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ORIGINALS

Molecular diagnosis of autosomal dominant renal polycystic disease in the Autonomous Community of the Canary Islands[1]

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SUMMARY

Adult dominant polycystic kidney disease is an hereditary condition responsible for 6% of end-stage renal failure in Spain. Two genes were located in chromosomes 16 and 4 as related to this age-dependent disease in the 90s (PKD1 and PKD2). The diagnosis can be easily achieved by sonographic study, but molecular analysis by means of linkage analysis has the advantage of an early diagnosis in asymptomatic genetic carriers, with a view to the preventive follow-up of these subjects and genetic counselling.

In this paper we present the results of molecular analysis of 30 families with Adult Dominant Polycystic Kidney Disease (from the province of Las Palmas Spain), carried out linkage analysis with two series of microsatellite markers located within or in the vicinity of PKD1 (D16S521, KG8, AC2.5, CW2, SM7) and PKD2 (D4S1538, D4S1534, D4S423, D4S414) genes. The objectives of the study were: first, to verify the informativeness, and therefore, the usefulness of these markers for family studies in our population; and second, to assess the sensitivity and specificity of the genetic analysis in our population.

Most of the markers showed a high heterozigosity, comparable to data in other studies. Considering the alleles of the different markers together in a chromosome as an haplotype increased the informativeness of the markers, and allowed the unequivocal identification of genetic data in 97.7% of patients and 88.7% of healthy subjects. The sensitivity and specificity of the genetic analysis were 90.7% (CI 95%: 85.7-95.7) and 86.8% (CI 95%: 80.6-93.0), respectively.

Key words: ***Polycystic disease. Early diagnosis. Genetic counselling. Microsatellite markers.***

DIAGNÓSTICO MOLECULAR DE LA POLIQUISTOSIS RENAL AUTOSÓMICA DOMINANTE EN LA COMUNIDAD AUTÓNOMA DE CANARIAS

RESUMEN

La poliquistosis renal autosómica dominante es una enfermedad hereditaria responsable del 6% de los casos de insuficiencia renal terminal en España. En la década de los 90 se identificaron los dos únicos genes relacionados con la enfermedad hasta el momento, en los cromosomas 16 y 4 (PKD1 y PKD2). El diagnóstico de esta enfermedad de desarrollo

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dependiente de la edad puede realizarse fácilmente mediante ecografía, pero el diagnóstico molecular mediante el análisis de ligamiento ofrece la ventaja de la detección precoz de individuos asintomáticos portadores del defecto genético, con vistas al seguimiento preventivo de estos individuos y al consejo genético.

En este trabajo presentamos los resultados del análisis molecular de 30 familias con poliquistosis renal de la provincia de Las Palmas, realizado mediante análisis de ligamiento con dos series de marcadores polimórficos localizados en las inmediaciones de los genes PKD1 (D16S521, KG8, AC2.5, CW2, SM7) y PKD2 (D4S1538, D4S1534, D4S423, D4S414). Los objetivos del trabajo fueron: primero, comprobar el grado de informatividad y, por tanto, la utilidad de estos microsatélites para los estudios familiares de la PQRAD en nuestra población; y segundo, determinar la sensibilidad y especificidad del análisis genético en nuestra población.

La mayoría de los marcadores mostró una alta heterocigosidad, comparable a la de otros estudios. Considerar los alelos de los distintos marcadores presentes en un mismo cromosoma conjuntamente, como un haplotipo, aumentó la informatividad de los marcadores y permitió la identificación inequívoca de los datos genéticos en el 97,7% de los pacientes y en el 88,7% de los individuos sanos. La sensibilidad y especificidad del análisis genético fueron del 90,7% (IC 95%: 85,7-95,7) y 86,8% (IC 95%: 80,6-93,0), respectivamente.

Palabras clave: ***Poliquistosis. Diagnóstico precoz. Consejo genético. Microsatélites.***

INTRODUCTION

Autosomal dominant polycystic renal disease (ADPRD) is a hereditary disease that accounts for 6% of end-stage renal failure cases in Spain[1]. It is one of the most frequent hereditary diseases with a prevalence ranging from one in 400 to one in 1000 individuals of Caucasian origin[2], and a dominant inheritance pattern. It is characterized by the formation of multiple renal cysts, which progressively impair renal function until reaching end-stage renal failure. Similarly, other cysts develop in other organs such as the liver, spleen, or pancreas. Extrarenal complications include gastrointestinal and/or cardiovascular disorders, the most severe ones being abnormalities of the cardiac valves, dissection of the thoracic aortic artery, and intracraneal aneurysm. Patients have severely impaired quality of life due to multiple complications from the disease. Arterial hypertension (AHT) affects 60% of the patients before the onset of renal failure[2].

In 1994, the European Consortium for Polycystic Renal Disease identified the first gene related with ADPRD, named PKD1 and localized within the chromosome 16[3]. A second gene (PKD2) was further localized within the chromosome 4[4,5]. In 85%-90% of affected families, the disease is associated with the PKD1 gene, whereas in the remaining 10%-15% it is associated with the PKD2 gene[6,7]. Although the disease in PKD1- and PKD2-positive individuals shows the same general clinical features, there are differences about the disease progression and mortality, the PKD1-associated disease being more severe and manifesting earlier.

Proteins encoded by the PKD1 and PKd2 genes are named polycystin-1 and polycystin-2, respectively. To date, it is known that they are membrane proteins involved in intracellular signal reception and transduction[8-10] in processes such as proliferation or apoptosis. Mutations within the genes encoding for polycystins give rise to functionally deficient proteins that altered the above-mentioned processes, besides the polarizing and trans-cellular transport properties of epithelial renal cells[11]. This leads to uncontrolled tissue growth and fluid retention within the cysts, characterizing polycystic renal disease.

ADPRD diagnosis is made by ultrasound. However, genetic testing may be used if ultrasound findings are not conclusive, as a complementary test, or if a definite diagnosis is required in a patient younger than 30 years. Molecular diagnosis cannot predict the time of onset, the severity, the type of symptoms, or the degree of disease progression. However, it allows for an early intervention on follow-up and management of AHT, infections, and lithiasis, and which may delay the onset of renal failure[12]. Another important use of molecular diagnosis is familial genetic counseling, which consists in informing the patient with the disease about it, its inheritance, and risk for passing the causative gene to his/her sibling, which is 50%.

Genetic diagnosis may be done by direct search of the mutation or indirectly by linkage analysis. The mutation analysis poses difficulties due to the big size and complexity of the PKD1 gene and to the high number of mutations and polymorphisms described for this gene, which makes difficult distinguishing pathogenic changes from neutral changes. With linkage analysis, transmission from parents to siblings of a series of polymorphic markers localized within the interest gene or in the neighborhoods, which allows identifying of carrier individuals before the onset of the disease symptoms.

In this work we present the results of the molecular analysis of 30 families with ADPRD from the province of Las Palmas, done by linkage analysis with markers of the chromosome regions where the PKD1 and PKD2 genes are located. The study goals were: firstly, to check the degree of informative capability and therefore, the usefulness of microsatellites for family studies of ADPRD in our population; and secondly, to determine the sensitivity and specificity of genetic analysis with these markers in our population.

MATERIAL AND METHODS

Families

Thirty families selected from patients with an ADPRD diagnosis seeing at the Nephrology Department of University Hospital of Gran Canaria Dr. Negrín (24 families) and at the Genetics Unit of the Complejo Hospitalario Materno-Insular (6 families), were included into the study. These are the reference centers for this disease within the province of Las Palmas. These families brought 248 individuals to the study, of whom 116 had a previous ADPRD diagnosis. Informed consent was obtained from all participants before their inclusion into the study.

Ultrasound Study

Ultrasound diagnosis criteria included the existence of at least two cysts (considering both kidneys as a whole) in individuals younger than 30 years, two cysts within each kidney in individuals aged 30 to 59 years, and four cysts within each kidney in individuals aged 60 years or older[13]. Individuals meeting these criteria were classified as patients. These criteria have been agreed by European laboratories and are virtually 100% sensitive for patients older than 30 years or for younger patients but with PKD1 mutations, and 67% sensitive for patients younger than 30 years with PKD2 mutations[14]. All the ultrasounds were performed at the Ultrasound Unit of the Radiology Department of the University Hospital of Gran Canaria Dr. Negrín by a single ultrasound operator (CRHS), with a high quality Color Doppler ultrasound, model Aplio 80 «SSA-770A» (Toshiba, Tokyo, Japan) with a 3.75 MHz sectorial convex transducer.

Genetic determinations

A 10-mL peripheral blood sample was obtained from each individual from which the DNA was extracted by saline precipitation[15]. For genetic analysis, five PKD1 (D16S521, KG8, AC2.5, CW2, SM7) and four PKD2 (D4S1538, D4S1534, D4S423, D4S414) markers were used, all nine markers being analyzed in all families. The markers were amplified using the primers previously described[16-18] by polymerase chain reaction (PCR) in a final volume of 15 µL, with 50 ng of DNA, $MgCl_2$ 1.5 mM (0.5 mM for KG8), dNTPs 200 µM, 4 pmoles of each primer, and 0.5 U EcoTaq polymerase (Ecogen, Barcelona, Spain). The amplification protocol was carried out in a GeneAmp PCR System 9600 thermocycler (Applied Biosystems, Foster City, USA), and consisted of an initial denaturation at 94 ºC for 5 minutes, followed by 25 cycles of 30 seconds at 94 ºC, 30 seconds at 58 ºC, and 30 seconds at 72 ºC. A final extension step was done at 72 ºC for 20 minutes.

The amplifiers for the different markers were mixed in order to obtain a single sample per patient. 0.2 µL of the marker with a molecular weight GeneScan-500-ROX (Applied Biosystems) and 20 mL of deionized formamide were added to 1 µL of each one of these samples. The samples prepared in this way were denatured at 95º C for 2 minutes and rapidly cooled in ice before being analyzed by capillary electrophoresis in an ABI PRISM™ 310 sequencer (Applied Biosystems).

Results Analysis

The percentage of heterozygotic individuals was calculated for a sample of 74 non-related individuals comprised by the spouses and one patient per family (index case). This percentage is considered to be as one of the measures of the informative capability of polymorphic markers[19].

For two-points linkage analysis, the MLINK software from the LINKAGE package (PC DOS v5.2, Columbia University, New York) was used. For *lod* score calculations[20] 0.001 and 0.0001frequencies were considered for PKD1 and PKD2, respectively. Three penetration classes were defined, with values of 0.64 (individuals < 20 years), 0.92 (20-30 years), and 1 (> 30 years) for PKD1, and 0.50, 0.85, and 0.95 values, respectively, for PKD2[21]. In order to increase the power of the linkage analysis a new marker was defined consisting in the whole set of alleles from different microsatellites within each chromosome (haplotype). By analyzing the pedigrees, an haplotype responsible of the disease transmission was identified for each family (transmitting haplotype). The order considered for the markers within the respective chromosomes was the one published in previous studies[16-18,22,23] and in the Jean Dausset Foundation-Centre d'Etude du Polymorphisme Humain database (http://www.cephb.fr).

The student's t test was used to compare mean ages.

RESULTS

One hundred and twenty out of 248 individuals in whom genetic analysis was performed presented cysts meeting the ultrasound criteria to be considered as patients, and 115 individuals were classified as healthy. Mean ages, sex distribution, and genetic classification for both groups are shown in table I. In four individuals, ultrasound was not possible, so that they were not included in none of the groups. Of them, two were not carriers of the transmitting haplotype, one was carrier of the transmitting haplotype, and the last one was classified as undetermined from a genetic point of view due to lack of informative capability of the markers. Of all patients, 13 are new diagnoses.

In 29 out of the 30 families analyzed PKD1 haplotypes were clearly identified, in 28 out of 30, the PKD2 haplotype was clearly identified. In the families in which the haplotype could not be determined (family n.º 13 for PKD1, and families n.º 15 and n.º 23 for PKD2) because of low informative capability of the markers and the small size of the families, the linkage analysis was not performed (table II). Twenty-eight families showed genetic data compatible with PKD1 linkage, and in one of them (n.º 15) the linkage was excluded. Table II shows the distribution of patients and healthy individuals by families and *lod score* values obtained with the linkage analysis for PKD1 and PKD2 markers. The sensitivity and specificity of the genetic analysis were 90.7% (95%CI: 85.7-95.7) and 86.8% (95%CI: 80.6-93.0), respectively.

Table I. Age, gender and genetic analysis of the study participants

	Patients (n = 129)	Healthy (n = 115)
Age*	37.5 ± 13.7	44.1 ± 18.6
Gender		
Male	61 (47.3%)	55 (47.8%)
Female	68 (52.7%)	60 (52.2%)
Genetics**		
(+)	118 (91.5%)	0
(-)	0	100 (86.9%)
recombinant	8 (6.2%)	2 (1.7%)
undetermined	3 (2.3%)	13 (11.4%)

*Is expressed as mean ± standard deviation.
**(+): carrier of the transmitting haplotype; (-): not carrier of the transmitting haplotype; recombinant: carrier of a part of the transmitting haplotype; undetermined: an unequivocal haplotype cannot be established due to the lack of markers informative capability.

Table II. Number of patients and healthy individuals and *lod score* values for the PKD1 and PKD2 haplotypes within the studied families

Family	Patients	Healthy	*Lod* PKD1	*Lod* PKD2
1	9	4	2.00	–10.42
2	11	9	2.84	–1.42
3	3	5	1.14	–0.18
4	7	7	2.95	–2.31
5	3	3	0.90	–2.31
6	4	5	1.55	–3.45
7	6	4	1.43	–2.61
8	8	3	1.84	–7.37
9	2	5	0.57	–0.20
10	5	5	1.74	–3.96
11	3	6	1.44	–5.78
12	4	2	0.44	–3.49
13	2	3	NC*	–0.22
14	3	2	0,60	0.58
15	2	2	<<–2.00	NC*
16	2	2	0.30	–1.02
17	2	1	0.30	0.00
19	2	1	0.30	–3.58
20	4	2	1.14	–7.62
21	3	1	0.60	–0.05
22	7	1	1.36	–3.40
23	4	1	0.90	NC*
24	2	3	0.60	–0.07
25	3	2	0.90	–4.38
26	7	3	2.21	–10.58
27	7	5	2.07	–8.16
28	7	17	3.01	–17.43
29	2	5	1.10	–3.89
30	3	3	0.60	–5.67
31	3	2	0.47	–0.18

*NC: not calculated.
Note: family nº 18 was finally excluded from the study because of having just one individual with cysts.

In the 21 families with a clear result of PKD1 linkage (families 1-8, 10-12, 19, 20, 22, 23 and 25-30), 15 different transmitting haplotypes were found. One of the three repeated haplotypes was present in four families, another in three, and the third one in two families. The number of different non-transmitting PKD1 haplotypes was 110 out of 125. Fifteen recombination events were detected among PKD1 markers, six of which involved the chromosome identified as transmitter. For PKD2 markers, 13 recombination events were detected. The range of allele size and percentages of heterozygotic individuals found for each markers are shown in table III.

About the clinical features of the patients belonging to the 21 families with PKD1 linkage, the most frequent cause for diagnosis was the family history (table IV). There were no differences by mean age at the time of disease diagnosis, hypertension, or onset of renal replacement therapy (RRT) (table V). The number of patients on RRT, and the prevalence of hypertension, vascular-cerebral, coronary, and vascular peripheral pathology are shown in table VI.

DISCUSSION

Renal polycystic disease is the fourth cause of chronic renal failure in the Canary Islands and accounts for 8% of all dialysis patients in our Community (Renal Patients Registry, Canary Society of Nephrology, 2004). Genetic analysis of families with a history of ADPRD is important for several reasons. Firstly, it makes genetic counseling possible. Secondly, it allows for preventive follow-up in individuals carrying the transmitting haplotype. Finally, unequivocally exclusion of the disease in relatives of patients allow them living without the uncertainty of developing the disease in the future and serving as potential renal donors in case of necessary.

Sensitivity and specificity values obtained (90.7 and 86.8%, respectively) confirm the validity of the genetic

Table III. Size range and percentage of heterozygotic individuals for PKD1 and markers in our study

Gen	Marker	Allele range (pb)*	% heterozygous (in this study)	% heterozygous (calculated)**
PKD1	D16S521	152 – 172	70.3	71.5
	KG8	106 – 124	55.4	56.4
	AC2,5	154 – 170	73.0	79.5
	CW2	107 – 127	79.7	82.9
	SM7	81 – 103	60.8	64.0
PKD2	D4S1538	145 – 171	68.9	69.9
	D4S1534	138 – 166	78.4	76.9
	D4S423	97 – 115	70.3	83.8
	D4S414	222 – 236	70.3	89.4

* Size of alleles found in this study, in basepairs.
** Data from *The GDB Human Genome Database* (http://www.gdb.org).

Table IV. Cause of disease diagnosis* in PKD1 patients

	N (%)
Family history	60 (69.1)
Arterial hypertension	13 (14.9)
Hematuria	5 (5.7)
Urinary tract infection	2 (2.3)
Other	7 (8.0)
Total	87 (100)

*Non available data in 23 patients.

analysis with the markers selected for this study in our population. Most of these markers are heterozygous in a percentage of individuals varying from 68.9% to el 78.4% (table III), a range that is considered of high informative capability[24]. Only the KG8 and SM7 (PKD1) markers present smaller percentages that do not represent a disadvantage for the analysis since they are used in combination with other markers. The percentage of heterozygotic individuals found is similar to that of other studies[16,21] and those published by the *Human Genome Database* (table III). This level of informative capability represents an unequivocal identification of PKD1 haplotypes in 96.7% of the families in our study, and in 93.3% for PKD2. Considering the individuals, the genetic data were clearly interpretable (table I: genetics (+), (-) or recombinant) in 97.7% of the patients and in 88.7% of individuals without cysts.

With the two-points linkage analysis, 18 families had a *lod score* value close to 1 for PKD1 (table II). Family n.º 15, in which the value is well below -2.00, PKD1 linkage is excluded. The possible linkage to PKD2 could not be confirmed due to the lack of informative capability of the markers. In the remaining cases, in which the *lod score* is clearly lower than 1 (≤ values 0.60), linkage to PKD2 is excluded in three families (n.º 12, 19, and 30). In the remaining families, the *lod score* values are within the -1.02; 0.58 range, these being families with 5 members or less in most of the cases and in which the individuals at risk of having inherited the transmitting haplotype are younger than 30 years, which decreases the power of the analysis. These families will continue their follow-up for the next years trying to incorporate into the study more members in order to obtain conclusive results with regards to the genetic study.

About the clinical characteristics of PKD1 patients in our study, we should note that the first cause for disease diagnosis is the family history, followed by AHT (table IV), similarly to what has been found in other studies performed in the Spanish population[25,26]. The patients age at the time of disease and hypertension diagnosis and at the time of RRT onset for PKD1 patients (table V) is similar to that found in the study by Torra *et al*[26]. The percentages of patients on RRT and with AHT are, however, higher in our study (table VI), although these data might change provided information would be available in all the cases.

The results from this study do not seem to point towards the existence of a founder effect of PKD1 mutations in our population, as has been shown in a study performed at the Seychelles Islands[27], and even in the Canary population for type 1 primary hyperoxaluria[28], since only a small number of transmitting haplotypes is repeated among the different families. However, in order to verify this fact, a mutational analysis would be required since many of the different haplotypes vary just in one or two markers and could be the result of a recombination that would maintain the mutation. As mentioned in the results, 6 recombined PKD1 transmitting haplotypes were detected but we could not rule out the presence of others that may not have been detected because of having occurred in a previous generation to the one studied. However, we should take into account that the Canary Islands have received a continuous migratory inflow, mainly from Europe and Africa, for the last five centuries so that it may not be considered as an isolated population in spite of its insularity.

Our study has identified no carrier of the asymptomatic transmitting haplotype (table I: healthy individuals with genetics (+)). However, we should consider that in 13 individuals without cysts the markers did not show sufficient informative capability to be unequivocally classified as carrier or not-carrier (undetermined genetics). Of them, 4 are younger than 30 years so that it is likely that there may be carriers among them that might have been detected provided the informative capability of the markers in these families had been higher.

In light of the results from this and other works[14], ultrasound still is a highly sensitive and affordable technique for pre-symptomatic diagnosis of ADPRD. Because of the rela-

Table V. Mean age and standard deviation at the time of disease diagnosis, hypertension onset, and RRT onset in PKD1 patients with available data

	Male (N)	Female (N)	p
Diagnosis	26.5 ± 11.2 (35)	25.8 ± 10.5 (47)	0.757
AHT	33.8 ± 9.0 (17)	31.3 ± 10.8 (19)	0.447
RRT	43.4 ± 6.6 (11)	48.1 ± 8.3 (13)	0.138

AHT: arterial hypertension; RRT: renal replacement therapy.

Table VI. Clinical features of the disease in PKD1 patients (N = 110)

	Present	Absent	NA*
RRT	33 (45.8%)	39 (54.2%)	38
AHT	73 (78.5%)	20 (21.5%)	17
Cerebro-vascular abnormalities	5 (13.5%)	32 (86.5%)	73
Coronary pathology	8 (21.1%)	30 (78.9%)	72
Peripheral vascular pathology	2 (5.6%)	39 (54.2%)	74

*ND: not available data. AHT: arterial hypertension. RRT: renal replacement therapy.

tively low usefulness of making the diagnosis at an early age and the lack of treatment currently, linkage analysis would be specially indicated when there is a need for genetic counseling. However, this may change in the future thanks to the advances in the knowledge of molecular mechanisms of the disease and modifying genes that may lead to at least the development of drugs slowing its course, although not curing it[29,30].

In summary, in spite of the limitations derived from the requirement of the participation of an adequate number of affected and healthy individuals in familial studies, the age of the subjects with a negative ultrasound diagnosis, and the informative capability of the markers for each individual family, linkage analysis with two series of polymorphic markers located in the neighborhoods of the PKD1 and PKD2 genes has shown to be a useful tool for genetic diagnosis of ADPRD in our population.

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