CKD and for subjects without advanced CKD, according to the presence or absence of established CHD. The frequencies of both the CC genotype and the C allele of rs2070744 were significantly higher among subjects with established CHD in the group with advanced CKD, but not in the group without advanced CKD. Also, in this case, the association between CHD and rs2070744 genotype was best explained by recessive pattern of inheritance (data

Table 2. Allelic and genotypic frequencies of rs2070744 and rs1799983 SNPs in analyzed study groups

	Type 2 diabetes with advanced CKD* (N = 152)	Type 2 diabetes without advanced CKD* (N = 110)	P**	General population without diabetes or CKD* (N = 292)	P**
rs2070744					
Genotypes					0.022
TT	43 (28.5)	29 (27.1)	0.021	70 (24.1)	
CT	62 (41.1)	60 (56.1)		158 (54.5)	
CC	46 (30.5)	18 (16.8)		62 (21.4)	
Alleles					0.504
T	148 (49.0)	118 (55.1)	0.170	298 (51.4)	
C	154 (51.0)	96 (44.9)		282 (48.6)	
rs1799983					
Genotypes					
GG	63 (42.9)	53 (50.5)	0.467	113 (42.0)	0.575
GT	66 (44.9)	42 (40.0)		131 (48.7)	
TT	18 (12.2)	10 (9.5)		25 (9.3)	
Alleles					
G	192 (65.3)	148 (70.5)	0.222	357 (66.4)	0.760
T	102 (34.7)	62 (29.5)		181 (33.6)	

*CKD: chronic kidney disease. **P values correspond to the comparison against the group with type 2 diabetes and advanced chronic kidney disease.

Table 3. Association between rs2070744 and type 2 diabetes-related advanced chronic kidney disease, according to genetic model of inheritance

		Type 2 diabetes with advanced CKD* (N = 152)	Type 2 diabetes without advanced CKD* (N = 110)	P**	OR (95% CI)	General population without diabetes or CKD* (N = 292)	P**	OR (95% CI)
Codominant	TT	28.5	27.1	-	1 (Ref)	24.1		1
	TC	41.1	56.1	0.230	0.70 (0.39 - 1.26)	54.5	0.067	0.64 (0.40 - 1.03)
	CC	30.5	16.8	0.139	1.72 (0.84 - 3.54)	21.4	0.492	1.21 (0.71 - 2.07)
Dominant	TT	28.5	27.1	0.808	1 (Ref)	24.1	0.322	1
	TC/CC	71.6	72.9		0.93 (0.54 - 1.62)	75.9		0.80 (0.51 - 1.25)
Recessive	TT/TC	69.6	83.2	0.014	1	78.6	0.036	1
	CC	30.5	16.8		2.17 (1.17 - 4.00)	21.4		1.61 (1.03 - 2.52)
Additive	-	-	-	0.182	1.26 (0.90 - 1.78)	-	0.504	1.10 (0.83 - 1.45)

*CKD: chronic kidney disease. **P values correspond to the comparison against the group with type 2 diabetes and advanced chronic kidney disease.

for other genetic models are not shown). No significant differences were found for rs1799983.

DISCUSSION

The present study found that Canarian subjects with advanced CKD secondary to type 2 diabetes have a higher prevalence of the CC genotype of the NOS3 rs2070744 polymorphism than subjects with long-standing type 2 diabetes without advanced CKD or than healthy controls without diabetes or CKD. On the whole, these findings are in accordance with the results of two previous meta-analyses (14, 24) that included studies performed on populations from different ethnic groups, although none of them from European countries. In particular, the results are consistent with studies conducted on populations with both type 1 and type 2 diabetes, in which subjects with end-stage or advanced CKD were primarily compared to controls without diabetic nephropathy (15, 17, 20). In contrast, the association between rs2070744 and kidney disease was found to be weaker, or even non-existent, when cases with CKD included subjects with milder forms of diabetic nephropathy (e.g., subjects with microalbuminuria) (18, 19). This suggests that this genetic variant of NOS3 may be a risk factor particularly for the development of severe and more progressive diabetes-related CKD. In line with this, different *in vitro* and *in vivo* evidences (25-27) suggest that the production of nitric oxide is increased in initial phases of diabetic nephropathy, which could contribute to the emergence of some of the characteristic alterations of early kidney disease secondary to diabetes, such as renal vasodilatation, hyperfiltration and microalbuminuria. In contrast, nitric oxide bioavailability decreases in subjects with long-standing diabetes and more advanced stages of CKD (27), so that the impact of genetic variants that reduce nitric oxide availability is likely to be greater in the setting of advanced CKD. In fact, while most murine models of diabetic nephropathy only develop manifestations of early human disease, the eNOS-deficient (eNOS

Table 4. Characteristics of subjects with type 2 diabetes and advanced chronic kidney disease, according to the previous diagnosis of coronary heart disease

	No CHD N = 98	CHD N = 53	P
Age, years	68.6 ± 12.5	71.7 ± 8.7	0.11
Sex male, %	54 (54.5)	33 (62.3)	0.359
Diabetes duration, years	20 (12 - 27)	17 (11 - 25)	0.243
Body mass index, kg/m^2	31.9 ± 7.0	32.5 ± 4.6	0.593
Waist, cm	109.8 ± 14.9	113.8 ± 11.0	0.089
Sedentary lifestyle, %	41 (41.8)	17 (32.1)	0.239
Current smoking, %	18 (18.2)	7 (13.2)	0.430
Systolic blood pressure, mmHg	150.1 ± 25.2	146.2 ± 22.9	0.341
Diastolic blood pressure, mmHg	80.1 ± 10.5	75.7 ± 12.1	0.022
Hypertension, %	98 (99.0)	52 (100)	1
LDL-cholesterol, mg/dL	85.3 ± 37.2	82.4 ± 31.3	0.631
HDL-cholesterol, mg/dL	43.6 ± 14.1	39.0 ± 10.0	0.038
Triglycerides, mg/dL	145 (113 - 207)	170 (130 - 226)	0.115
Metabolic syndrome, %	89 (91.8)	50 (100)	0.051
Diabetic retinopathy, %	59 (59.6)	30 (56.6)	0.721
Severe diabetic retinopathy, %	49 (50.5)	24 (45.3)	0.540
HbA_{1c}, %	7.2 (6.6 - 8.1)	7.5 (6.4 - 8.6)	0.376
eGFR, mL/min/1.73 m^2	21.7 (18.1 - 25.6)	23.3 (19.5 - 27.0)	0.128
Serum creatinine, mg/dL	2.7 (2.4 - 3.0)	2.6 (2.3 - 2.9)	0.297
Urine albumin/creatinine ratio, mg/g	929 (166 - 3084)	386 (74 - 1015)	0.026
Albumin, g/dL	3.9 (3.7 - 4.1)	4.0 (3.7 - 4.2)	0.108
Serum potassium, mmol/L	4.7 (4.3 - 5.1)	4.7 (4.3 - 5.2)	1
Serum calcium, mg/dL	9.2 (8.9 - 9.8)	9.4 (8.9 - 9.7)	0.354
Serum phosphate, mg/dL	4.1 (3.5 - 4.7)	3.9 (3.4 - 4.4)	0.138
PTH, pg/mL	147 (100 - 203)	154 (95 - 243)	0.557
25OHD, ng/mL	12.0 (7.3 - 22.2)	12.0 (7.5 - 19.0)	0.744
Uric acid, mg/dL	6.6 (5.8 - 7.4)	7.1 (5.9 - 8.5)	0.202
Hemoglobin, g/dL	11.4 (10.4 - 12.8)	11.4 (10.5 - 12.8)	0.829

*eGFR: estimated glomerular filtration rate. Data are means ± SD, medians (IQR) and frequencies (%).

−/−) diabetic mice exhibit pronounced albuminuria, renal insufficiency and histological changes typical of advanced diabetic nephropathy (28).

Our results also indicated that the association between diabetes-related CKD and the rs2070744 is best explained by a recessive inheritance model. This finding is in agreement with a meta-analysis that examined the association between rs2070744 and diabetic CKD, analyzing different genetic models separately by ethnic group (24). In this study, an association was found in the recessive model for white population, while the association was significant for other types of inheritance (additive, dominant and co-dominant) for African population.

Regarding rs1799983, similar to the results of meta-analyses of studies performed on white populations (14, 24), we found no association between this variant and advanced type 2 diabetes-related CKD. For unknown reasons, however, this polymorphism has been found to be strongly associated with diabetic nephropathy in other ethnic groups, such as Africans and Asians (14, 24).

Additionally, as a secondary objective, the present study assessed whether rs2070744 and rs1799983 could have a different degree of association with CHD in people with type 2 diabetes, depending on the presence or absence of advanced CKD. Patients with CKD typically have endothelial dysfunction and increased oxidative stress, which also progress as kidney function deteriorates (29). The availability of nitric oxide is one of the main causes of endothelial dysfunction and can occur in CKD through several mechanisms, including a deficiency in the availability of L-arginine, the substrate necessary for its production, an increase in the circulating levels of substances that inhibit its synthesis, such as asymmetric dimethylarginine, and a decrease in the abundance of neuronal nitric oxide synthase in the renal cortex (30). Since endothelial dysfunction increases the risk of cardiovascular complications, even independently of the effect of other conventional risk factors (1), this could be one of the causes of the elevated rate

Table 5. Characteristics of the diabetics without chronic kidney disease, according to the previous diagnosis of coronary heart disease

	No CHD N = 89	CHD N = 21	P
Age, years	68.5 ± 8.3	70.6 ± 22.3	0.471
Sex male, %	28 (31.5)	12 (57.1)	0.028
Diabetes duration, years	25 (21 - 32)	28 (23 - 29)	0.425
Body mass index, kg/m^2	33.0 ± 6.4	31.6 ± 5.2	0.362
Waist, cm	107.4 ± 13.9	106.7 ± 11.2	0.830
Sedentary lifestyle, %	26 (29.2)	8 (38.1)	0.428
Current smoking, %	3 (3.4)	0 (0.0)	1
Systolic blood pressure, mmHg	141.2 ± 22.5	135.7 ± 20.3	0.305
Diastolic blood pressure, mmHg	73.9 ± 9.9	68.3 ± 11.5	0.028
Hypertension, %	79 (88.8)	18 (85.7)	0.711
LDL-cholesterol, mg/dL	79.8 ± 23.5	68.5 ± 24.1	0.051
HDL-cholesterol, mg/dL	47.6 ± 11.4	42.2 ± 11.0	0.050
Triglycerides, mg/dL	133 (101 - 159)	118 (96 - 148)	0.486
Metabolic syndrome, %	76 (87.4)	19 (95.0)	0.457
Diabetic retinopathy, %	50 (56.2)	17 (85.0)	0.017
Severe diabetic retinopathy, %	40 (44.9)	12 (57.1)	0.314
HbA_{1c}, %	7.6 (7.0 - 8.4)	7.5 (7.0 - 8.2)	0.706
eGFR, mL/min/1.73 m^2	74 (67 - 82)	67 (55 - 79)	0.108
Serum creatinine, mg/dL	0.9 (0.8 - 1.0)	1.0 (0.9 - 1.1)	0.012
Urine albumin/creatinine ratio, mg/g	0 (0 - 17.0)	8.6 (0.0 - 30.0)	0.292
Albumin, g/dL	4.3 (4.1 - 4.5)	4.2 (4.0 - 4.3)	0.195
Serum potassium, mmol/L	4.4 (4.2 - 4.7)	4.5 (4.3 - 4.8)	0.500
Serum calcium, mg/dL	9.7 (9.4 - 10.0)	9.5 (9.2 - 9.9)	0.207
Serum phosphate, mg/dL	3.4 (3.1 - 3.6)	3.4 (3.2 - 3.8)	0.822
PTH, pg/mL	46.4 (34.7 - 61.9)	42.6 (37.3 - 56.2)	0.962
25OHD, ng/mL	22.0 (15.2 - 30.0)	23.8 (19.1 - 28.4)	0.485
Uric acid, mg/dL	5.2 (4.2 - 6.3)	5.7 (5.0 - 6.6)	0.131
Hemoglobin, g/dL	13.2 (12.4 - 14.0)	12.5 (11.4 - 13.2)	0.209

*eGFR: estimated glomerular filtration rate. Data are means ± SD, medians (IQR) and frequencies (%).

of cardiovascular disease among people with CKD, even more so in subjects with diabetes-related CKD, as diabetes, per se, is also accompanied by endothelial dysfunction (31).

However, while the evidence linking CHD to NOS3 polymorphisms associated with decreased nitric oxide production has been definitely established in recent meta-analyses analyzing tens of thousands of subjects (6, 7), only a handful of studies have evaluated this association specifically in populations with type 2 diabetes, and their results are conflicting. The rs1799983 polymorphism has been the most studied. A prospective (32) and two cross-sectional studies (33, 34) found an association between this variant of

Table 6. Allelic and genotypic frequencies of rs2070744 and rs1799983 SNPs in subjects with diabetes-related advanced chronic kidney disease, according to the presence or absence of coronary heart disease

		No coronary heart disease N = 98	Coronary heart disease N = 53	p
rs1799983				
	Genotypes			0.129
	GG	46 (48.9)	17 (32.1)	
	GT	37 (39.4)	29 (54.7)	
	TT	11 (11.7)	7 (13.2)	
	Alleles			0.112
	G	129 (68.6)	63 (59.4)	
	T	59 (31.4)	43 (40.6)	
rs2070744				
	Genotypes			0.009
	TT	36 (36.4)	7 (13.5)	
	CT	38 (38.4)	24 (46.2)	
	CC	25 (25.3)	21 (40.4)	
	Recessive genetic model			0.055
	TT or CT	74 (74.7)	31 (59.6)	
	CC	25 (25.3)	21 (40.4)	
	Alleles			0.002
	T	110 (55.6)	38 (36.5)	
	C	88 (44.4)	66 (63.5)	

Data are frequencies (%).

Table 7. Allelic and genotypic frequencies of rs2070744 and rs1799983 SNPs in subjects with diabetes without advanced chronic kidney disease, according to the presence or absence of coronary heart disease

		No coronary heart disease N = 89	Coronary heart disease N = 21	P
rs1799983				
	Genotypes			0.319
	GG	41 (48.8)	12 (57.1)	
	GT	33 (39.3)	9 (42.9)	
	TT	10 (11.9)	0	
	Alleles			0.198
	G	115 (68.5)	33 (78.6)	
	T	53 (31.5)	9 (21.4)	
rs2070744				
	Genotypes			0.568
	TT	22 (25.6)	7 (33.3)	
	CT	48 (55.8)	12 (57.1)	
	CC	16 (18.6)	2 (9.5)	
	Recessive genetic model			0.516
	TT or CT	70 (81.4)	19 (90.5)	
	CC	16 (18.6)	2 (9.5)	
	Alleles			0.326
	T	92 (53.5)	26 (61.9)	
	C	80 (46.5)	16 (38.1)	

Data are frequencies (%).

NOS3 and CHD. The first of them (32) was carried out specifically in subjects with CKD. In contrast, as occurred in our study, other investigations failed to confirm a relationship between CHD and rs1799983 among subjects with type 2 diabetes (35-37). The rs2070744 has been less studied. Zhang *et al.* (35) found no relationship between rs2070744 and CHD in US men with type 2 diabetes, while Narne *et al.* (36) observed that the C allele was associated with the presence and severity of CHD in Indian patients. No studies, to our knowledge, have analyzed the association between rs2070744 and CHD in patients with diabetes, depending on whether or not they had CKD. Our data suggest that the impact of this NOS3 variant on the risk of CHD might be greater in subjects with advanced CKD.

In conclusion, this study suggests that, in population with type 2 diabetes from Gran Canaria island, the NOS3 rs2070744 is associated with advanced CKD. Additionally, this genetic variant could be also associated with CHD, but only when advanced CKD is present. The rs1799983 variant of NOS3 was not associated with CKD or CHD in this population.

Conflict of interest

The authors declare that they have no conflict of interest.

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