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AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE IN UNIVERSITY CLINIC OF NEPHROLOGY AND HAEMODIALYSIS OF COTONOU: CLINICAL AND GENETICAL FINDINGS

Y A. LALEYE[1], B AWEDE[2], B. AGBOTON[3], S. AZONBAKIN 1, O. BIAOU[4], G. SAGBO[5], M. ADJAGBA[1] , M.P. AUDREZET[6], C. FERE[5] AND R. DARBOUX[1]

Summary: *Autosomal dominant polycystic kidney disease in University Clinic of Nephrology and Haemodialysis of Cotonou: Clinical and genetical findings:* Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease, but poorly studied in Africa. Its frequency in the University Clinic of Nephrology and Hemodialysis of Cotonou during the ten last years was 7 cases per year with a hospital prevalence estimated at 18 per 1000. The mean age of patients was 47.2 years extending from 29 to 70 years. Males were predominant with a sex ratio of 1.13. Family history was found in 47% of patients. The most common manifestations were lumbar pain (62%), high blood pressure (59%) urinary tract infections (53%), hematuria (46%), and abdominal masses (43%). Hepatic cysts were the most extra renal manifestations, found in 34% of cases. Renal failure was observed in 72% of patients of our series, six of them were under dialysis. Direct sequencing of polycystin 1 gene enabled us to identify some new mutations: 4 nonsense mutations (p.Q2824X exon 23, p.Q1651X exon 15, p.W1666X exon 15, p.R966W exon 12), duplication (c _1761.1745 dup exon 9), a deletion (c.9397+1_9397+8del intron 26) and a deletion-insertion (c.7290_7291delins CTGCA exon 18).

Key-words: Autosomal dominant polycystic kidney disease – Polycystin 1 – Mutations – Renal failure.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is a monogenic dominant inherited disease characterized by progressive development of intra-renal cysts associated with extra-renal cysts manifestations and intracranial aneurysms (7). It is estimated that 7 to 10% of chronic renal failure cases are due to ADPKD (9, 15). It is a genetically heterogeneous disorder with two genes identified: PKD1 in 85% and PKD2 in 15% (20) associated with an allelic heterogeneity with different mutations (http://pkdb.mayo.edu). A third unidentified gene is involved in few families (5). This disease, described in all races with a similar incidence in both Caucasian and black of USA, is poorly known in sub-Sahara Africa (8, 14, 17, 25). In the practice of nephrology in Benin, while the diagnosis of ADPKP is evocated, there is not yet a specific study on the disease. Thus, we undergo the present study involving epidemiological and clinical aspects of ADPKD and genetic mutations in the PKD1 in our population.

(1) UFR de Biologie Humaine, Faculté des Sciences de la Santé (FSS). Université d'Abomey-Calavi (UAC), Cotonou, Bénin.

(2) UFR de Physiologie Humaine, Faculté des Sciences de la Santé (FSS). Université d'Abomey-Calavi (UAC), Cotonou, Bénin.

(3) Clinique Universitaire de Néphrologie et d'Hémodialyse, CNHU-HKM, Cotonou, Bénin.

(4) Service de Radiologie, CNHU-HKM, Cotonou, Bénin.

(5) Service de Pédiatrie et Génétique Médicale, CNHU-HKM, Cotonou, Bénin.

(6) Laboratoire de Génétique Moléculaire et d'Histocompatibilité, CHU Brest, France.

PATIENTS AND METHODS

The study extended from January 2000 to June 2010. The study population consisted of patients followed-up for ADPKD in the University Clinic of Nephrology and Hemodialysis of Cotonou. All ADPKD patients with a completed medical record and who have agreed to blood sampling were included. Diagnosis of ADPKD was made on the basis of a family history of cystic renal disease, clinical manifestations involving kidney or other organs and on ultrasound criteria of RAVINE (21). The epidemiological and clinical data were collected by counting record, interview and family survey. Renal function of patients was assessed by creatinin dosage. A total genomic DNA extraction was performed by the conventional phenol-chloroform technique from venous blood. After PCR using specific primers, mutations in PKD1 were screened by direct sequencing of regions of the gene. In practice, we used the kit "Big Dye Terminator V1" (Applied Biosystems) and a capillary sequencer (16 capillaries) ABI Prism 3130 XL. Sequences of the primers used for PCR and sequencing are described in Table I. All patients included in this study gave their informed consent. The confidentiality of all information received in this context was strictly respected.

RESULTS

Among 3783 patients followed in University Clinic of Nephrology and Hemodialysis Clinic of Cotonou during the study period, 70 cases were identified as ADPKD. The hospital prevalence of ADPKD in that service was 1.8% with 7 new cases per year.

Thirty two of these 70 ADPKD patients, belonging to 25 different families were interviewed, examined and enrolled. The other patients were either dead or out of the hospital setting.

The mean age of patients was 47.2 years, extended from 29 to 70 years. Males were predominant (17 patients) with a sex ratio of 1.13. A family history of ADPKD was found in 47% of cases. Lumbar pains were the main reason of consultation, found in 20 patients among 32 (62%), Hypertension (59%), urinary tract infections (53%), hematuria (46%) and lumbar mass (43%) were also observed. The most common extra-renal manifestations were liver cysts present in 11 patients (34%). Ovarian cysts were found in 5 patients (33% of women). In our study, the renal failure, defined by a creatinin level greater than 14 mg/l, was observed in 23 patients or 72% of cases. Table II and figures 1 and 2 summarize the clinical observations in ADPKD patients.

Table I: Primers used for PCR and sequencing of PKD1 regions

Exons	Primers for PCR	Sequences of primers used for PCR	TM(°C)	Amplicon's length	Séquences of primers used for sequencing		
1	PKE1F	CGGGGCCCGCACTGCAGCGCCAG	84.3	587	Exon 1	PKE1F	PCR's primer
	PKE1R	AGGCGTTCCTTATTTAGCAGGGCCGCC	75.2			PKE1R	PCR's primer
2 to 5	PKE2-5F	CCGCCGATTTGGGTCTTCCATCAGAAAGT	75.4	1937	Exons 2 and 3	PKE2-3seqF	AGCTCAAGGGTGGGAGGCCATA
	PKE2-5R	CGGTAGCAGTGCCCGTTGCCAGG	75.2		Exon 4	PKE4seqF	GTGCTGTCAGGGTGGCTCCAG
					Exon 5	PKE5seqF	GCATGGGAGCCTGTGAGTGCG
						I5p2	CCCACACTGACCGTTGACAC
22 to 23	PKE22-23F	CCGCCTCTCCTCTCTCCCCTCCT	71.2	1798	Exon 22	I21P7	GGGGTGGGAGCCAGGTGAGG
	PKE22-23R	CGTGGCCCCCAGCTCCTCTCT	71.3		Exon 23	I22P5	AGCTTCCCCCTTCCTTCTGC
						PKE22-23R	PCR's primer
24 to 26	PKE24-26F	GGCTGCTGGGGGCCTGGCCA	77.7	1408	Exon 24	I23p1	CGTGGCAGAGGGTGGGCTCA
					Exon 25	I24P2	GGGTATGGGCTCTGAwGACTG
	PKE24-26R	GTCAGGATCGCGGGTGGATGCACA	76.0		Exon 26	I25p1	TCTGCAGAGTCGAGGAGGGC

Table II: clinical manifestations observed in ADPKD patients

		Number	Percentage (%)
Sex	Male	17	53
	Femal	15	47
Age (years)	<30	2	6
	30-50	20	63
	> 50	10	31
Familial history	Present	15	47
	Absent	11	34
	No available	6	19
Renal manifestations	Lumbar pains	20	62
	HTA	19	59
	Urinary Infection	17	53
	Hematury	15	46
	Abdominal mass	14	43
Extra-renal Manifestations	liver cysts	11	34
	Ovarian cysts	5	33
	Ombilical hernia	1	34
	cerebral haemorrhage	1	3
	Pancreatic cysts	1	3
	Splenic cysts	1	3
Renal function	Normal	9	28
	failure	23	72

The ultrasound diagnosis of ADPKD in our series was based on the Ravine criteria and on the presence of extra-renal cysts, especially in the liver, spleen or pancreas, as shown in Figures 3-6.

Among the 320 patients having dialysis for ESRD, 6 were carriers of ADPKD. The ADPKD represents 2% of cases of dialysis for ESRD in Cotonou. The mean age of patients submitted to dialysis in our series was 51.4 years.

A direct sequencing in the polycystin 1 gene (PKD1) had found seven different mutations. Figure 6 showed some of genetic profile of mutational analysis and Table III summarized clinical manifestations found in patients with PKD1 mutations.

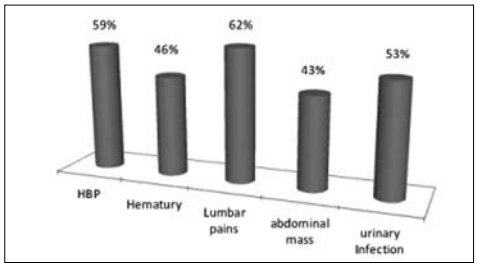

Figure 1: Renal manifestations of ADPKD.

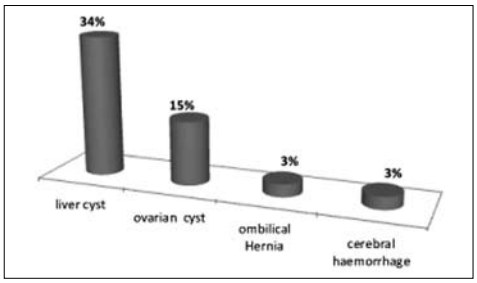

Figure 2: Extra renal manifestations of ADPKD.

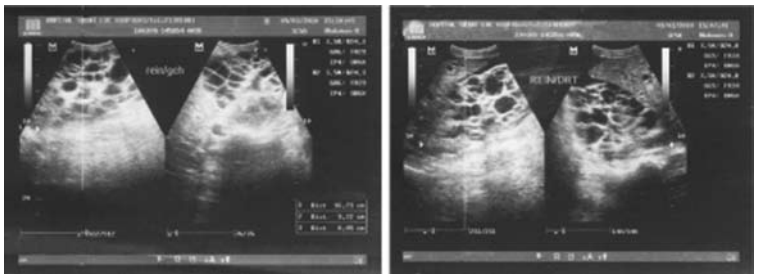

Figure 3: Sonographic pictures of ADPKD patient kidney.

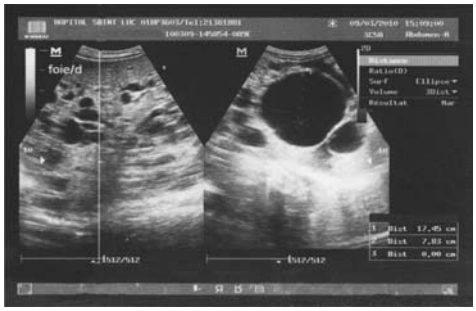

Figure 4: Sonographic pictures of ADPKD patient liver.

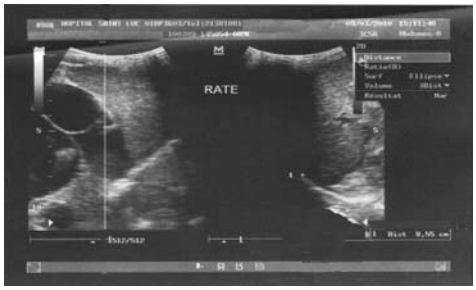

Figure 5: Sonographic pictures of ADPKD patient spleen.

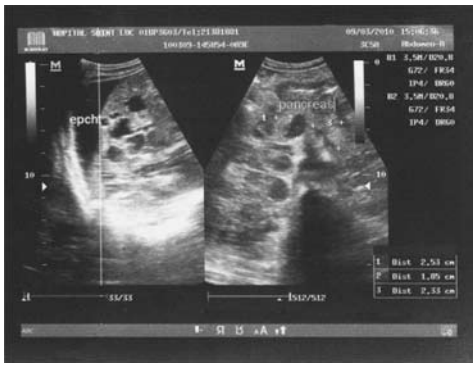

Figure 6: Sonographic pictures of ADPKD patient pancreas.

Table III: Clinical manifestations in patients with PKD1 mutations

PATIENT	Clinical manifestations	Mutations
PKR 008 AJo	Age: 65 High Blood Pressure, Renal Failure , Haematuria Aneamia Severe oedema	p.Q 1651X Exon 15
PKR 010 HSi	Age: 42 Lombar pain, Hight Blood Pressure	p.Q 2824X Exon 23
PKR011 MMi	Age: 55 Familial History of ADPKD , Hight Blood Pressure Lombar pain End Stage Renal Disease Under dialysis 8 years ago	c.1745_1761dup Exon 9
PKR012 HGi	Age: 34 Haematuria Hepatic and ovarian cyst	c.9397+1_9397+8del Intron 26
PKR 013 HGil	Age: 42 Hight Blood Pressure Haematuria Lombar pain	c.9397+1_9397+8del Intron 26
PKR 020 DCo	Age: 42 Hight Blood Pressure Cerebral vascular Hemorrhage, Lombar mass Hepatic and ovarian cyst	p.W 1666X Exon 15
PKR 024 MKe	Age: 55 Familial notion of ADPKD Hight Blood Pressure Lombar pain	C.7290_7291delinsCTGCA Exon 18
PKR 026 BZe	Age: 51 Hight Blood Pressure Renal Failure Lombar Mass	c.6575_6581del Exon 15

DISCUSSION

The ADPKD exists in all races, with a variability in prevalence from a region to another. In the general population of western countries such as France and United States of America, the prevalence is estimated at 1/1000 (13, 23). In few studies performed in Africa particularly in Ethiopia, Senegal and Nigeria, the actual prevalence of the disease in the population could not be assessed from only sporadic cases that have been described (3, 6, 10). During our investigation, we counted 70 cases of ADPKD during 10 years with a hospital prevalence of 18 cases per 1000 in the University Clinic of Nephrology and Hemodialysis of Cotonou. Our study had been carried out in the reference center of Nephrology of Benin, so this hospital prevalence obtained could not be applicable to the general population. Due to the cost of ultrasound necessary for routine screening of ADPKD, the prevalence in the general population would be underestimated. Moreover, the high proportion of infectious diseases and other important causes in renal insufficiency in sub-sahara Africa make ADPKD among etiologies of ESRD easily forgotten.

The mean age of patients in our study was 47 ± 5 years with a minimum of 29 years and a maximum of 70 years. In the series of Bourquia A. in Morocco, the mean age was comparable (46 ± 3 years) (2). Conversely, Idriz et al. found a mean age different from our patients (40 ± 4.2 years) in Albania (12). Everywhere, the ADPKD appears especially within the fourth decade of life.

A male predominance was observed with a sex ratio of 1.13. Al-Muhanna et al. reported a female predominance with a sex ratio of 1.30 in Saudi Arabia (1). This great male predominance observed in this study could be explained by the tendency of men to expose themselves to worsening and rapid growth factors of cysts that are alcohol, tobacco, coffee, and intensive physical activity.

Among the 32 patients of this study, 15 (47%) had a family history of ADPKD. Diouf et al. in Senegal had a record percentage of 52% (6). Corradi et al. found that 86% of patients had a known family history of ADPKD in Italy (4). Thus, the lack of systematic family screening of ADPKD cases and the absence of information on family diseases justify this gap.

The clinical manifestations of ADPKD i.e. lumbar pain, hypertension, urinary tract infections and hematuria, were present respectively in 62%, 59%, 53%, 46% of patients.

Hypertension is raised from the activation of the renin-angiotensin system, due to local renal ischemia caused by renal cysts (7). The hematuria, it is the result of the rupture of cysts in the urinary tract.

Nunes et al. reported in Brazil that the renal manifestations like hypertension, abdominal pain, urinary tract infection and hematuria were present in respectively 50%, 11.9%, 7.5% and 6% of the patients (16). The high rate of renal manifestations in our series could be explained by the late resort of patients to the specialist, the delay in diagnosis and especially the fact that most patients are already in advanced stages of the disease.

Liver cysts are the most common extra-renal manifestations of ADPKD. They are usually asymptomatic and become clinically significant only at an advanced stage of the disease. Other extra-renal manifestations such as ovarian cysts, umbilical hernia, and intracranial aneurysms are rare and probably underestimated as they have not been systematically searched.

In ADPKD, hypertension is a major factor in worsening renal function (22), which was altered in 23 patients or 72% of them. This result differs from that of Parfrey et al. (18). The high rate of renal function failure is due to the long time wasted before the treatment by a specialist.

In this study, six patients were at the end stage of renal disease (ESRD); this had justified a haemodialysis implementation. ADPKD represents 2% of cases of dialysis in University Clinic of Nephrology and Hemodialysis of Cotonou. Higashi et al. reported that ADPKD is responsible for 2.5 to 3.2% of cases of ESRD in Japan (11). This contrasts with the results obtained by Melander et al. in France (2), York et al. in the U.S. (19), who reported that in the white population, the PKAD represented 8% and 5% of cases of 'IRCT. This difference can be attributed to genetic variations between populations and environmental factors. The low proportion of ADPKD among dialysis patients in Cotonou was due to the lack of family diagnosis of ADPKD.

All mutations searched in our patients were in the PKD1 gene. This is justified by the fact that mutations in this gene account for the majority (85%) of cases of ADPKD. In addition, the PKD1 gene is associated with significant mortality and morbidity. Direct sequencing of the genes of polycystin 1 enabled us to identify new mutations: 4 nonsense mutations (p.Q2824X exon 23, p.Q1651X exon 15, p.W1666X exon 15 and p.R966W exon 12), duplication (c _1761 .1745 dup exon 9), a deletion (c.9397+1_9397+8del intron 26) and a deletion-insertion (c.7290_7291delins CTGCA exon 18). None of those mutations identified have yet been described in the database of mutations ADPKD (http://pkdb.mayo.edu). They are therefore new mutations to be included in the international database of the polycystic kidney disease.

Autosomal dominant transmitted with complete penetrance, the ADPKD has a variability in expression.

More ever, there is no parallelism between the evolution of the disease among members of the same family. No correlation between phenotype and genotype has been yet clearly established (24). The observation of a type of mutation in a family does not predict the severity of the disease within this family.

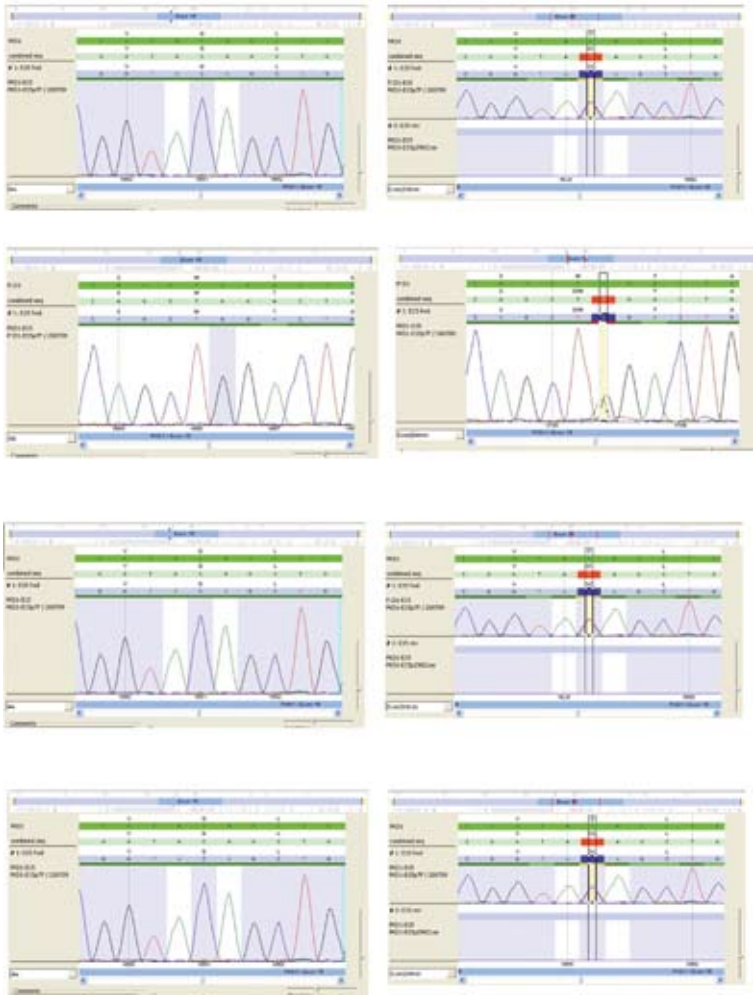

Figure 7: Mutational analysis of PKD1 gene mutations of a) p.Q1651X and p.W1666X in exon 15; b) c.7290_7291delinsCTGCA in exon18 and c) p.Q2824X in exon 23.

CONCLUSION

The ADPKD is not so rare in our population as previously thought. It is a major cause of renal failure and is associated with new mutations not yet described in the PKD1 gene. To our knowledge, mutational studies in the genes of ADPKD in sub-Sahara Africa have been yet undertaken. Also, our results suggest that the mutational basis of ADPKD in black African might be different from those of Caucasian and American.

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ADDRESS FOR CORRESPONDENCE:

Laleye Anatole
FSS/UAC
01 BP 188 Cotonou
Bénin e-mail:
E-mail: laleyec@yahoo.fr