Clinical and genetic bases of hypertensive nephrosclerosis. NEFROSEN Study

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ABSTRACT

Background: Hypertensive nephrosclerosis is a chronic kidney disease (CKD) associated withto essential hypertension. The lack of correlation between hypertension control and progression to end-stageof CKD suggests an intrinsic and primitive disease. New evidence suggests that *MYH9* gene alterations are associated with polymorphisms in African Americans. The aim of this study is to investigate whether a polymorphism of *MYH9* in Caucasians is linked to essential hypertension and nephrosclerosis. The secondary objective is to identify the clinical risk factors of progression to end-stage renal disease (ESRD)end-stage CKD. This is a retrospective study that will compare patients with nephrosclerosis *versus* and essential hypertensives without renal disease, and also patients with nephrosclerosis and impaired renal function *versus* with those that are stable. **Method:** Between October 2009 and October 2010, 500 patients with stages 3-5 CKD attributed to nephrosclerosis according to usual clinical criteria, and 300 essential hypertensives (eGFR>60mL/min/1.73m^2; microalbuminuria <300mg/g) are to be recruited. A total of 200 healthy controls from the general population are also to be included for the genetic study. There are two study sections, being the first and final visits to the clinic (for stage 5 cases, the start of replacement therapy will be the end of follow-up). Clinical and laboratory data will be recorded, and blood samples will be collected. **Discussion:n** Our study will aim to determine if there exists is a relationship between the diagnosis of nephrosclerosis and the *MYH9* gene in Caucasiansthe Caucasia race, and to study possible risk factors for progression to ESRDend-stage CKD, on both clinical and genetic baseis.

Key words: Hypertensive nephrosclerosis. Essential hypertension. MYH9 gene. Chronic kidney disease.

Bases clínicas y genéticas de la nefroesclerosis hipertensiva. Estudio NEFROSEN

RESUMEN

Justificación: *Se conoce como nefroesclerosis la enfermedad renal crónica (ERC) que complica la hipertensión arterial (HTA) esencial. La ausencia de correlación entre el control de la HTA y la progresión a ERC terminal sugiere la existencia de una enfermedad intrínseca y primitiva. Recientemente se ha asociado con polimorfismos del gen MYH9 en individuos afroamericanos. El objetivo del trabajo que presentamos es determinar si algún polimorfismo de dicho gen se relaciona en raza caucásica con la asociación de HTA esencial y nefroesclerosis y, además, conocer los marcadores de progresión a ERC terminal. Será un estudio retrospectivo que comparará a pacientes con nefroesclerosis frente a pacientes con HTA esencial sin enfermedad renal y, además, se incluirán pacientes con nefroesclerosis y progresión de la enfermedad renal frente a los que se mantienen estables.* ***Métodos:*** *Entre octubre de 2009 y octubre de 2010 se incluirán 500 pacientes con ERC (estadios 3-5) atribuida a nefroesclerosis según criterios clínicos habituales, y 300 pacientes afectados de HTA esencial (FGe >60 ml/min/1,73 m^2; microalbuminuria <300 mg/g). Para el estudio genético también se incluirán 200 controles sanos de población general. Habrá dos cortes del estudio, la primera visita en el hospital y la visita final (en esta-*

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dio 5 el inicio del tratamiento sustitutivo constituirá el final del seguimiento). Se registrarán datos clínicos y analíticos, y se recogerán muestras de sangre para el estudio genético. ***Discusión:*** *Nuestro estudio, con la doble vertiente genética y clínica, tratará de determinar si en la raza caucásica existe relación entre el diagnóstico de nefroesclerosis y el gen MYH9, y estudiará, además, los posibles marcadores de progresión.*

Palabras clave: *Nefroesclerosis. Hipertensión arterial esencial. Gen MYH9. Enfermedad renal crónica.*

INTRODUCTION

The term nephrosclerosis is usually used for kidney disease which complicates arterial hypertension and that primarily affects the preglomerular microvasculature.[1,2] In practice, it is an entity with vague clinical profiles that groups hypertensive patients with chronic kidney disease (CKD) without other identifiable causes of disease.[3,4]

In nephrosclerosis, also known as benign nephroangiosclerosis or hypertensive nephropathy, the most characteristic microscopic lesion is hyalinosis of the afferent arterioles. Vascular changes produce vasoconstriction, glomerular ischaemia (retraction of glomerular tuft, focal or global sclerosis) and in some areas, interstitial fibrosis and tubular atrophy. Other authors point out that the hyalinisation of afferent arterioles would initially cause vasodilatation, glomerular hypertrophy and, in the long-term, segmental glomerulosclerosis lesions that overall favour the appearance of proteinuria and progression of the disease. These alterations are more frequent and severe in black patients, unrelated to the control of blood pressure or the degree of proteinuria. In fact, nephrosclerosis is a form of intrarenal renovascular disease, and in some cases may represent a magnification of the changes of kidney aging, from both a histological and clinical standpoint.[5-7]

Its causal relation with essential hypertension is still a subject of debate. It is not clear that treated arterial hypertension can lead to end-stage CKD.[8-11] Therefore, some authors have postulated that renal structural alterations may precede hypertension, and that it would be an intrinsic process of the renal microvasculature with loss of self-regulation that would mainly result in excessive preglomerular vasoconstriction[4] or persistent vasodilation of the afferent arteriole.[6,7] A chronically impaired renal plasma flow, in the long run, leads to hypertension and CKD. Nephroangiosclerosis could have the same clinical significance as atherosclerosis in coronary or cerebral vessels.[2,3]

In the US, Europe and Spain, vascular kidney disease is the second most common cause of terminal CKD. However, the diagnosis of nephrosclerosis is usually made by exclusion, when no data for another type of kidney disease is found, and in very few cases is based on histological findings. There are no specific signs or symptoms, but there are some suggestive clinical findings (men aged 55-60 years, long-standing hypertension, left ventricular hypertrophy [LVH], mild CKD and proteinuria less than 0.5-1g/24 hours). As with diabetic kidney disease, a kidney biopsy is almost never performed. This attitude may be reasonable in many cases, but is a source of misdiagnoses.[12]

Some studies with small samples suggest that the degree of proteinuria may be variable, and may even reach the nephrotic range. However, there is general consensus that subnephrotic proteinuria values are typical of hypertensive nephrosclerosis. Clinical inclusion criteria for the African American Study of Kidney Disease and Hypertension (AASK) required a proteinuria level lower than 2.5 (UP/Cr).[4,13,14] End-stage CKD onset age for African-Americans varied between 45 and 64 years, while for Caucasian Americans it is over 65 years.[4]

Compared with early glomerular or diabetic kidney diseases, the progression of kidney failure is slow in most cases, especially in whites. Renal function can remain stable for years if hypertension is well controlled. However, in a few cases the disease progresses to end-stage CKD.[5,12,15] Vascular kidney disease is the most common cause of hospital visits for CKD in nephrology departments in Spain. Up to 39% of cases have this aetiology, higher than diabetic or glomerular kidney disease (20%).[16] Despite the small percentage of patients with progression, its high prevalence makes it the second leading cause of end-stage CKD.

The factors for progression of nephrosclerosis are not well recognised, which hampers the implementation of preventive measures. Usually, firstly the black race is cited, and later age, the degree of kidney failure at diagnosis, the level of systolic blood pressure (SBP) and the degree of proteinuria.[1,13-20] In the AASK study, patients with proteinuria below 0.3g/24 hours, who had received ramipril, an angiotensin-converting enzyme inhibitor (ACEI) from the start had slower progression after an 11 year follow-up. This same study showed that old age (over 70 years) was inversely correlated with kidney failure.[20,21]

Only some cases of whites, perhaps those who are genetically predisposed, have an unfavourable clinical evolution. The progression of the disease has been associated with the concomitant presence of atherosclerotic lesions in the aorta and main renal arteries, and with processes such as Type 2 diabetes mellitus, hyperuricaemia and dyslipidaemia.[3,5,10,19] In the Norwegian HUNT 2 population study, progression to end-stage CKD was associated with the estimated glomerular filtration rate

(eGFR) and basal microalbuminuria in the multivariate analysis after 10 years of follow-up.[22]

In the last decade, the disease is being diagnosed mainly in patients older than 65-70 years of age and with vascular disease in other parts of the body. In these cases, nephrosclerosis is observed as a diffuse atherosclerosis of the renal arterioles.[19,23,24] Recently, a lesser-known fact has been pointed out: the presence of concomitant cardiovascular disease is a factor for progression to kidney failure.[25-29] Levin et al studied a group of 313 patients with kidney failure (eGFR of 10-75ml/min) and a mean follow-up of 23 months. They found that patients with cardiovascular diseases (ischaemic heart disease, cerebrovascular disease, peripheral artery disease or heart failure) increased the risk of progression to end-stage CKD (relative risk [RR] 1.58, *P*=.047).[25] Hillegas et al's study of 298 patients who had an acute myocardial infarction (AMI), found that the eGFR had declined 5.4ml/min after the first year, much higher than the usual 1ml/min/year reported for the general population.[28] Elsayed et al studied 13,826 subjects, of which 18.5% were African American, enrolled in the Atherosclerosis Risk in Communities Study and Cardiovascular Health Study trials. They found that after 9 years of follow-up, patients with cardiovascular disease at baseline were significantly more likely to develop CKD (odds ratio [OR] 1.75, *P*<.001).[29]

In Spain there is an ongoing prospective study supported by the Spanish Society of Nephrology (SEN), the "*ESTUDIO PRONEFROS*", which aims to determine the proportion of patients with nephrosclerosis showing progression to kidney failure. "Historical" nephrosclerosis cases have been excluded and only incident cases (n=430) over one year have been included. After 2 years of follow-up, preliminary data show that there is impaired renal function in only 3.9% of cases. Progression markers include the presence of higher baseline SBP and a higher rate of associated cardiovascular events.[30]

The explanation is complex and probably multifactorial: the deterioration of myocardial function implies a reduction of renal blood flow that would be added to the presence of microvascular and macrovascular renal injury. Furthermore, until just a few years ago, the presence of renal failure has led to underuse of cardioprotective drugs or more conservative application of coronary revascularisation or valvular prostheses.[31]

For years, the relationship between hyperuricaemia and CKD in patients with essential hypertension has been widely debated. While it is true that up to 40% of individuals with gout develop CKD, the fact remains that almost all have hypertension and arteriolosclerosis and glomerulosclerosis lesions similar to those seen in patients with nephrosclerosis. There is no evidence that hyperuricaemia is a risk factor for CKD. However, two recent studies have offered new perspectives: Iseki et al have shown that hyperuricaemia is an independent marker of renal failure for subjects at baseline who had normal renal function, especially women.[32] Furthermore, Siu et al studied 54 patients and found that when uric acid is decreased with allopurinol progression to CKD is postponed.[33] It seems necessary to promote studies specifically designed to clarify whether hyperuricaemia may be an independent risk factor for vascular and renal risk.[34]

The lack of correlation between the degree of hypertension control and the prevention of disease progression, from the clinical and histological standpoint suggests that this process may be an intrinsic and early renal disease. In some cases the clinical context maintains some similarity with focal segmental glomerulonephritis (FSGP). For many years, genetic markers have been sought that could explain the onset and progression of the disease. Arterial hypertension, hyperuricaemia, dyslipidaemia and metabolic syndrome were frequently associated phenotypic factors, but not the cause of the process.[2,3]

Some studies carried out a decade ago verified a direct relationship between nephrosclerosis and ACE DD genotype. The D allele appears to be predominant in hypertensive patients with nephrosclerosis and could be a progression marker. Although the proportion of patients was small, studies were conducted on Caucasians, and included histological support and control groups of hypertensives without kidney disease and subjects from the general population.[35,36]

American nephrosclerosis genetic studies have been conducted primarily in African-Americans because the disease is more frequent and aggressive in this race.[37-42] Two independent studies published recently have led to a new approach to the pathogenesis of nephrosclerosis. The study by Kao et al, which included 1372 patients, revealed a close relationship between the presence of end-stage CKD secondary to hypertensive nephrosclerosis in non-diabetics, and some polymorphisms of the *MYH9* gene, located on chromosome 22, which encodes the non-muscle myosin heavy chain IIA.[37] The study by Kopp et al compared 190 black individuals with 222 healthy controls. It found the same association with the presence of FSGP of idiopathic origin or secondary to infection with human immunodeficiency virus (HIV), but no relationship was found with progression.[38]

Furthermore, a study by Freedman et al confirmed the presence of the *MYH9* gene polymorphisms in 696 African-American subjects with hypertensive kidney disease and end-stage CKD, compared with 948 control subjects without kidney disease, of whom 34% were hypertensive. However, not all individuals who were homozygous

dominant for *MYH9* risk alleles developed the disease, which suggests that other factors, such as environment or interaction with other genes, add to individual genetic susceptibility.[39] Meanwhile, the same group recently reported an association between the *MYH9* E1 haplotype and the presence of microalbuminuria in 1458 African-American patients with essential hypertension but no kidney disease. Increased risk has been observed for *MYH9* E1 haplotype, which consists of the polymorphisms rs4821480, rs2032487, rs4821481 and rs3752462. However, the strength of the association is weaker than in subjects with FSGP and terminal CKD of hypertensive origin.[40]

Further complicating the issue, an association of that gene has been found in 751 diabetic black end-stage CKD patients compared with 227 diabetic controls without kidney disease and 925 healthy controls, but there is no clear correlation with the development of diabetic kidney disease because there is no histological confirmation. It has even been suggested that it may coincide with kidney disease related to baseline *MYH9* polymorphisms.[41]

It appears that during the early stages, myosin IIA is primarily situated at the podocyte level and induces structural alterations. Recently, the role of podocyte loss and dysfunction has been reported as part of disease development. Wang et al conducted a study on 41 patients with biopsy diagnosis of hypertensive nephrosclerosis, finding less podocytes and a reduced intrarenal expression of protein genes such as nephrin, podocin and synaptopodin, which is also related to the decrease in eGFR, and inversely proportional with the degree of renal fibrosis.[43]

A bold new hypothesis on this issue states that polymorphisms of this gene would be markers of various kidney diseases that fall into one histological group, FSGP, and any of its variants, such as nephrosclerosis, which would be a disease akin to primary kidney disease with idiopathic FSGP and with the collapse observed in HIV.[44] A renal disease associated with macrothrombocytopaenia has even been reported. Lastly, there are some rare entities associated with mutations of that gene, which are transmitted by autosomal dominant inheritance, known as *MYH9*-related disorders. These include the May-Hegglin anomaly and the Sebastian, Epstein and Fechtner syndromes, which are in many cases present with kidney disease, although its mechanism and that of *MYH9*-related hypertensive nephrosclerosis polymorphisms in blacks is currently unknown.[45] However, not all cases of nephrosclerosis are associated with *MYH9* haplotypes.[46]

For all these reasons, further studies are needed to provide a better understanding of its pathogenesis, and the potential role of detections of *MYH9* polymorphisms in the diagnosis and evolution of CKD secondary to nephrosclerosis (Table 1).

HYPOTHESIS

Hypertensive nephrosclerosis is a CKD that rarely progresses.

1 Clinical progression markers would be related to:

a) The presence of associated cardiovascular disease, especially cardiovascular events appearing on a recurring basis.

b) The degree of proteinuria.

c) Initial renal function.

d) The patient being less than 70 years old.

2 Histological progression markers would be related to:

a) The degree of global and segmental glomerulosclerosis.

b) The degree of interstitial fibrosis.

3. Genetic markers would be related to:

a) *MYH9* gene polymorphisms.

b) I/D polymorphisms of the ACE gene.

OBJECTIVES

Primary Objective

To determine if any *MYH9* gene polymorphism is associated in Caucasians:

a) With the association of essential hypertension and nephrosclerosis.

b) With progression of the disease.

Secondary Objectives

1. To compare the clinical characteristics of nephrosclerosis patients with essential hypertension and hypertensives with no kidney disease, and to determine clinical progression markers.

2. To recognise the importance of proteinuria, associated cardiovascular disease, sex, age (over and under 70

years), the presence of hyperuricaemia or gout, and concomitant treatments (antihypertensives, statins and antiplatelet agents).

METHOD

Study Design

Multicentre retrospective study, which involves four hospitals in Asturias (Central Asturias University Hospital, Valle del Nalón Hospital, Cabueñes Hospital, and San Agustín Hospital), and the Marques de Valdecilla University Hospital in Cantabria.

A basic study comparing patients with essential hypertension with nephrosclerosis patients versus hypertensive patients without kidney disease (control group). Furthermore, patients with nephrosclerosis and impaired kidney function are compared with those who are stable. For the genetic study, healthy controls from the general population with similar age and sex as the cases are also included.

PATIENTS

Cases

The cases were selected between October 2009 and October 2010 from nephrology, kidney transplantation, haemodialysis and peritoneal dialysis units of the participating hospitals.

Patients recruited have eGFR <60ml/min/1.73m^2 (measured by the MDRD formula) in stages 3-5, attributed to nephrosclerosis according to standard clinical criteria (Table 2), with or without histological documentation. Diagnosis of CKD of unknown origin will not be valid, and patients with secondary, renovascular or accelerated hypertension will be excluded.

Patients with stage 5, on dialysis or to undergo kidney

Table 1. Main studies on the polymorphisms of the MYH9 gene and the presence of kidney disease

Authors, journal and year	n	Type of patient	Results
Kao et al, Nat Genet 2008[37]	1372 ESRD cases, 806 controls	**African-Americans**	Association with **ESRD in all non-diabetic subjects,** particularly in hypertensive nephrosclerosis, FSGP and nephropathy secondary to HIV
Kopp et al, Nat Genet 2008[38]	891 CKD cases, 1024 controls	1569 **African-Americans** and 346 Caucasians	Association with African Americans with idiopathic FSGP or secondary to HIV, and hypertensive nephrosclerosis, but not in diabetic kidney disease
Freedman et al, Kidney Int 2009[39]	871 ESRD cases, 948 controls	Non-diabetic **African-Americans**	Association with **ESRD in all patients without diabetes,** and hypertensive nephrosclerosis E1 haplotype
Freedman et al, Am J Nephrol 2009[40]	2903 individuals (HyperGEN study)	**With essential hypertension** without kidney disease	E1 haplotype association and the presence of microalbuminuria in African-Americans
Freedman et al, Nephrol Dial Transplant 2009[41]	751 ESRD diabetics, 227 diabetic patients without kidney disease, 925 controls	**African Americans**	Association with **ESRD** in diabetic patients compared to healthy controls and diabetics without kidney disease There does not seem to be a clear relationship with diabetic kidney disease because there is no histological confirmation.
Behar et al, Hum Mol Genet 2010[42]	997 ESRD cases, 448 controls	African-Americans and those of Hispanic origin	Association with **ESRD in non-diabetics.** The Hispanic population studied had varying degrees of African descent.
Pattaro et al, Kidney Int 2009[49]	2859 individuals	**Europeans without kidney disease**	Association with **serum creatinine** levels, primarily **in non-diabetics**

Table 2. Inclusion-exclusion criteria

Inclusion criteria	Exclusion criteria
Age 18-80 years	BP <140/90mm Hg without treatment
Caucasian	Diabetes mellitus based on fasting glucose ≥126mg/dl
BP ≥140/90mm Hg or on antihypertensive medication	Proteinuria ≥3.0g/24 hours, except in cases in which there is histological confirmation by kidney biopsy
Creatinine ≥1.5mg/dl in men	One kidney
Creatinine ≥1.4mg/dl in women	Malignant or accelerated arterial hypertension (opthalmoscopy grade III or IV)
eGFR <60ml/min/1.73m^2)	Secondary arterial hypertension, including renovascular
There are no other identifiable causes of kidney disease, including ischaemic kidney disease	Serious systemic disease
	Morbid obesity

Adapted from the AASK study: Agodoa et al JAMA 2001,[13] Wright et al JAMA 2002.[14]

transplant, may be admitted, provided that developmental data are available from their first contact with the hospital. The start of replacement therapy marks the end of follow-up.

Cases younger than 80 years will be valid for the study. However, the selection will be made mainly of patients younger than 60 years, to minimise the effect of age-related gene denaturation.

Controls

The control group is from Central Asturias University Hospital and Valle del Nalón Hospital, to simplify data collection, given that there are few differences between the subjects as they are from the same geographic area. Patients with essential hypertension are included with eGFR>60ml/min/1.73 m^2 and microalbuminuria <300mg/g.

Data for Each Patient

There will be two sections of the study, one for the first hospital visit and one for the last («current visit»). Clinical data are to be collected from all patients including socio-demographic variables, comorbidity and cardiovascular risk, medical treatment and physical examination, weight in kilograms, height in centimetres, systolic (SBP) and diastolic (DBP) blood pressure in millimetres of mercury, and heart rate. Analytical data will be recorded including renal function by serum creatinine, eGFR, creatinine clearance, microalbuminuria in urine alone (mg/g creatinine) and proteinuria in urine for 24 hours and also glucose, uric acid, cholesterol and calcium-phosphorus metabolism. Furthermore, the performance of imaging techniques (ultrasound, CT angiography, MR angiography or arteriography), and renal biopsy diagnosis (Table 3) are also included.

Laboratory Analysis

Routine laboratory tests shall be performed by the usual methods in the laboratory of each hospital. Blood samples for genetic studies will be submitted to the Laboratory of Molecular Genetics, Central Asturias University Hospital. DNA was obtained from leukocytes from 10ml of EDTA anticoagulated peripheral blood. The samples will be stored for 5 years.

Major *MYH9* gene polymorphisms to be identified are those which showed an association with hypertensive nephrosclerosis in non-diabetics (rs4821480 and rs3752462, belonging to E-1 haplotype), and with the ACE gene, by the polymerase chain reaction (PCR) test.

DEFINITIONS

Progression of Kidney Disease

Doubling of baseline creatinine, eGFR decline >50% above baseline or a reduction of 25ml/min/1.73m^2 or onset at end-stage CKD (defined by the need for renal replacement therapy).

Arterial Hypertension

SBP≥140mm Hg and/or DBP≥90mm Hg, or treatment with diet or antihypertensive agents. The percentage of patients with SBP≥130mm Hg and/or DBP≥80mm Hg will also be analysed.

Diabetes Mellitus

Fasting glucose≥126mg/dl or ≥200mg/dl 2 hours after oral glucose or antidiabetic treatment (diet, oral antidiabetic agents or insulin).

Table 3. Clinical and analytical data sheet

Vital statistics:			
Local No.:	Clinical history no.:	Centre:	
Initials:	D.O.B.:	Sex:	
Comorbidity and cardiovascular risk factors:			
Family history kidney disease:	Yes☐	No ☐	Unknown ☐
Smoker:	Yes☐	No ☐	Ex-smoker ☐
High cholesterol:	Yes☐	No ☐	
Gout:	Yes☐	No ☐	
Heart disease:	Yes☐	No ☐	
Heart failure:	Yes☐	No ☐	
Atrial Fibrillation:	Yes☐	No ☐	
Degenerative valve disease:	Yes☐	No ☐	
Left ventricular hypertrophy:	Yes☐	No ☐	Unknown ☐
Stroke:	Yes☐	No ☐	
Peripheral artery disease:	Yes☐	No ☐	
CKD stage:	3 ☐	4 ☐	5 ☐
Renal replacement therapy:	Yes☐	No ☐	
Type:	Haemodialysis ☐	Peritoneal dialysis ☐	Transplantation ☐
Baseline data. Date:			
Creatinine (mg/dl):	Ccr (ml/min):	eGFR (ml/min/1.73m^2):	
Proteinuria (g/24h):	Microalbuminuria (mg/g):	Hb[1] (g/dl):	
Glucose[1] (mg/dl):	Uric acid[1](mg/dl):	LDL-cholesterol[1] (mg/dl):	
Final visit. Date:			
Weight (kg):	Height (cm):		
Systolic BP (mm Hg):	Diastolic BP(mm Hg):	Heart rate (bpm):	
Creatinine (mg/dl):	Ccr (ml/min):	EGFR (ml/min/1.73m^2):	
Proteinuria (g/24 h):	Microalbuminuria (mg/g):	Hb (g/dl):	
Glucose (mg/dl):	Uric acid (mg/dl):	LDL-cholesterol (mg/dl):	
Calcium[2] (mg/dl):	Phosphorus[2] (mg/dl):	Albumin[2] (mg/dl):	
Treatment at the final visit:			
ACEI:	Yes☐	No ☐	
ARB:	Yes☐	No ☐	
DRI:	Yes☐	No ☐	
Calcium agonists:	Yes☐	No ☐	
Diuretics:	Yes☐	No ☐	
Beta blockers:	Yes☐	No ☐	
Alpha blockers:	Yes☐	No ☐	
Alpha-beta blockers:	Yes☐	No ☐	
Other anti-hypertensives:	Yes☐	No ☐	Unknown ☐
Number of anti-hypertensive drugs:			
Statins:	Yes☐	No ☐	
Antiplatelet therapy:	Yes☐	No ☐	
Anticoagulants:	Yes☐	No ☐	
Erythropoietin[3]:	Yes☐	No ☐	
Renal morphology study and diagnosis[4]:			
Renal ultrasound:	Yes☐	No ☐	
Hyperechogenic kidneys	Yes☐	No ☐	
Kidneys <9cm	Yes☐	No ☐	
Renal arteriography	Yes☐	No ☐	
Angio-CT:	Yes☐	No ☐	
Angio-MR:	Yes☐	No ☐	
Renal biopsy:	Yes☐	No ☐	
Blood sample for genetic study. Date:			

[1] In the hypertensive controls, only initial data concerning renal function were recorded. Baseline metabolic or anaemia data were not collected.
[2] In hypertensive controls, data were not collected on calcium-phosphorus metabolism at the final visit.
[3] In the hypertensive controls, administration of erythropoietin was not recorded.
[4] In the hypertensive controls, no diagnostic studies were recorded.

Hypercholesterolaemia

LDL cholesterol≥100mg/dl, or treatment with lipid lowering agents.

Smoker

Tobacco consumption (cigarettes, cigars, pipe) during the last month. Former smoker: absence of tobacco use for a consecutive year.

Obesity

BMI≥30kg/m^2.

Cardiovascular Comorbidity

Must be properly documented. Presence of one or more of the following: ischaemic heart disease by acute myocardial infarction (AMI), unstable angina or stable angina, atrial fibrillation, heart failure, degenerative mitral or aortic valve disease, ischaemic, hemorrhagic, or transient stroke, or peripheral arterial disease.

Left Ventricular Hypertrophy

Diagnosis made by echocardiography, which is a more specific method than the electrocardiogram.

ETHICAL ASPECTS

The investigator must inform the patient about his or her participation in the study, which is voluntary and will not cause any change in the treatment or medical care to be received. Subsequently, the investigator must obtain the patient's informed and voluntary consent. The highest levels of confidentiality must always be maintained, as well as compliance with national legislation on data protection.

The study protocol was approved by the Research Ethics Committee of the Regional Clinical Research of Asturias.

SAMPLE SIZE

It is estimated that the size of the sample needed to observe the effects of *MYH9* gene polymorphisms according to their allele frequencies for an alpha error of 0.05 and a beta error of 0.2, will be 500 cases with CKD and 500 controls. The subjects will be distributed as such: 500 cases, 300 hypertensive controls with no kidney disease and 200 general population subjects (healthy controls).

We will attempt to control the sample for sex and age (Figure 1).

STATISTICAL ANALYSIS

There will be a descriptive analysis of continuous variables, giving the mean, median, standard deviation and range, and in case of discrete variables, the frequency distribution and percentages. When necessary, 95% confidence intervals are calculated. The description of the main variables are based on age, sex, level of baseline renal failure and proteinuria, degree of hypertension control and dyslipidaemia and associated cardiovascular risk factors. The association between qualitative variables will be assessed with the chi-square or Fisher's exact test and quantitative variables using parametric tests (t-test, Pearson correlation coefficient and ANOVA). If a normal distribution cannot be assumed, non-parametric tests will be used such as Mann-Whitney U or Kruskal-Wallis. Comparison tests will be bilateral and considered significant when $P<.05$. A logistic regression model will be used to evaluate the association of those variables in which the result of the P comparison in the "raw" analysis is less than 0.15. Statistical analysis of the study will be conducted with SPSS for Windows, version 15.0 (SPSS, Chicago, IL).

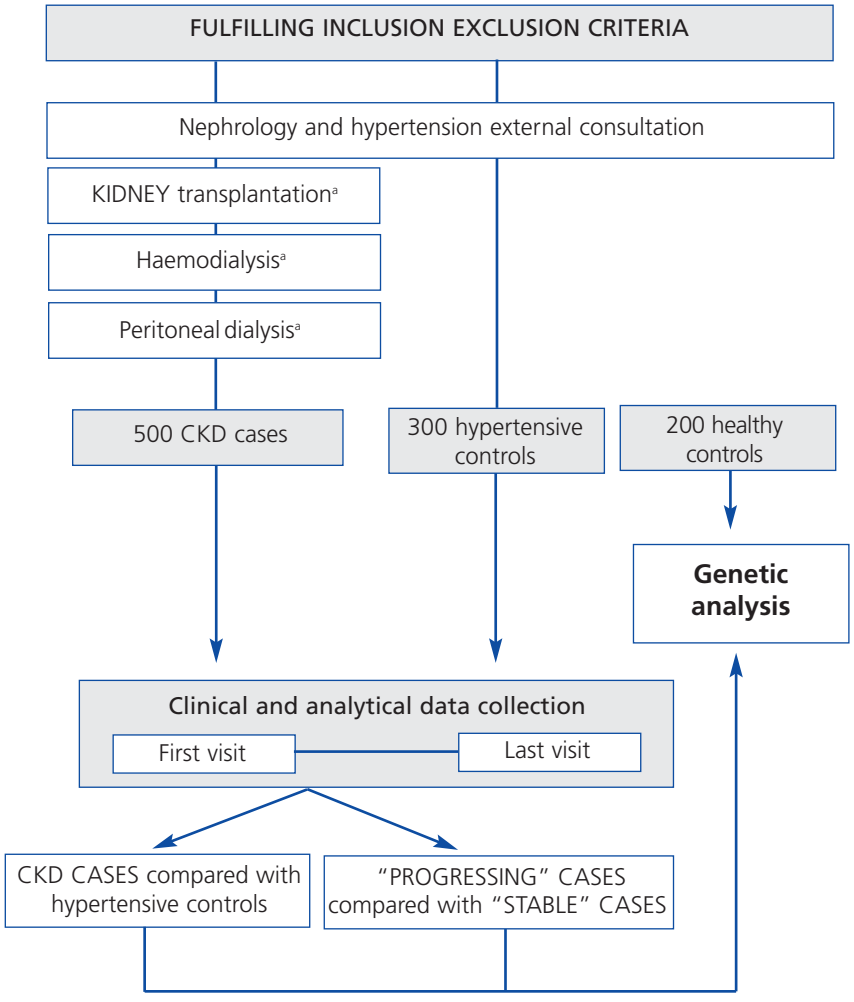
FINANCIAL SUPPORT

The NEFROSEN Study is a typical clinical practice study that does not involve extra costs. It is being carried out with the sponsorship of the Spanish Society of Nephrology (SEN) through its "Kidney and Hypertension" Work Group.

The cost for each case analysed in the genetic substudy of the *MYH9* gene polymorphisms is about 10 euros. It is being financed with funds from the *Fundación Renal Iñigo Álvarez de Toledo* (Iñigo Alvarez de Toledo Kidney Foundation) in the Molecular Genetics Group.

DISCUSSION

Following the publication of genetic studies on nephrosclerosis, some documents have indicated that it should no longer be regarded as a disease that is secondary to essential hypertension. At least in the black race, it seems to be a genetically based disease closely related to FSGP, and it is estimated that there may be an association with *MYH9* gene polymorphisms in 43% of African-American individuals who progress to end-stage CKD.[47] Of these, control of hypertension does not stop progression, while in



[a] In stage 5 cases, the start of replacement therapy will be the end of follow-up.

Figure 1. Case and control recruitment.

the white race it seems more effective, so it is speculated that the treatment of hypertensive nephrosclerosis should be approached from a different perspective that not only includes blocking RAS and strict control of blood pressure.[48]

However, much is still unknown about these findings. The referred studies have been conducted on patients with nephrosclerosis not supported by renal biopsies. This indicates the opportunity to reassess the cases of the AASK study (n=1094 patients), which is the only one that has been conducted with histological confirmation.[13,14]

In the white race there are no studies on whether these or other *MYH9* polymorphisms might be involved in the disease. Only the Pattaro et al group found an association of this gene with serum creatinine values in 2859 European individuals without kidney disease, coming from three different populations, while the genes related to renal function showed great heterogeneity.[49]

The proposed study is both clinical and genetic. Their relationship seems logical and complementary. The limitations are those related to a retrospective study design, together with the lack of a centralised laboratory for routine analysis, because the data are historic. Nephrosclerosis cases that maintain periodic visits over a variable time period, and patients that have progressed to end-stage CKD are included. In this respect, we have significant selection bias when calculating the percentage of patients whose disease has progressed, since many will be obtained from dialysis and transplant units. We will probably not be able to draw clear conclusions about disease progression but, despite these

limitations, we believe a study such as this will be interesting because there are very few publications to date on this topic.

It seems necessary to design prospective genetic studies to determine the relationship with this gene, both in terms of diagnosis and the possibility that it could be a progression marker.

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