Molecular diagnosis of autosomal dominant polycystic kidney disease

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ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited renal disorder. Its estimated prevalence is 1 per 800 individuals. ADPKD patients constitute 8% of the population on dialysis or kidney transplantation. The disease can be diagnosed using radiological or genetic procedures. Direct genetic diagnosis of the disease can now be performed in Spain; however, it is not an easy or cheap test. This is why every case should be considered individually to determine whether genetic testing is appropriate, and to determine which genetic test is most adequate. Genetic testing in ADPKD is of special interest for living donors and neonatal and sporadic cases. Genetic testing offers the chance of performing prenatal or pre-implantation testing of embryos in families with severe cases of the disease. Also, this will enable the disease to be treated, when specific treatment becomes available, in cases that would not be candidates for treatment without genetic confirmation.

Key words: ADPKD. Autosomal dominant polycystic kidney disease. Genetic diagnosis. *PKD1*. *PKD2*. Mutations.

Diagnóstico molecular de la poliquistosis renal autosómica dominante

RESUMEN

La poliquistosis renal autosómica dominante (PQRAD) es la enfermedad renal hereditaria más frecuente. Su prevalencia estimada es de uno cada 800 personas. Los pacientes con PQRAD constituyen un 8% aproximadamente de la población en diálisis o trasplante renal. El diagnóstico de la enfermedad es radiológico y/o genético. La posibilidad de realizar un diagnóstico genético directo de la PQRAD es actualmente una realidad en nuestro país, aunque por las características del gen PKD1 no es un análisis sencillo ni económico. Debe estudiarse cada caso de forma individualizada con el fin de determinar la idoneidad de realizar un estudio genético y determinar qué tipo de estudio es el adecuado. El diagnóstico genético es de especial interés para los donantes vivos, para casos neonatales y para casos esporádicos. El diagnóstico genético permite ofrecer diagnóstico prenatal o preimplantacional en familias con casos severos de la enfermedad y también permitirá tratar la enfermedad, cuando exista un tratamiento específico, en aquellos casos dudosos que sin confirmación genética no serían candidatos a tratamiento.

Palabras clave: *PQRAD. Poliquistosis renal autosómica dominante. Diagnóstico genético.* PKD1. PKD2. *Mutaciones.*

INTRODUCTION

Clinical and epidemiological background

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease. Its prevalence is estimated at 1 in 800 people. About 8% of patients on dialysis or undergoing kidney transplantation have ADPKD. It is characterised by the progressive development of kidney cysts that often lead to end-stage kidney disease (ESKD), usually in adulthood. Most patients usually have liver cysts, but only a minority develops massive polycystic liver disease. The presence of cerebral aneurysms in these patients is also rare (approximately 10%). A familial clustering of cerebral

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aneurysms has been described.[1,2] Therefore, cranial magnetic resonance angiography is currently recommended when an affected family member has brain aneurysms or an incident compatible with ruptured aneurysms. There is considerable clinical variability both within and between families, with the former being much more common.[3] The phenotypic spectrum of the disease ranges from severe prenatal cases to elderly patients with normal kidney function.

Genetic background

It is well known that the disease ADPKD has a genetic cause. It has genetic heterogeneity, i.e. more than one gene causes the disease. The genes responsible are: *PKD1* (located on chromosome 16, at 16p13.3)[4] and *PKD2* (located on chromosome 4, at 4p21).[5] Mutations in the *PKD1* gene are responsible for 85% of ADPKD cases, while mutations in the *PKD2* gene give rise to the remaining 15%.[6] The disease is more severe in cases caused by *PKD1* mutations. The mean age at onset of dialysis is 54.3 years for people with *PKD1* and 74 for people with *PKD2*.[7] For the same age, kidney size is significantly higher if the gene responsible is *PKD1* than for *PKD2*, which fully justifies the different evolution of the disease according to the CRISP study.[8] There is, however, considerable variability in the age of dialysis onset for each of the genes.[9]

PKD1 gene

The *PKD1* gene consists of 46 exons and encompasses a 54kb genomic region. It is transcribed into an mRNA of approximately 14kb with a 12,909bp coding sequence (Figure 1A).[10,11] Analysis of this gene is very complex, because the region between exons 1 and 33 has undergone intrachromosomal duplication throughout human evolution. It now contains 6 pseudogenes (*PKD1*P1-P6), located between 13 and 16Mb proximal to the *PKD1* gene. These pseudogenes are expressed but have stop codons preventing translation, so they are expected to give rise to small non-functional proteins. By comparing the sequences of these pseudogenes with *PKD1* pseudogenes, it can be seen that they contain mutations but they have a sequence identity of 98-99% in regions homologous to *PKD1*. The minor differences between these pseudogene sequences and *PKD1* have been fundamental in designing strategies for the selective amplification of *PKD1*, thereby preventing the amplification of these pseudogenes.[12-14]

The *PKD1* gene encodes an integral membrane protein called polycystin 1.

PKD2 gene

The *PKD2* gene contains 15 exons and covers a genomic region of 68kb with a coding sequence of 2,904pb.[5,15] This gene encodes the protein polycystin 2, which is a TRP calcium channel family (transient receptor potential). This is why it is also called TRPP2 (Figure 1B).

Both polycystins are located in the primary cilia, among other places. Therefore, ADPKD is considered a ciliopathy, like all cystic diseases of the kidney. It seems that the polycystins have a function related to the detection of flow,[16] pressure[17] and modulation of centrosome duplication, and/or cell cycle regulation.[18,19] They have also been implicated in the preservation of planar polarity.[20] All these mechanisms may be involved in cystogenesis, but the precise key mechanism is still unclear.

## DIAGNOSIS OF AUTOSOMAL DOMINANT POLYCYSTIC KINDEY DISEASE

Since ADPKD is an autosomal dominant disease with high penetrance, the offspring of affected parents have a 50% chance of developing the disease. In addition, given its high penetrance, it would be highly unlikely to clinically skip a generation. Currently, the disease is usually ruled out or confirmed after ultrasound in a person with an affected relative. The diagnosis of the disease is at present radiological and/or genetic.

Radiological diagnosis

Ultrasound is the most widely used technique for diagnosing the disease in subjects at risk. The classic criteria for the diagnosis of *PKD1* were described by Ravine et al. in 1993,[21] and more recently, the ultrasound criteria for ADPKD when the gene causing the disease is unknown have been published.[22]

There is no family history of the disease in 10% of cases found.[23,24] In these cases, the disease is diagnosed by chance, but it is often difficult to determine if it is really an ADPKD. The general population can progressively develop kidney cysts with age, so diagnosis is more complex for cases without a family history or in elderly adults. Despite the relative accuracy of ultrasound criteria, they are not very sensitive or specific in the young and in elderly individuals with a mild form of the disease.

CT and MRI are more expensive techniques than ultrasound but are more sensitive, with smaller cysts being detectable (2mm instead of 10mm with ultrasound). There are no radiologic criteria for diagnosis of ADPKD by CT or MRI.

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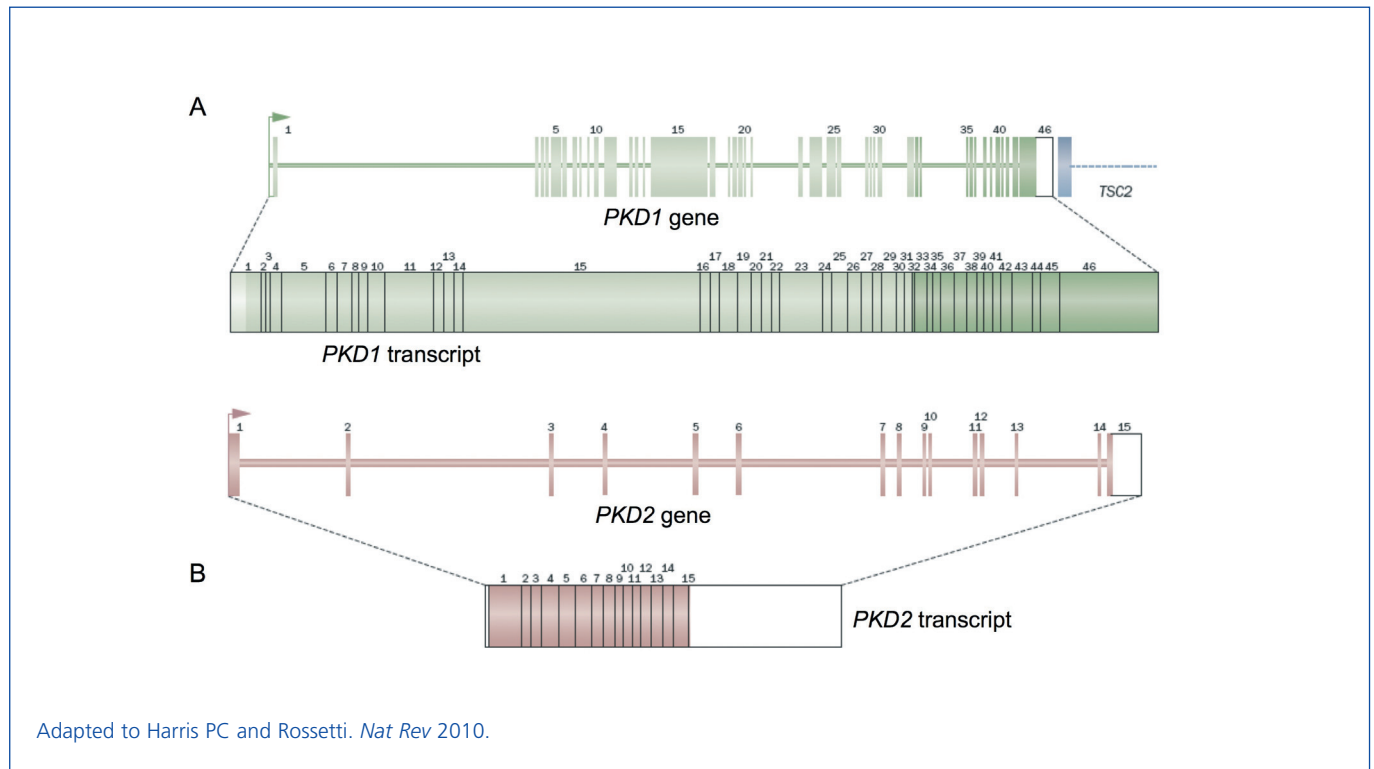
Figure 1. Structure of the *PKD1* (A) and *PKD2* (B) genes and their transcripts

The difficulty of diagnosing ADPKD via radiology in certain cases, such as the young or those with no family history, have necessitated the development of genetic diagnosis.
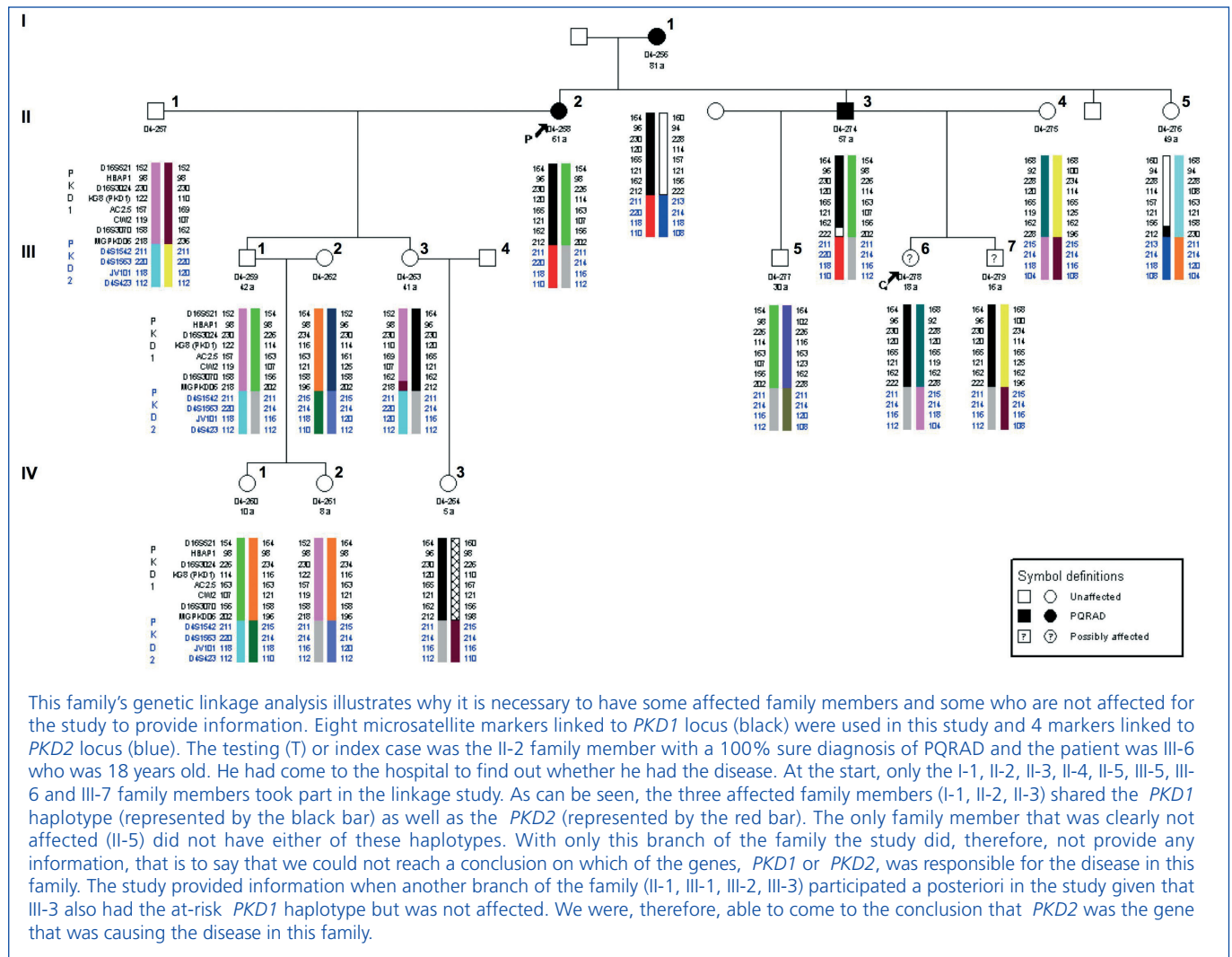
Genetic diagnosis

Genetic linkage analysis

Locating the genes *PKD1* and *PKD2* allowed ADPKD to be diagnosed by genetic linkage analysis in the 1990s. This study is indirect and is based on the analysis of genetic markers located in the regions of the *PKD1* and *PKD2* genes (Figure 1). It can determine whether it is the *PKD1* or *PKD2* haplotype which segregates with the disease in a given family and, consequently, which gene is responsible for the illness in the family. Currently, there are databases available locating a minimum of 15 microsatellite markers useful for genetic linkage to *PKD1* and 8 markers for *PKD2*. The major drawback of this type of diagnosis is that it can only be used in family cases. Also, due to the genetic heterogeneity of the disease, there must be several other affected and unaffected family members available for radiological study as well as the patient for an accurate ascertainment of the disease. It is also imperative that one member of the family is diagnosed with ADPKD with absolute certainty (this person is called the testing or index case). Only certain families are large enough for the study to confirm the linkage to one of the genes and discard the linkage to the other gene. In some cases, all affected family members share both the *PKD1* and the *PKD2* haplotype, and none of the unaffected family members carry these. Therefore, the study does not provide any information (as it cannot be discerned if the *PKD1* or *PKD2* is responsible for the disease, Figure 2). However, it is not only family size or how informative the markers may be that make this such a complicated diagnostic approach. There are also other phenomena, such as the presence of *de novo* mutations, mosaicism, the presence of hypomorphic alleles, etc. All this indicates that one must be very cautious with this type of diagnostic approach.

Mutation analysis

Although there are various possible approaches for the mutation study of *PKD1* and *PKD2* genes, exon sequencing is the most commonly used technique today. Many studies have shown the allelic heterogeneity of these genes, such that the same mutation is not found in more than 2% of families. This fact greatly complicates the search for mutations. Therefore, all exons in the *PKD1* and *PKD2*

This family's genetic linkage analysis illustrates why it is necessary to have some affected family members and some who are not affected for the study to provide information. Eight microsatellite markers linked to *PKD1* locus (black) were used in this study and 4 markers linked to *PKD2* locus (blue). The testing (T) or index case was the II-2 family member with a 100% sure diagnosis of PQRAD and the patient was III-6 who was 18 years old. He had come to the hospital to find out whether he had the disease. At the start, only the I-1, II-2, II-3, II-4, II-5, III-5, III-6 and III-7 family members took part in the linkage study. As can be seen, the three affected family members (I-1, II-2, II-3) shared the *PKD1* haplotype (represented by the black bar) as well as the *PKD2* (represented by the red bar). The only family member that was clearly not affected (II-5) did not have either of these haplotypes. With only this branch of the family the study did, therefore, not provide any information, that is to say that we could not reach a conclusion on which of the genes, *PKD1* or *PKD2*, was responsible for the disease in this family. The study provided information when another branch of the family (II-1, III-1, III-2, III-3) participated a posteriori in the study given that III-3 also had the at-risk *PKD1* haplotype but was not affected. We were, therefore, able to come to the conclusion that *PKD2* was the gene that was causing the disease in this family.

Figure 2. Genetic linkage analysis for *PKD1* and *PKD2*.

genes must be analysed systematically. Given the size and complexity of *PKD1*, this is particularly expensive. To analyse the *PKD1* region homologous to pseudogenes, polymerase chain reaction (PCR) of the sequences where *PKD1* differs from these pseudogenes has been designed. Thus, the genomic region that includes exons 1 to 33 is amplified in 5 long PCR, with these initial products then used as a template in the subsequent amplification of these 33 exons.[13] The remaining 13 *PKD1* and 15 *PKD2* exons can then be amplified conventionally using the patient's genomic DNA. Once the 46 exons in *PKD1* and/or the 15 in *PKD2* are sequenced, the potential pathogenicity of the different sequence variants identified must be correctly assessed.

A very useful tool for this is the database developed and maintained by the Mayo Clinic (http://pkdb.mayo.edu/cgi-bin/mutations.cgi), which includes all the sequence variants described in these genes. Table 1 shows the types of

mutations that cause ADPKD. As can be seen in Figure 3A, there are mutations that are clearly pathogenic, while a considerable percentage of sequence variants identified in these genes are probably neutral. Variants expected to result in a truncated protein (smaller than the wild-type protein), and those that affect canonical splicing sequences that are 100% conserved, are generally considered to be clearly pathogenic and require no further study. On the other hand, the in-frame variants that do not interrupt the translation process of the protein (amino acid change variants, deletions/insertions of base numbers which are multiple of 3, mutations in non-coding regions) require further evaluation. If we add to this the fact that each patient has an average of 10 neutral variants in the *PKD1* gene, we can begin to see how difficult diagnosing the disease is in many cases. There are bioinformatic tools used to classify these sequence variants, thus determining their pathogenicity.[12,25-28] To date, there are 436 different *PKD1* mutations and 115 for *PKD2* on the database. Figure 2B shows the different types of

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Table 1. Mutations in *PKD1* and *PKD2* that cause autosomal dominant polycystic kidney disease

Clearly pathogenic mutations: Those that truncate the protein or alter the canonical splicing sequences (AG/GT).

- *Nonsense*: A nucleotide substitution resulting in a stop codon.
- *Frameshift*: Insertion or deletion altering the reading frame of the mRNA.
- *Canonical splicing*: Change in canonical nucleotides flanking the exon, which change the way the intron is cleaved from the exon.
- *Large chromosome rearrangements*: Deletion or insertion of a considerable region of the gene, usually involving more than one exon.

Mutations of uncertain clinical significance: Those that do not alter the reading frame or have uncertain consequences. Their pathogenicity is unclear and require further analysis.

- *Missense*: Substitution of one amino acid for another.
- *In-frame* (deletions and insertions maintaining the reading frame): Gain or loss of a number of multiple of three nucleotides, thus maintaining the reading frame.
- *Non-canonical splicing*: Changes in the excision of introns not affecting the canonical nucleotides flanking the exon.

***De novo* mutations**: A mutation occurring in a family for the first time.

Mosaicism: A mutation that occurs in an early embryonic stage, but does not affect all the cells (the affected patient is a chimera). Mosaicism may also affect germ cells (the patient produces germ cells with and without mutation).

Hypomorphic alleles or alleles with incomplete penetrance: A sequence variant alone does not lead to a typical phenotype of ADPKD, because it is generated with the protein partially functioning. Patients with these mutations in both alleles have a common or severe form of the disease.

sequence variants identified for both *PKD1* and *PKD2*. Sometimes, there are large deletions that remove up to 10 genes near the 5' region without phenotypic consequences, apart from ADPKD. Moreover, deletions may occur in the 3' region which also includes the TSC2 gene (which causes one of two types of tuberous sclerosis), giving rise to a contiguous gene syndrome which clinically results in early onset polycystic kidney disease and tuberous sclerosis. As can be seen, the percentage of sequence changes likely to be highly pathogenic is much higher in *PKD2* than in *PKD1* (Figure 2A). Likewise, the percentage of missense mutations (change of direction) is much higher in *PKD1*, which makes diagnosis difficult as their pathogenicity must be proved (Figure 2B).

In total, sequence variants with a high probability of pathogenic mutations can be identified in 91% of families. Of these, 65% are protein-truncating mutations and can therefore be directly used for diagnosis, but 26% are in-frame variants that require careful analysis before they can be used in testing.[29] The segregation of these in-frame variants in a given family is a great help in definitively establishing their pathogenicity. It is also very important to consider whether they alter highly conserved amino acids in homologous proteins. Also, the inclusion of these mutations in databases will give a more accurate determination of pathogenicity when the same mutations are found again in other families.
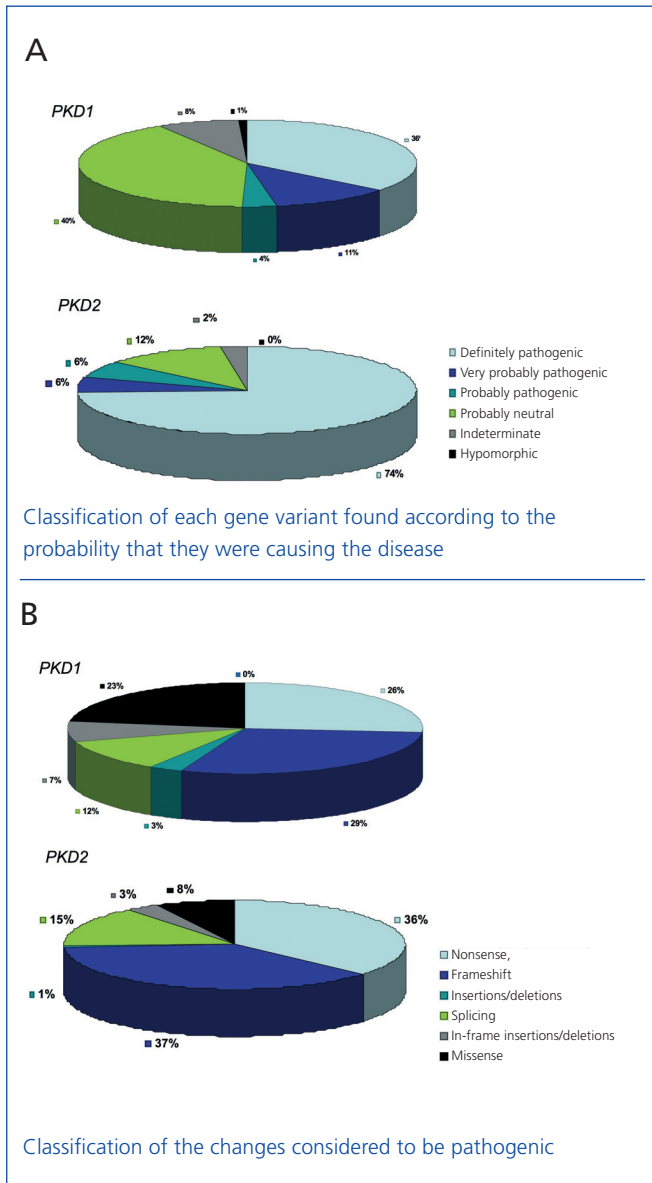
Instead of or in addition to bioinformatic tools, functional studies can be performed for other genes to determine the functional consequences of sequence variation, and therefore, their pathogenicity. Although this is theoretically possible for *PKD2* and for some *PKD1* mutations, these are extremely time-consuming techniques, which are not sufficiently sensitive or specific and not applicable for routine diagnosis. Therefore, although a functional test would be helpful, bioinformatic tools are likely to be much more important in the genetic diagnosis of ADPKD than functional studies.

Additional difficulties with genetic diagnosis by detection of mutations in autosomal dominant polycystic kidney disease.

Individuals with autosomal dominant polycystic kidney disease with no mutation detected

No pathogenic mutation in either *PKD1* or *PKD2* is found in about 9% of patients with ADPKD.[29] These patients usually have a milder form of the disease, and usually do not have a family history of the disease. Thus, the non-detection of mutations is not only a lack of sensitivity of the technique but could in fact be indicative of another disease. Firstly, there may be changes in the introns that affect splicing

A

PKD1

PKD2

Definitely pathogenic
Very probably pathogenic
Probably pathogenic
Probably neutral
Indeterminate
Hypomorphic

Classification of each gene variant found according to the probability that they were causing the disease

B

PKD1

PKD2

Nonsense,
Frameshift
Insertions/deletions
Splicing
In-frame insertions/deletions
Missense

Classification of the changes considered to be pathogenic

Figure 3. Classification of sequence variants found in the *PKD1* and *PKD2* genes according to the mutation database in *PKD1* and *PKD2* (http://pkdb.mayo.edu/cgi-bin/mutations.cgi).

(which could be detected by intron sequencing or mutation studies using RNA). There may be mutations in the regulatory regions, such as the promoter (so far no mutations in these regions have been described); or there might be changes that may alter the protein assessed as non-pathogenic *in silico* (using bioinformatic tools), that may, however, produce a subtle alteration of the protein causing the disease. Secondly, families have been described with no linkages to *PKD1* or *PKD2* although, after fully exploring these families, there are doubts about the linkage analysis being correct.[30] Finally, there are cystic diseases with a similar phenotype/clinical course which can behave like

phenocopies, due to the genes HNF1B, PRKCSH, SEC63 or PKHD1.

Mosaicism

Mosaicism (the coexistence of normal and mutant cell populations in an individual) is common in genetic diseases with a high rate of *de novo* mutations. Only 10% of cases are *de novo* in ADPKD, so this phenomenon is not expected to be very common. Mosaicism can be germinal (when it affects only the germline), somatic (if the genetically distinct cell populations are only somatic) or gonosomic (when it involves both somatic and germ cells).

Two ADPKD families with mosaicism have been described.[31,32] To detect it, the first generation of the family with one or more relatives affected by the disease (*de novo* case) has to be available. Mosaicism may explain the large phenotypic variability within a family, and it must be taken into account when offering genetic counselling. Thus, a patient with apparently healthy parents, and therefore considered a *de novo* or sporadic case, may have affected siblings if one parent suffers from germinal mosaicism (having germ cells with and without the mutation). The presence of mosaicism is one of the reasons why genetic linkage analysis is not conclusive and may be negative for *PKD1* and *PKD2*. In addition, when there is somatic mosaicism, there may be different levels of a mutant allele in different tissues. Therefore, a determination in peripheral blood may not be representative of what is happening in the kidneys.[31]

Genotype-phenotype correlation and hypomorphic alleles

Current data shows poor genotype-phenotype correlation for both *PKD1* and *PKD2*. Although some reports show some correlation for a mutation type or location, the results are insufficient to establish a prognosis based on the detected mutation.[33,34]

It was believed that missense mutations in the PKD genes were inactivating. However, recently[28,35] it was seen that some of these changes result in hypomorphic alleles or incomplete penetrance that behaves as if the ADPKD were a recessive disease. This phenomenon has also been observed in animal models of ADPKD.[36]

Recently, members of families with ADPKD have been detected who are affected very differently by the disease, due to the presence of hypomorphic alleles.[28,35] Thus, individuals with the two hypomorphic *PKD1* alleles in trans have a severe phenotype, whereas individuals in the family with only one of these alleles have a much milder form of the disease, or may

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not even have detectable kidney cysts. As a result, the disease may be erroneously labelled in these families as recessive polycystic kidney disease or as sporadic cases of ADPKD. In both cases, the error may have serious consequences for genetic counselling and will result in linkage analysis errors. To determine the pathogenicity of these hypomorphic alleles resulting in an amino acid change in polycystin 1, the available bioinformatic tools must be consulted.

It is estimated that between 43% and 50% of the clinical variability in ADPKD, regarding the age of patients with end-stage kidney disease (ESKD), stems from modifying genetic effects.[37-39] It is not currently known to what extent the hypomorphic alleles are involved in the phenotypic variability of ADPKD.

The current status of genetic diagnosis of autosomal dominant polycystic kidney disease

In general, genetic testing for ADPKD is not recommended when the clinical and imaging diagnosis is clear, because it is very expensive and in many cases, does not provide relevant information. Table 2 summarises the current indications for genetic diagnosis of ADPKD.

Genetically testing patients to determine whether the gene causing the disease is *PKD1* or *PKD2* is questionable, as there is a significant clinical variability within each gene,[40] although it is clear that being a mutant *PKD2* gene carrier leads to a better outcome than for *PKD1*.[41]

The genetic diagnosis of ADPKD is not extensively indicated due to the cost and current technical complexity of determining whether the changes detected in the DNA sequence are actually pathogenic mutations. As sequencing techniques become cheaper and the pathogenicity of the detected changes can be better determined, the technique may be used much more widely.

Moreover, there is currently no effective treatment for ADPKD, although there are several ongoing clinical trials. It is hoped that in a few years there will be treatment for the disease, and then, it will be necessary to be sure about the diagnosis of a patient. Therapies may be initiated in younger patients where imaging diagnosis is inconclusive.

CONCLUSIONS

Direct genetic diagnosis of ADPKD is now possible in Spain, although the analysis is not easy or cheap due to the characteristics of the *PKD1* gene. Each case must be considered individually to determine whether genetic testing should be carried out and what type of study is appropriate. This type of diagnosis is of particular interest to living donors and in neonatal and sporadic cases. Genetic testing offers the chance of performing prenatal or pre-implantation testing in families with severe cases of the disease. Also, this will enable the disease to be treated, when specific treatment becomes available, in unconfirmed cases that would not be candidates for treatment if it was not for genetic confirmation.

Table 2. Genetic diagnosis indications for autosomal dominant polycystic kidney disease

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Potential living donor: Each case must be evaluated individually, taking into account age and severity of illness in the family, as well as imaging tests.

Patients without a family history of ADPKD. Especially indicated:

- When radiographic findings are atypical (eg, very marked kidney asymmetry, multiple small cysts, kidney disease in the presence of normal size kidney cysts).
- In mildly affected patients.
- In patients with atypical extrarenal symptoms of ADPKD.
- For relative prognostic information, as *PKD1* is associated with a worse prognosis than *PKD2*.

Families with many members with kidney cysts with radiographic pattern not typical of ADPKD, candidates for a differential diagnosis of other cystic kidney diseases.

Patients with very early onset of the disease.

- In families with typical presentation of ADPKD, but with one member with very early presentation, genetic studies may identify a hypomorphic allele in addition to an allele with a pathogenic mutation.
- In patients with no family history of ADPKD and no detected mutations in the PKHD1 gene (cause of autosomal recessive polycystic kidney disease) or with radiological features of ADPKD.

Patients with or without family history who want a prenatal or pre-implantation genetic diagnosis.

KEY CONCEPTS

1. Molecular diagnosis of ADPKD is increasingly feasible and provides increasing information.

2. The most important uses are currently determining whether a living donor candidate has the disease or not, and for couples who want a pre-implantation genetic diagnosis.

3. Genetic studies confirm the suspected diagnosis of the disease in sporadic patients or those with atypical symptoms.

4. It is of interest in determining if a family is *PKD1* or *PKD2*, as this is more indicative of the clinical course of the disease in the family than the genotype itself.

5. Hypomorphic alleles significantly alter the *PKD1* phenotype and have important significance in prognosis and genetic counselling.

6. When ADPKD treatment is available, a precise diagnosis of the disease must be available.

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