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Short communication

Association between a *MYH9* polymorphism (rs3752462) and renal function in the Spanish RENASTUR cohort

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ABSTRACT

The *MYH9* gene encodes a protein that is expressed in the kidney glomerular podocytes. *MYH9* single nucleotide polymorphisms (SNPs) have been linked to the risk for chronic kidney disease (CKD) and end stage renal disease. Our aim was to determine whether *MYH9* SNPs were associated with renal disease in Spanish Caucasians. The RENASTUR cohort consisted of 592 Spanish Caucasians, aged 55–85 years. They were genotyped for SNPs rs3752462 and rs4821480, which tagged haplotype E. The main values between individuals with a glomerular filtration rate (eGFR) <60 and ≥60 ml/min/1.73 m^2 were statistically compared. The next variables were significantly associated with the eGFR in the univariate analysis: age, gender, type 2 diabetes, total cholesterol, total LDL-cholesterol, and the *MYH9* rs3752462 (TC + TT genotypes; $p = 0.003$). This SNP remained significantly associated with the eGFR in the multivariate analysis.

In conclusion, SNP rs3752462 was an independent predictor of reduced eGFR in the Spanish RENASTUR population. The genotyping of this *MYH9* SNP could help to identify individuals at risk of developing CKD.

1. Introduction

The estimated glomerular filtration rate (eGFR) is commonly used to define renal function and identify individuals with chronic kidney disease (CKD). The Kidney Disease Outcome Quality Initiative (K/DOQI) defined CKD as the presence of renal impairment with an eGFR < 60 (National Kidney Foundation, 2002). Based on this definition the EPIRCE study (Epidemiology of Chronic Kidney Disease in Spain) estimated that approximately 10% of the Spanish adult population had some degree of CKD, and similar values were also estimated from other epidemiological studies (Coresh et al., 2007; Otero et al., 2010). CKD is a strong predictor of end stage renal disease (ESRD), cardiovascular morbidity, and mortality. In addition to well known risk factors such as hypertension and diabetes, recent genome wide association studies (GWAs) linked several single nucleotide polymorphisms (SNPs) to eGFR and prevalent CKD (Köttgen et al., 2009, 2010). Some of these SNPs were also associated with the progression to ESRD (Böger et al., 2011).

The *MYH9* gene encodes a myosin heavy chain protein expressed in glomerular podocytes and mesangial cells (Marini et al., 2006). Recent studies linked *MYH9* SNPs/haplotypes to the risk of developing focal segmental glomerulosclerosis (FSGS), hypertensive nephropathy, and non-diabetic ESRD among African and Hispanic Americans (Behar et al., 2010; Freedman et al., 2009a, 2009b; Kao et al., 2008; Kopp et al., 2008). This association was not replicated among American Indians, a fact that could be partly explained by differences in the prevalence of risk factors for CKD between the populations (Franceschini et al., 2010).

Among individuals of European ascent, *MYH9* polymorphisms have been linked to kidney function, non-diabetic and diabetic nephropathy, and idiopathic FSGS (Cooke et al., 2012; Kao et al., 2008; O'Seaghdha et al., 2011; Pattaro et al., 2009). Our objective was to define

Abbreviations: *MYH9*, myosin heavy chain 9 gene; SNPs, single nucleotide polymorphisms; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; BMI, body mass index; MDRD, Modification of Diet in Renal Disease; RFLP, restriction fragment length polymorphism; HTA, hypertension; T2DM, type 2 diabetes mellitus.

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the effect of common *MYH9* SNPs on renal function in a Spanish cohort of healthy elderly individuals.

2. Material and methods

2.1. Study population and data collection

This study was approved by the Ethical Committee of Hospital Universitario Central de Asturias (HUCA) and all the participants signed an informed consent. The RENASTUR was a cross-sectional study to evaluate the association between gene polymorphisms and renal function in elderly people. A total of 592 individuals aged 55–85, all Caucasian, non-related, and from the region of Asturias (Northern Spain, total population one million), were randomly chosen from the general population in the period 2010–2012. All the participants were evaluated by a qualified physician, who was also responsible for obtaining the main analytical and clinical values (Table 1).

Smoking (ever/never smoked) was self reported. Body mass index (BMI) was defined by weight and height measured at the exam. Blood pressure values were obtained at examination. Blood glucose, serum creatinine (SCr), total serum cholesterol, high and low density lipoprotein cholesterol, and triglycerides were measured from blood samples collected by venipuncture. Individuals with a documented history of hypertension or diabetes or who were treated with antihypertensive or antidiabetic drugs were classified as hypertensives and diabetics, respectively. The biochemical profiles from fasting blood samples were obtained by routine methods. The estimated glomerular filtration rate (eGFR) was calculated with the Modification of Diet in Renal Disease (MDRD) formulae (Levey et al., 1999).

2.2. MYH9 genotyping

DNA was obtained from 5 ml of blood leukocytes, and two *MYH9* SNPs (rs3752462 and rs4821480) were genotyped through a Polymerase Chain Reaction-restriction fragment length polymorphism (PCR-RFLP) method (Supplementary Fig. 1). These SNPs defined four haplotypes (E1–E4) that have been linked to nephropathies (Kao et al., 2008; Kopp et al., 2008; Oleksyk et al., 2010). Briefly, for the rs3752462 a 421 bp fragment was amplified with primers CCAGGAGCATCCGGGCTCTA (forward) and CACCTCCACAACCAACACAGAGCT (reverse). After digestion with *RsaI*, reactions were electrophoresed on 3% agarose gels and the two alleles were visualized as bands of 243 + 178 bp (C allele) and 243 + 92 + 86 bp (T allele). For the rs4821480 the DNA was PCR amplified with primers CCGCTGGGCAGGGGTGTT (forward) and TCTTCTGTG AGGTTGGT GGTG (reverse), followed by digestion with *DraI* and electrophoresis on agarose gels to visualize the two alleles of 537 bp (G allele) and 322 + 215 bp (T allele). To confirm the accuracy of this genotyping method, PCR fragments from several individuals with each of the RFLP-genotypes were sequenced using BigDye chemistry in an ABI3130 automated system (Applied Biosystems).

2.3. Statistical analysis

CKD cases were defined as those with an eGFR < 60 ml/min/1.73 m^2, while healthy controls were those free of CKD at baseline. All the statistical analyses were done with the SPSS statistical software (version 17.0; SPSS Inc., Chicago, IL). The Fisher's exact and student's t tests were used to compare categorical and continuous variables between the groups, respectively. The multivariate regression analysis was performed to determine the independent effect of the variables. p values < 0.05 were considered statistically significant. Haplotype frequencies were calculated online (cubic exact solutions for the estimation of pairwise haplotype frequencies; http://www.oege.org/software/cubex).

3. Results

Table 1 summarizes the main characteristics in the whole RENASTUR cohort, and in those with eGFR < 60 (n = 77) and ≥60 (n = 515) ml/min/1.73 m^2. The genotype frequencies for the two *MYH9* SNPs did not deviate from Hardy–Weinberg equilibrium in the two groups.

In the univariate analysis the next variables were significantly associated with an eGFR < 60: age ($p < 0.001$), male ($p = 0.04$), type 2 diabetes ($p < 0.001$), total cholesterol ($p = 0.03$), LDL-cholesterol ($p = 0.05$), and *MYH9* rs3752462 T allele (dominant effect, TC + TT genotypes; $p = 0.003$). Hypertensives and smokers were non-significantly more frequent in the eGFR < 60 group. In the multivariate logistic regression analysis age, diabetes, gender, and the *MYH9* rs3752462 genotype remained as independent risk factors for eGFR < 60 ml/min/1.73 m^2 (Table 2). *MYH9* rs3752462 T-carriers had lower mean eGFR compared to CC homozygotes (77 ± 18 vs. 83 ± 19; $p = 0.02$). The *MYH9* rs4821480 G allele was more frequent in the eGFR < 60 group (0.12 vs. 0.08), although the difference was not statistically significant. The most common E2 haplotype (rs4821480 T/rs3752462 C) showed a significantly higher frequency in the eGFR ≥ 60 group ($p = 0.04$) (Table 3). This was in agreement with the reported protective effect for this haplotype among Caucasians.

To determine whether the rs3752462 SNP had an effect on pre-mRNA splicing we amplified the cDNA from six heart tissues with primers that matched exons 12 and 14. Only a single PCR band was visualized from all the tissues, including the two that were heterozygotes for this SNP. Sequencing of the amplified fragments showed a normal transcript in all the samples (Supplementary Fig. 2). This suggested that the association between rs3752462 and CKD was likely not due to a direct effect pre-mRNA splicing.

4. Discussion

CKD is a major cause of morbidity and mortality, and is thus considered an important community health problem. According to the EPIRCE study approximately 10% of the Spanish adult population had some degree of CKD (Otero et al., 2010). This was in agreement with our findings in the RENASTUR cohort, with 10% of the participants showing an eGFR < 60 ml/min/1.73 m^2. The search for markers associated with CKD is important to identify individuals at risk for ESRD and cardiovascular events, which could benefit from preventive approaches. It is a well established fact that renal function declines with age (Fox et al., 2004). In addition, several gene polymorphisms have been linked to impaired renal function and CKD. Mutations in the *MYH9* gene cause the Fechtner and Epstein syndromes, both resulting in nephritis to varying degrees (Arrondel et al., 2002; Bostrom and Freedman, 2010; Seri et al., 2000).

Recent studies linked *MYH9* SNPs/haplotypes to the risk of developing FSGS, hypertensive nephropathy, diabetic and non-diabetic nephropathy, and lupus nephritis (Behar et al., 2010; Cooke et al., 2012; Freedman et al., 2009a, 2009b; Kao et al., 2008; Kopp et al., 2008; Lin et al., 2012; O'Seaghdha et al., 2011; Pattaro et al., 2009). We found a significant association between rs3752462 and a reduced eGFR among individuals without a previous diagnosis of CKD. In the multivariate analysis this association was independent of other risk factors, such as age and diabetes. The *MYH9* SNP rs4821480 was previously linked to CKD among individuals of African ancestry but was not associated with a reduced eGFR in our cohort. Due to the very low frequency (0.08) of the risk allele in our population, to exclude an association to CKD a much large number of individuals with impaired renal function should be required: for a power of 80 at a $p = 0.05$, a total of 4213 individuals (548 with an eGFR < 60). Haplotype frequencies were similar to that reported from other Caucasian populations (Oleksyk et al., 2010). In agreement with previous findings the most common E2 haplotype showed a protective effect in our population.