CD36 Gene Polymorphism and Susceptibility to Nephropathies

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ABSTRACT: Introduction: Renal diseases affect huge populations in Morocco and abroad. Genetic polymorphisms (SNPs) in scavenger receptors such as CD36 have been implicated in the pathogenesis of diabetes, hypertension, atherosclerosis and cardiovascular diseases. Since CD36 gene expression contributes to the leading causes of kidney disease, we proposed to study the association of two of its polymorphisms to the occurrence of nephropathy. Methods: A population of 130 controls and 132 renal disease patients from the nephrology department of Fez university hospital was analyzed according to clinical parameters and genotyped for two SNPs namely rs1527479 (C > T) and rs1761667 (G > A) in the CD36 gene using polymerase chain reaction and restriction fragment length polymorphism (PCR–RFLP) followed by statistical analysis. Results and discussion: At least one out of the two CD36 gene polymorphisms tested rs1527479 (P1) and rs1761667 (P2) was found in 96.2% of renal patients (45.5% in the homozygous state) and 97.7% of controls (34.6% in the homozygous state). Both polymorphisms (P1 and P2) were present simultaneously in the homozygous state in 38.64% of nephrology patients and only 1.54% of controls (p <0.001). Conclusion: Co-existence of the two polymorphisms rs1527479 (C > T) and rs1761667 (G > A) in the homozygous state would be a powerful predictor of the onset of kidney disease in the Moroccan population.

KEYWORDS: Genetics, Nephropathy, Polymorphism, Renal disease.

I. INTRODUCTION

Kidney disease is the consequence of various injuries of the renal parenchyma that may initially affect the glomeruli, vessels or tubular and interstitial structures. These lesions result in a reduction in the number of functional nephrons. The speed of progression varies considerably from one patient to another for the same kidney disease, suggesting that independent of the origin of renal disease factors play a key role in the evolutionary process.

We distinguish schematically: factors that increase the susceptibility of developing chronic kidney disease (advanced age, family history of kidney disease...); factors that directly initiate kidney damage (diabetes, hypertension, autoimmune disease, inherited disease...); factors of progression that may worsen renal damage and accelerate the decline in renal function, modifiable through appropriate management; and factors that increase morbidity and mortality in end-stage renal failure.

However, genetic factors appear to play a decisive role. Epidemiological studies suggest that susceptibility to kidney damage is genetically determined (1). These studies have proposed various polymorphisms of genes implicated in the physiopathology of kidney disease as risk factors of initiation and progression of the disease.

CD36 is a membrane glycoprotein that is present on various types of cells, including monocytes, macrophages, microvascular endothelial cells, adipocytes and platelets (2). Numerous studies have highlighted the role of genetic linkage of the CD36 gene in the onset of diabetes and hypertension that are the two leading causes of kidney disease in Morocco and in the world (3,4). The role of CD36 protein is also described in the control of lipid metabolism, atherosclerosis and oxidative stress (2,5,6). Polymorphisms of the CD36 gene also appears to be involved in more
direct mechanisms of impaired renal function such as tubular damage (7) and the hemolytic uremic syndrome (8). CD36 polymorphism thus appears as a good candidate gene for susceptibility to kidney disease.

The aim of our study was to check the link between the polymorphism of the CD36 gene and the onset of nephropathy in the Moroccan population regardless of known predisposing factors for kidney disease.

II. MATERIALS AND METHODS

In this case-control study, we compared two groups of patients according to the presence or not of nephropathy. The study was conducted in collaboration between the Faculty of Sciences Dhar El Mahraz Fez (Morocco) on one hand and the unity of Medical Genetics and the Nephrology Department of the University Hospital Hassan II Fez (Morocco) on the other.

We first prospectively identified all inpatients and outpatients in whom the diagnosis of nephropathy have been confirmed in the nephrology department of Hassan II University Hospital of Fez (Morocco) during a period of six months, ranging from 02 September 2012 to 28 February 2013. The diagnosis of the kidney disease was based on the presence of renal failure (eGFR <60 ml / min / 1.73m²) and / or abnormalities of the urinary sediment (proteinuria> 300 mg / 24h and / or hematuria of non-urological causes).

We excluded patients aged less than 16 years and over 65 years, patients with liver disease, pregnant women, patients with obstructive or functional renal failure, and patients who have not given their informed consent to participate in the study.

Thus, 132 unrelated patients were included randomly according to simple randomization method after a draw among 265 patients. A control group of 130 persons was randomly selected from a panmictic population devoid of nephropathy. The two groups were matched for age, gender and ethnicity (Arab or Berber).

The study protocol complied with the ethical guideline for the 2013 Declaration of Helsinki and was approved by the local Hospital Ethical Committee (Faculty of Medicine and Pharmacy and University hospital of Fez - Morocco). All participants filled out a written informed consent for participating in the study and for storage of their DNA.

From the medical record of each patient included in the study, we collected: demographic data (age, gender, and ethnicity), medical history (personal history, family history and consanguinity), nephropathy category (glomerulonephritis, interstitial nephropathy, vascular nephropathy, diabetic nephropathy, polycystic kidney disease, or nephropathy of unknown origin), and additional relevant clinical and biological data. A pedigree was also drawn for each patient including familial cases of nephropathy, deceased subjects and causes of death. This allowed us to determine the degree of consanguinity and potentially the mode of transmission.

We also collected blood samples from each patient for molecular study: two dry tubes each containing 5 ml of blood for serum storage; and an EDTA tube of 5 ml sent to the medical genetics unit for performing a DNA extraction and contribute to the DNA-bank. The DNA extraction was performed by a salting out procedure followed by removal of the proteins and any cellular debris, and then precipitation of the DNA by alcohol (9).

The DNA collected was then numbered, labeled and stored in a freezer at -80°C. The samples remained anonymous and the subjects could not obtain any personal benefit. Before using the DNA stored we made a determination and an appreciation of the quality of the extracted DNA. This quality of the extraction is of major importance, as it directly influences the quality molecular biology techniques (PCR, PCR-RFLP, and sequencing) used to search for polymorphisms of the promoter and the intron of the CD36 gene.

The amount of DNA extracted was determined by spectrophotometry. DNA concentrations ranged from 125 to 2564 ng/µl. Since the proteins absorb near 280 nm, while the nucleic acids absorb near 260 nm, the value of the absorbance at 280 nm determined any protein contamination. The DNA was considered as pure extract since the ratio of absorbance at 260 nm and 280 nm was between 1.8 and 2. Moreover, an optical density unit corresponds to 50 mcg/ml of double-stranded DNA. The "NanoDrop" was used to perform the assay.

To study the polymorphism of CD36 gene, we used the primer sequences for genotyping of two SNPs (single nucleotide polymorphism): rs1761667 (G > A) and rs1527479 (C > T) (10,11). The former is at the level of the promoter region whereas the latter is located at the intron level. These two markers were detected by the Restriction
Fragment Length Polymorphism technique (PCR-RFLP) using two restriction enzymes. NEB cutter software is the free online software that has been applied (12) to search for the possible cleavage sites of restriction enzymes in the sequence of the CD36 gene. The rs1527479 (called P1) and rs1761667 (called P2) CD36 gene polymorphisms were detected and read by a Real-Time PCR System (Applied Biosystems GeneAmp ® PCR System 2700). DNA was used at a final concentration of 70 ng. Conditions of the polymerase chain reaction were 95°C for 5 min and 30 cycles of denaturation at 95°C for 30s and annealing/extension at 72°C, for 1 min. Genotyping was verified by using positive controls of the DNA samples corresponding to the three possible genotypes in each 96- well plate as well as rerunning 10% of the samples, which were 100% concordant.

The prevalence of polymorphisms rs1527479 (C > T) (P1) and rs1761667 (G > A) (P2) of the CD36 gene in the group of kidney disease patients was compared to that of the control group. Statistical analysis was performed in the laboratory of Epidemiology and Community Health of Faculty of Medicine and Pharmacy of Fez, using Epi Info 3.3.2 software. Quantitative variables were expressed as mean ± standard deviation and compared using the Student's test. Categorical variables were expressed as percentages and compared using Chi-square test. A p value <0.05 was considered significant.

### III. RESULTS

We included 265 patients with nephropathy of which 153 were hospitalized within the nephrology department and 112 were seen as outpatients. The average age of our patients was 46.7 ± 13.3 years with a sex-ratio M/F of 0.85. Patients were of Arab origin in 80% and Berber in 20%. The analysis of the medical history of the patients noted that 35% were hypertensive and 21% were known diabetics. Only 7% had a history of renal disease (Table 1).

**Table 1: Major medical history of the study population**

<table>
<thead>
<tr>
<th>Comorbidities</th>
<th>Percentage of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>High blood pressure</td>
<td>35%</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>21%</td>
</tr>
<tr>
<td>Smoking</td>
<td>13%</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>1.5%</td>
</tr>
<tr>
<td>History of kidney disease</td>
<td>7%</td>
</tr>
<tr>
<td>History of heart disease</td>
<td>8.7%</td>
</tr>
<tr>
<td>History of systemic disease</td>
<td>6.5%</td>
</tr>
<tr>
<td>Absence of any medical history</td>
<td>23%</td>
</tr>
</tbody>
</table>

Nephrotic syndrome was the main reason for hospitalization (33%), while renal failure was discovered in the biological examination in 83% of patients. Oliguria/anuria was found in 11% of patients. Hematuria and proteinuria were present in 83% and 53% of cases. Renal biopsy was performed 42% of patients. Following the clinical, biological and histological assessments, five categories of kidney disease have been identified (figure 1). However, the cause of the renal disease has not been identified in 17% of patients.
Our results showed that 46.8% of renal patients come from a consanguineous marriage, mainly a first degree marriage (23%) (figure 2).

Patients with renal disease (n = 132) showed a higher prevalence of diabetes and hypertension than the control group (n = 130) despite a younger age (Table I). In addition, among the 132 patients with renal disease, 121 (91.7%) had renal failure and 35 (26.5%) underwent a kidney biopsy.

Table I: Renal risk factors in the renal disease and the control groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients (n=132)</th>
<th>Control (n=130)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47.23±13.8 (16-65)</td>
<td>52.54±16.7 (17-88)</td>
<td>0.005</td>
</tr>
<tr>
<td>Sex-ratio (M/F)</td>
<td>59/73 (0.81)</td>
<td>68/62 (1.09)</td>
<td>0.2</td>
</tr>
<tr>
<td>Hypertension n, (%)</td>
<td>49 (37.1%)</td>
<td>10 (7.7%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes n, (%)</td>
<td>27 (20.5%)</td>
<td>9 (6.9%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Smoking n, (%)</td>
<td>15 (11.4%)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Analysis of rs1527479 (P1) and rs1761667 (P2) polymorphisms of the CD36 gene showed the presence of at least one polymorphism (P1 or P2) in 96.2% of renal patients (45.5% in the homozygous state) and 97.7% of controls (34.6% in the homozygous state) (Table II).

Nephrology patients had more often a homozygous polymorphism compared to controls (40.9% vs 16.9% for P1; 43.2% vs. 19.2% for P2). Both polymorphisms (P1 and P2) were present simultaneously in the homozygous state in 38.64% of nephrology patients and only 1.54% of controls (p <0.001).

Table II: Results of the CD36 gene polymorphisms (patients versus controls)

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=132)</th>
<th>Controls (n=130)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homozygous</td>
<td>79/132 (59.8%)</td>
<td>51/130 (39.2%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>50 (37.8%)</td>
<td>30 (23.1%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homozygous</td>
<td>96/132 (72.7%)</td>
<td>79/130 (60.8%)</td>
<td>0.049</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>63 (47.7%)</td>
<td>54 (41.6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P1+P2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35.6%</td>
<td>2.3%</td>
<td>&lt;0.001</td>
</tr>
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</table>

IV. DISCUSSION

Despite progress in understanding the mechanisms of kidney disease, the prevalence of chronic kidney disease is growing at an alarming rate. Individuals with this disorder are characterized by an atherogenic lipid profile, and are likely to develop oxidative stress and chronic inflammation. All these factors contribute to the rapid progression to renal dysfunction (13,14). Clinical and experimental evidence suggest the existence of genes that modulate the progression of kidney damage.

To identify genes that may alter the renal phenotype, two main strategies can be used: genome scan and search for candidate genes. The first approach analyzes hundreds of microsatellite polymorphisms or SNPs equally distributed over all chromosomes. The link of each polymorphism with the presence of renal disease is tested. The great advantage of this procedure is the ability to identify unknown genes that might contribute to the disease process, but the cost in time and money is very high. The second approach analyzes the relationship between the occurrence of the disease and the microsatellite polymorphism or SNP located near or in the sequence of a gene which, according to the pathophysiological knowledge, could potentially be involved. The limits of this strategy, relatively simple, are the difficulties of choosing the "good gene" and interpret the results as part of a multigene predisposition. In this study, we chose the second approach to find a genetic link between the existence of nephropathy and CD36 gene. The reasons for choosing this candidate gene have been listed above.

The CD36 gene is a good candidate for its intervention in the pathophysiological process of kidney damage. Indeed, its role has been well documented in the pathogenesis of diabetes and hypertension that are the leading causes of renal failure (10,11,15), but also atherosclerosis, inflammation, and lipid metabolism (16,17). A case-control study conducted on metabolic syndrome patients in Egypt showed that CD36 rs1761667 SNP is positively associated with increased risk of metabolic syndrome and its components (higher systolic blood pressure, wider waist circumstance, and higher degree of dyslipidemia) (17).

In this study, we sought to verify whether the polymorphism of the CD36 gene is involved in kidney disease among the Moroccan population. It is an association study that compares the frequency of two genetic markers SNPs in affected subjects and controls. A positive result (allelic distribution difference between cases and controls) suggests that there is an association between the phenotype (renal disease) and the marker studied.

The results of our work have shown that the prevalence of both polymorphisms (rs1527479 and rs1761667) were significantly higher in our patients compared to the control group with no significant difference regarding the state
CD36 could also play a direct role in susceptibility to kidney disease and its progression. Kim et al. studied the expression of factors involved in lipid metabolism in the kidneys of nephritic and control rats. They showed that proteinuria results in accumulation of cholesterol in the kidney and that this is associated with upregulation of CD36, but also SRA-1 and ACAT1 and activation of SREBP-2 which can collectively raise tissue cholesterol and promote renal injury by facilitating the influx, synthesis and retention of cholesterol in the nephrotic kidney (18). The findings of a recent study suggest that oxidized HDL enhances the pro-inflammatory properties and impairs the function of Human renal proximal tubule epithelial cells which play an important role in the progression of CKD, mainly through the scavenger receptor, CD36, as well as through the Src, MAPK and NF-kB pathways (19). Finally, Kennedy et al. suggested that ligands generated in hyperlipidemic states activate CD36 and the Na/K-ATPase and potentiate an inflammatory signaling loop involving proximal tubule cells and their associated macrophages, which facilitates the development of chronic inflammation, oxidant stress, and fibrosis underlying the renal dysfunction common to proatherogenic, hyperlipidemic states (20).

Chronic accumulation of plasma advanced oxidation protein products (AOPPs) promotes renal fibrosis (21). However, the mechanism at the cellular level has not been clarified. Iwao et al. suggested that AOPPs-human serum albumin (HSA) may cause renal tubular injury via the CD36 pathway (21). Given the fact that accumulation of AOPPs is prevalent in diabetes and CKD, targeting AOPPs could be a strategy for the therapeutic intervention of CKD (22).

V. CONCLUSION

Our study showed that polymorphism of the CD36 gene has significant effect in the development of kidney disease. This effect is stronger when it comes from the association of two polymorphisms in the same patient.

The CD36 gene polymorphisms in patients with hypertension, diabetes, dyslipidemia and/or kidney failure could even be proposed as an item in the evaluation of cardiovascular and renal risks in such patients.

VI. ACKNOWLEDGMENT

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