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Review Article

Autosomal Dominant Polycystic Kidney Disease and Genetic Counseling

Suraksha Agrawal*

Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, India

Abstract

Autosomal polycystic kidney disease (ADPKD) is a progressive, adult-onset disease instigating end-stage renal disease (ESRD). Pre-symptomatic diagnosis is carried out among family members who are at risk. Abdominal imaging is used to see the cyst development in ADPKD. Cystsare mostly associated with age, even genetically unaffected individuals can develop few simple cysts as they age hence criteria have been developed to define whether at-risk individuals are affected based on number of cysts by age.

This disease displays high degree of allelic heterogeneity, carrying a germline mutation in one of the alleles i.e. PKD1 or PKD2 or loss of heterozygosity resulting into cyst formation. There are other epigenetic mechanisms that may play an important role in the causation of ADPKD. Genetic counseling among these patients create awarenessand also helps the patients to receive the integrated patient support. ADPKD is a monogenic disorder but due to high degree of allelic variability makes genetic counseling quite complex. However, in the last decades, the remarkable advances in the knowledge of genetic aspects of ADPKD based on genetic and molecular research, as well as the development of new molecular diagnostic tools, has substantially changed this situation. Nowadays, it is estimated that using the currently available molecular tests, a potential underlying genetic cause can be identified. Combined with clinical assessment, prenatal history evaluation and investigation of other physiological aspects, an etiological explanation for the disease can be found. Therefore, in view of the current knowledge about the genetic architecture of ADPKD, has contributed for a more precise genetic counseling, and of the potential benefits that an etiological investigation can bring to patients and families, molecular genetic investigation has become increasingly important. The diagnosis of PKD is based upon genetic testing that helps in the proper diagnosis of ADPKD which helps to improve the therapeutic options.

Keywords: Polycystic kidney disease; PKD1; PKD2; Genetic testing; Genetic counseling

Introduction

Autosomal Polycystic kidney disease [MIM#173900] (APKD) affects kidneys and other organs. The distinguishing feature of this disease is the development of variable number of renal cysts which may amount to the presence of thousands of cysts and can weigh up to 20 to 30 pounds. This is one of the most common monogenic autosomal dominant diseases with a penetrance of almost100 percent. This disease has two extremes on one hand are those that live up to old age and develop only few cysts with no renal insufficiency, whereas at the other end of the spectrum are neonates that die shortly after birth with hugely enlarged cystic kidneys.

It has been estimated that ADPKD affects I in 500 to 1000 births. This disease has a range of renal cysts that become distended due to fluid retention and kidneys are not able to filter the waste product from the blood resulting into renal failure. It is related to hypertension at young age i.e. 20 to 30 years of age, pain in the back or sides, hematuria, repeated urinary tract infections, kidney stones, and heart valve abnormalities. PKD can affect other organs like liver, pancreas, lung etc. There are approximately 5% of the patients that require dialysis or kidney transplantation and may be associated with PKD. PKD patients also suffer from congenital anomalies, such as intracranial aneurysms, thoracic aortic aneurysms and impaired function of the heart valves that can be lethal.

PKD was recognized as early as 1533 by Stefan Bathory, the King of Poland, who lived from 1533 to 1588. Polycystic kidney disease exists in two forms dominant and recessive. Dominant form is further characterized into PKD1 (MIM: 601313) and PKD2 (MIM: 173910). PKD1 encodes a gene having the 14-kb transcript and was discovered in 1994. Two years later in 1996 PKD2 was discovered by Mochizuki et al, [1]. In 1997 Ariza et al. [2], described two generation Spanish family with PKD and demonstrated that PKD1 and PKD2 were not present in this family but some other variant form was seen which was labeled as PKD3 however, this has not yet been mapped. The pathophysiology of autosomal dominant polycystic kidney diseases reveals that the primary abnormality leading to cyst formation in both the autosomal dominant and recessive forms of PKD is due to defects in cilia-mediated signaling pathway [3]. In the kidney, primary cilia are found on most of the cells of the nephron, projecting from the apical surface of the renal epithelium into the tubule lumen [4]. In response to fluid flow over the renal epithelium, the primary cilium is bent, resulting in a flow-induced increase in intracellular calcium [5]. Polycystin 1 and 2 proteins are localized to the primary ciliathat are involved in the tubulogenesis,

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***Corresponding author:** Suraksha Agrawal, Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Ex-Head, Lucknow 226014 (UP), Srimann Superspecialty Hospital, NH44, Jalandhar Pathankot Road, Near Reru Chowk, Jalandhar-144012, India, Tel: 91-9839604343; Email: sur_ksha_agrawal@yahoo.co.in

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maintenance of tubular structure and sensing of urinary flow to maintain normal orientation. The cilia-associated disorders include a variety of different entities, ranging from autosomal dominant and autosomal recessive forms of polycystic kidney disease (ADPKD and ARPKD, respectively) to nephronophthisis, Senior–Løken syndrome, Joubert syndrome, Meckel–Gruber syndrome, Bardet–Biedl syndrome, von Hippel–Lindau syndrome and tuberous sclerosis complex [6-9].

Glomerular filtration rate continues to decline gradually among PKD patients, eventually leading to ESRD by 55–75 years of age in patients with *PKD1* mutations and around 20 years later in patients with *PKD2* mutations who exhibit a milder course of disease [10,11].

Diagnosis

Patients with the positive family history of ADPKD are diagnosed using ultrasonography. Initial guidelines stated that two renal cysts are suitable to diagnose ADPKD. Recently different algorithms have been developed to predict renal survival in autosomal dominant polycystic kidney disease. A recent cross-sectional study conducted on 1341 patients from the Genkyst cohort studied the influence of clinical and genetic factors on renal survival. Multivariate survival analysis was carried out and authors have identified four variables that were significantly associated with the age at ESRD onset, and a scoring system from 0 to 9 was developed depending upon: being male: 1 point; hypertension before 35 years of age: 2 points; first urologic event before 35 years of age: 2 points; PKD2 mutation: 0 points; no truncating PKD1 mutation: 2 points; and truncating PKD1 mutation: 4 points. Based upon these criteria patients were classified into low risk (0-3 points), intermediate risk (4-6 points), and high risk (7-9 points) of progression to ESRD, with corresponding median ages for ESRD onset of 70.6, 56.9, and 49 years, respectively. Whereas a score ≤3 eliminates evolution to ESRD before 60 years of age with a negative predictive value of 81.4%, a score >6 forecasts ESRD onset before 60 years of age with a positive predictive value of 90.9%. This new prognostic score accurately predicts renal outcomes in patients with ADPKD and may enable the personalization of therapeutic management of ADPKD [12]. In some of these cases who belong to the age group <35 years of age with no or incomplete clinical data the above scoring pattern (PROPKD) was not applied, authors evaluated the predictive ability of a genetic score that constituted the genetic data and the sex only. Patients with *PKD2* mutations: 1 point; patients with non-truncating *PKD1* mutations: 2 points; women with truncating *PKD1* mutations: 3 points; and men with truncating *PKD1* mutations: 4 points. A genetic score ≥2 points, which incorporates the presence of a truncating *PKD1* mutation, predicted ESRD onset before age 65 years with a sensitivity of 73.8%, a specificity of 74.3%.

Mayo clinic classification system is based on age-banded height-adjusted total kidney volume (TKV) [13] PROPKD and (TKV) scores depend upon the genotype and the age of onset of clinical symptoms. Mayo clinic classified the autosomal dominant PKD patients into three categories i.e. low, intermediate, or high risk for progression to ESRD. This scoring system is applicable to patients >35 years hence cannot be applied to the younger patients especially without symptoms. This reflects that there is a need to improve the scoring system through imaging, clinical scoring and genetic testing this will lead to the better assessment of the patients and genetic counseling.

Imaging methods

Imaging methods are based on ultrasound that is used as an imaging modality to diagnose the ADPKD. ADPKD is suspected when patients present with symptoms related to the large kidneys associated with hypertension, flank pain or hematuria, palpable kidneys,liver or subarachnoid hemorrhage. ADPKD is also diagnosed incidentally when an abdominal ultrasound is carried out for other reasons, such as pregnancy. When there is no family history of ADPKD the presumptive diagnosis can be made if more than 10 cysts in each kidney are found more so absence of any other disease associated with cysts formation, such as tuberous sclerosis complex, von Hippel-Lindau disease, and acquired cystic disease. Repeat ultrasound or magnetic resonance imaging scanning may be useful to detect new cysts [14]. According to the new unified criteria presented by Pei *et al.*, the presence of less than two renal cysts has a negative predictive value of 100% and is enough to exclude the disease in high risk individuals who belong to ≥40 yrs of age [15]. In 2010 Barua et al., have shown that the negative predicted values, positive predicted values and specificity of PKD1 and PKD2can help to find out the diagnoses ofunknown cases where otherwise no diagnosis was possible [16].

Genetics of autosomal dominant kidney disease

Autosomal dominant polycystic kidney disease (ADPKD) causes progressive loss of renal function in adults around 60 years of age because of the accumulation of cysts. It affects only 1/400 or 1/1000 individuals [6]. Among family members autosomal dominant kidney disease is caused due to the mutation in two genes i.e. PKD1, PKD2. PKD1 is located on chromosome 16p13.3 it is consisting of 46 exons with an open reading frame of approximately 13 kb and encodes a protein of 4302 amino acids. The 50 Kb of PKD1 region encodes a large transcript with an open reading frame of 12,909 bp. PKD1 has 46 exons. The exon 33 has been duplicated six times on chromosome 16p, and the presence of these highly homologous pseudo genes has made the genetic analysis and genetic counseling of PKD1 quite challenging [17].

PKD1 mutations account for around 85% of the ADPKD cases in clinically identified populations. Disease progression of ADPKD is highly variable due to strong gene locus effect [17]. Two recent studies have documented higher rate of mutation of PKD2 from Toronto (26%) and Manchester (36%) (16) PKD2 is a single-copy gene located on chromosome 4 (4q21) consisting of 15 exons (70,133bp) with an open reading frame of approximately 3 kb and is predicted to encode a protein of 968 amino acids [6]. Mutations in PKD2 are accounted for the remaining 15% [8]. Recently a comprehensive analysis has revealed a high degree of genetic heterogeneitymultiple bilateral renal cysts that replace normal renal parenchyma, causing end-stage renal disease (ESRD) in approximately 50% of the individuals with ADPKD by

the age of 50 however; it varies depending upon the presence of PKD1 or PKD2 gene mutation. Both APKD1 and APKD2 genetics is quite complex as many modifier genes also play an important role in causation of APKD1 and APKD2 disease (Figure 1). It has been shown that there are approximately 9% of the cases which remain unsolved at genetic level [18].

Mutations in protein coding region i.e. polycystin-1 occur in 87% of cases of ADPKD and mutations in polycystin-2 are found in 12% of ADPKD patients. Both these proteins are of variable size i.e. (~460 and ~110 kDa,) [19]. Polycystin-1 is a receptor for an unidentified ligand, whereas polycystin-2 forms a nonselective calcium channel [8]. These proteins are involved in the ion channel complex and have an important function in tubular differentiation and maintenance [9]. Both proteins localize to primary cilia required for the proper functioning. The complexity of ADPKD has not allowed in identifying the mechanisms underlying its pathogenesis. Heterogeneity between families and "within-family" has been noticed in ADPKD. Reasons proposed for such a diversity are: (i) spontaneous mutations —which may arise due to *de novo* mutations superimposed on existing mutations giving rise to new mutations. Approximately 10% of patients show spontaneous mutations. Early-onset ADPKD may be due to these mutations [20,21]. Another hypothesis is that there may be hypomorphic mutation of PKD1 inherited in trans configuration manner [9,22-25]. The average age of onset of ESRD is 54 years in patients harboring PKD1 gene mutations while it is 74 years for patients having PKD2 gene mutations PKD1 and PKD2 encode two distinct proteins these are polycystin-1 and polycystin-2 (~460 and ~110 kDa) respectively [19]. Polycystin-1 is a receptor for an unidentified ligand, whereas polycystin-2 forms a nonselective calcium channel [26-31]. These proteins are involved in the ion channel complex and play an important role in tubular differentiation and maintenance.Both proteins are localized to primary cilia that are essential for their correct function.

Mutations in both the genes i.e. PKD1 and PKD2 result into reduction in intracellular calcium, increase in cyclic adenosine monophosphate (cAMP), activation of protein kinase A, and an increase in sensitivity of collecting duct principal cells causing vasopressin (Figure 2). This result into enhanced cAMP signaling that activatedownstream signaling pathways responsible for impaired tubulogenesis, cell proliferation, increased fluid secretion, and interstitial inflammation. Abnormal epithelial chloride secretion occurs throughthe cAMP-dependent transporter encoded by theCFTR gene and plays an important role in generatingand maintaining fluid-filled cysts in ADPKD. Otherpathogenic pathways may include activation of mTOR, Wnt, or hedgehog signaling; direct effects ofPC-1 fragments on gene transcription; and increasedaerobic glycolysis.

Known genetic mutations in autosomal ADPKD

The PKD mutation database is available at http://pkdb.mayo.edu/index.html. According to this data base there are 81 % PKD1 mutations while PKD2 mutations are seen only in 19% of cases. PKD1 mutations are categorized into in frame in/del mutations which are found in only 8% of the cases, splice site mutations (9%), nonsense mutations (24%), mis-sense mutations (27%) in frame shift mutations (3%) while large deletions are seen only in small percentage of patients (1%). PKD2 mutations are classified into non-sense mutations (42%), missense mutations (10%), frame shift (27%), large deletions (3%), in frame in/del (2%) and splice site mutations are seen in 16% of PKD2 cases. Mutations in *PKD1*, *PKD2*, or *PKHD1*, function of the primary cilium is impaired, resulting in the interruption of several intracellular signaling cascades that create dedifferentiation of cystic epithelium, increased cell division, increased apoptosis, and loss of resorptive capacity [32,33] (Figure 3).

Signaling pathways include cAMP activated, Wnt signaling, and mammalian target of rapamycin (mTOR) pathways, the discoveries of which have greatly expanded the number of potential

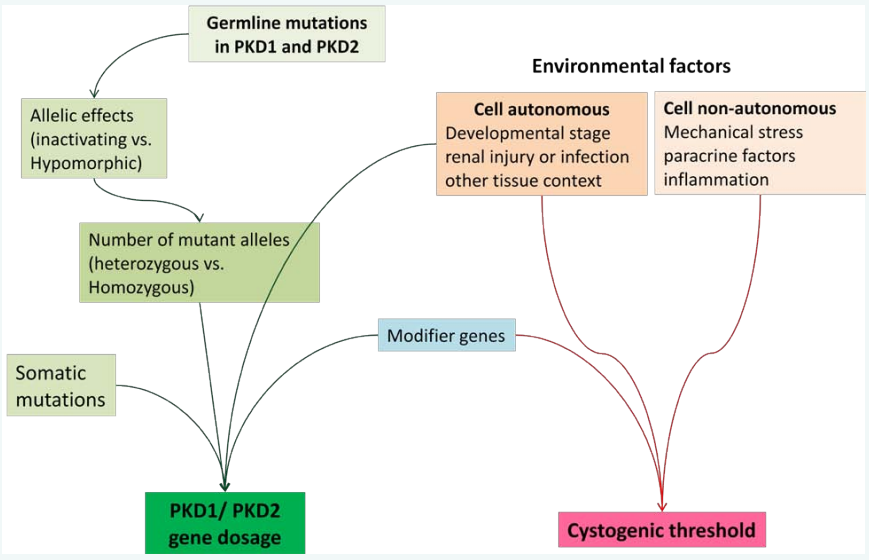


Figure 1 Role genetic and environmental factors in APKD.

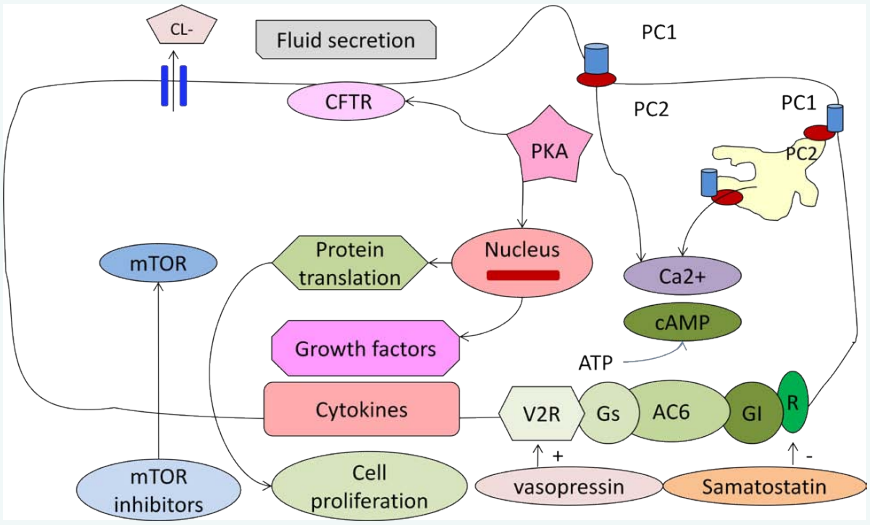
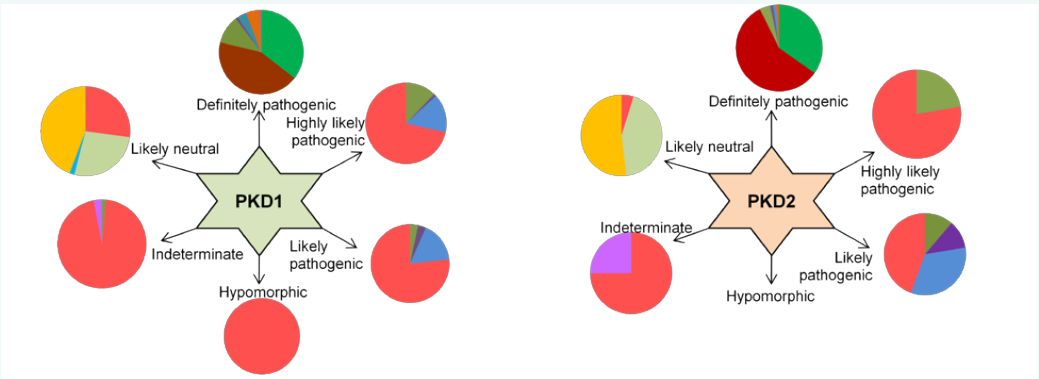


Figure 2 ADPKD pathophysiology.
Polycystin 1 and 2 localize to primary cilia. These are involved intubulogenesis, maintenance of tubular structure and sensing of urinary flow to maintain normal orientation
Abnormalities in these genes and the resultant loss of polarity can result in cyst formation
What is clear in PKD is that intracellular cAMP levels are increased, and of its many effects, two that are relevant to the disease process occur:
Increase cell proliferation, including through the mTOR pathway
Activation of the CFTR chloride channel leading to calcium secretion at the apical membrane.



	Definitely pathogenic	Highly likely pathogenic	Likely pathogenic	Hypomorphic	Indeterminate	Likely neutral
PKD1						
Nonsense	35.70	0.00	0.00	0.00	0.00	0
Frame shift	43.50	0.00	0.00	0.00	0.00	0
Splice	10.90	12.20	3.30	0.00	1.50	0
Insertion	0.97	1.00	3.30	0.00	0.00	0
Deletion	3.60	15.30	16.60	0.00	0.00	0.28
Large deletion	4.90	0.00	0.00	0.00	0.00	0
Large duplication	0.32	0.00	0.00	0.00	0.00	0
Substitution	0.65	71.40	76.70	0.46	95.50	26.9
IVS Silent	0.00	0.00	0.00	0.00	0.00	26.9
IVS unknown	0.00	0.00	0.00	0.00	2.98	0
3' UTR silent	0.00	0.00	0.00	0.00	0.00	1.7
Synonymous	0.00	0.00	0.00	0.00	0.00	44.3
PKD2						
Nonsense	30.9	0.00	0.00	0.00	0.00	0
Frame shift	51.5	0.00	0.00	0.00	0.00	0
Splice	3.5	22.20	2.60	0.00	0.00	0
Insertion	1	0.00	2.60	0.00	0.00	0
Deletion	1	0.00	7.70	0.00	0.00	0
Large deletion	1	0.00	0.00	0.00	0.00	0
Substitution	0	77.80	10.30	0.00	75.00	2.8
IVS Silent	0	0.00	0.00	0.00	0.00	25
IVS unknown	0	0.00	0.00	0.00	25.00	0
Synonymous	0	0.00	0.00	0.00	0.00	30

Figure 3 Mutations seen in PKD-1 and PKD2.

therapeutic targets for the disease [34]. Ultimately, cyst growth and expansion compress renal vessels and leads to intrarenal ischemia and activation of the renin-angiotensin-aldosterone system (RAAS), producing progressive cyst expansion, increased systemic vascular resistance, sodium retention, and renal fibrosis [35]. Vascular manifestations of ADPKD may result into abnormal functioning of polycystin-1 and polycystin-2, which additionally have been found to be expressed in vascular smooth muscle and endothelium [36,37]. Polycystin-1 and polycystin-2 form a 'receptor-ion channel complex' on the membrane of primary cilia of renal epithelial cells as well as endothelial cells. The luminal sheer stress is recognized by polycystin-1, resulting into the calcium-permeable channel to open. Polycystin-2 is involved in the calcium-dependent signaling cascades [38,39]. When this mechanosensory function is lost in ADPKD, calcium signaling is disrupted, contributing to cyst formation and numerous vascular alterations [40]. More recently, studies have suggested a role for polycystins in pressure-sensing within arterial myocytes, showing that the ratio of polycystin-1 to polycystin-2 regulates the opening of stretch-activated cation channels, modulating the arterial response to changes in intraluminal pressure in turn resulting into increased systolic blood pressures [41] (Figure 2).

The ADPKD mutation database revealed a high degree of allelic heterogeneity (Figure 3). This data base has reported 1266 pedigrees showing 932 PKD1 mutations and 167 PKD2 mutations in 322 pedigrees [42]. The commonest mutation described at PKD1 is C.5014_5015 del AG. It has been shown that more than 2% of PKD1 families show this mutation. A recent paper describes a novel mutation c.8791+1_8791+5delGTGCG which have been created due to splice site mutation resulting into frameshift mutation.There are approximately 500 mutations in PKD1 and > than 120 mutations in PKD2. Most of these mutations result into the protein truncation and unique to single family. Mutations result into the protein truncation that increases the severity. Mutations can be due to large genomic deletions these are rare and seen in only 4% of the patients. Recently a novel PKD1 variant (p.H1769Y) has been found which reveal the disease-modifying role, in Trans with the PKD1 truncating mutation, among ADPKD patients. This variant shows a well-conserved histidine, which is replaced by tyrosine, a non-conservative change, which suggests that this position is important for proper protein function. Another study with early onset presentation in siblings and a negative family history, this was diagnosed as ARPKD, a variant p.R3277C was found to be in trans with a second PKD1 variant, p.R2220W, in the severe disease condition [42].

A study on knockout mice model have shown that this variant act as a hypomorphic [14]. However, if it is combined with a null allele it leads to progressive kidney disease. There are other hypomorphic variants seen in both PKD1 and PKD2 loci which may occur in homozygous or compound heterozygous form. These variants may cause mild or early onset ADPKD.

Most of the truncated mutations are associated with the early onset (12 years) of ESRD as compared to non-truncated

Table 1: Characteristic features of two forms of polycystic kidney disease.

	Two forms of Polycystic disease	
	Dominant (ADPKD)	**Recessive (ARPKD)**
Incidence	1/500 to 1/1000	1/6000 to 1/40000
Gene (chromosome)	PKD1 (Chr 16); PKD2 (Chr 4)	PKHD1 (Chr 6)
Age of onset of ESRD	53 yr (PKD1); 69 yr (PKD2)	Infancy/childhood usually
Extra renal manifestations	Hepatic cysts/pancreatic cysts	Biliary dysgenesis
	Cerebral & aortic aneurysms	Hepatic fibrosis
	Cardiac valvular abnormalities	Portal hypertension
	Systemic hypertension	Systemic hypertension
Protein name	Polycystin-1; Polycystin-2	Fibrocystin/Polyductin
Protein size	Polycystin-1: 4302 amino acids	4074 amino acids and alternative shorter forms
	Polycystin-2: 968 amino acids	Polycystin-2: 968 amino acids
Protein structure	Polycystin-1: Integral membrane protein, multiple Ig-like domains, similar to egg jelly receptor	Transmembrane protein (and possible secreted forms), multiple TIG/IPT domains, as occur in hepatocyte growth factor receptor and plexins
	Polycystin-2: Integral membrane protein, similar to TRP channel	
Tissue distribution	Polycystin-1 and -2: Widespread	Kidney, pancreas, and liver
Sub-cellular localization	Polycystin-1: Plasma membrane, ciliab	Unknown
	Polycystin-2: Endoplasmic reticulum, cilia	
Function	Polycystin-1: ? Receptor, forms ion channel when co expressed with polycystin-2	? Receptor
	Polycystin-2: Calcium-activated cation channel	

mutations where the onset of ESRD is 55.6-67.9 years. This has indicated that there is lot of intra-individual variability assuming that there are many modifying factors other than genetic factors which may influence the polycystic kidney disease and its association with ESRD. Unfortunately, not much is known about the impact of allelic heterogeneity on the severity of ADPKD. In general, patients with mutation in the 5' region of PKD1 reach ESRD slightly earlier than patients with mutation in the 3' region (53 vs. 56 years). There is certain modifier genes associated with the ADPKD. The modifier genes are ACE, CFTR, and *ENOS* NO. *ENOS* NO inhibits platelet activation, regulates angiogenesis and controls microvascular permeability. The influence of *ENOS* on hypertension, coronary vasospasm, atherosclerosis and, most importantly, progression of diabetic nephropathy led to the hypothesis that it could be a modifier gene in ADPKD. There are reports showing the co-occurrence of autosomal dominant polycystic kidney disease (ADPKD) and connective tissue disorders. However, co-occurrence of osteogenesis imperfecta (OI) type I and ADPKD has not been observed so far. Hoefele etal., have shown that PKD1 and COL1A1 in the index patient. Mutational analysis of the parents indicated the mother as a carrier of the PKD1 mutation and the father as a carrier of the COL1A1 mutation. The simultaneous occurrence of both disorders has an estimated frequency of 3.5:100 000 000. These finding highlights the ADPKD can occur in combination with other rare disorders, e.g. connective tissue disorders etc [43].

Genetic counseling and Genetic analysis of ADPKD

Steps involved in the genetic counseling are an option for the individuals who have a family history of ADPKD. Genetic testing might be useful only in selected patients.Hence disease history in all the available family members should be taken this helps in identifying the disorder, risk of recurrence in future births.Counselor provides the alternatives and help to the family members to take the rational decisions. After genetic counseling genetic testing becomes important for the diagnosis of ADPKD that could influence family planning decisions, insurability and/or emotional stability. Individuals with a clinical presentation of ADPKD and no known family history of this disease, inconclusive ultrasonography, and renal ultrasound abnormalities are detectable from the 20th week of pregnancy or perhaps from the 13th week of pregnancy when there is an established diagnosis in an affected sibling. In this subgroup, the ultrasound reveals enlarged bilateral hyperechogenic kidneys, with or without cysts associated with oligohydramnios. However, this applies to 40% of the affected patients that have a more severe form of the disease [44]. Before focusing on this nephropathy, renal tract abnormalities (obstructive cystic dysplasia and multicystic dysplastic kidney) should be excluded. Other hereditary nephropathies (e.g., Bardet-Biedl syndrome) leading to this clinical picture are associated with other anomalies, which set them apart [45]. In some of the cases the diagnosis remains inconclusive. A pregnant woman with fetus affected by Adpkd usually has a normal amniotic fluid volume. Patients in this subgroup (40%) develop Potter sequence and die due to respiratory failure shortly after birth. When the result of the ultrasound is inconclusive, MRI, being more sensitive, can provide additional information. However, MRI also detects small simple cysts (without consequence), which have their number and size increase with age, and its role in this context has not been formally evaluated [46]. CT or MRI findings, or below 30 years of age with no detectable cysts (especially if they are being evaluated as potential kidney donors) could be potentially benefitedby genetic testing.

As seen above hereditary polycystic kidney disease is important because it is relatively common and show defined genetic mechanisms. Genetic counseling is useful in the proper management of autosomal dominant PKD cases. It is evident from the preceding paragraph that most of the PKD mutations are known. However, large amount of mutations still needs to be identified. Families where no mutation were identified and further investigated, and a gene encoding glucosidase II subunit alpha was identified that needs be screened [47].

Principles of genetic testing

Genetic testing provides information about diagnosis, treatment and the prevention, however, it has limitations. If an individual is tested for genetic testing and he turns out to be positive, but he does develop the disease he will suffer with unnecessary anxiety. On the contrary an individual with negative test may develop the disease both the situations are devastating. Positive results show that gene is disease causing. On the contrary negative results show no APKD is there. Whenever target gene is present, it means a true positive (TP) positive result every negative result indicate true negative (TN). Sometime interpretation is difficult. False results can cause serious consequences. A normal person will suffer from anxiety in case of false positive result hence undergo unnecessary treatments or depression. In practice if positive test is seen the person should be reassessed by scoring system as disused above and by repeating the test or finally confirming it by more advanced tests. A false negative result can have very grim consequences as some time the APKD may be missed. To overcome these adverse effects clinical tests should be adjusted to reduce the incidence of false negatives however this leads to the increase in the cost.

Various indices, like sensitivity and specificity, have been calculated to know the clinical relevance of diagnostic tests various parameters which should be used are shown below.

Parameters which should be calculated in a screening test are shown below.

	Fetus +ve for NTD	Fetus –ve for NTD
+ on test	A	C
-ve on test	B	D

Sensitivity = Proportion of affected picked up = a /a+ b

Specificity = True negative = d/c+d.

False positive = c/(a+b+c+d)

False negative = b/(a+b+c+d)

Positive predictive value = a/(a+c)

Odds ratio: It is defined as odds of being a case after a positive test (a:c) compared to odds of being case after a negative result. b:d) = (a/c) / (b/d) = ad/bc

Relative risk: This is the risk after a positive test is compared with the general population risk or risk after a negative test

= [(a/(a+c)] / [a+b+c+d]

Or = [a/(a+c)] / [b/(b+d)]

So far PKD data base has shown that at PKD1 locus there are 2323 known mutations, 868 revealed clear pathogenic significance. At PKD2 locus only 278 mutations have been identified and 168 show clear pathogenic significance. These results highlight the importance of genetic testing and counseling in these patients [48]. The ADPKD genetic mutations have been classified into 12 distinct forms (frameshift mutations, nonsense mutations, splice-site substitutions, IVS (intervening sequence) variations, silent changes, silent 3' untranslated changes, synonymous changes, and rearrangements (deletions and duplications) based upon their characteristic features both at DNA and protein level. (http://www.pkdb.mayo.edu/). Further these variations have been classified into six categories depending upon the clinical significance i.e. pathogenic, highly likely pathogenic, likely pathogenic, hypomorphic, indeterminate and likely neutral. There are 864 changes for PKD1. The percent distribution of various mutation in the PKD1 gene the PKD2 mutations are shown in Figure (3). These figures illustrate PKD1 with a higher percentage of definite pathogenic mutations. PKD1 is more polymorphic compared to PKD2: 424 polymorphisms of 864 total changes documented in PKD1 (49%), whereas only 24 polymorphisms of 139 changes (17%) were shown for PKD2. The high number of polymorphisms observed in PKD1. The reasons could be high GC content, presence of a long polypyrimidine tract in intron 21 due to multiple reiterations of the 5' region on chromosome 16, predisposing it to unequal recombination and gene conversion events

A more recent study has reported 188 pathogenic mutations in 186 of 220 (84.5%) families, including two families with bilineal ADPKD. *PKD1* PT mutations were the most common (72 of 188 or 38.3%) followed by *PKD2* mutations (57 of 188 or 30.3%) *PKD1* NT mutations (51 of 188 or 27.1%) and *PKD1* IF indels (8 of 188 or 4.3%) Among the identified mutations, 40% (75 of 188) were novel, and 19 mutations were detected in two or more families which were not related. and III:5, whereas p.Ser4054Phe originated from II:4 and segregated with the disease in III:3, III:4, and III:5. One affected member (III:5) who was transheterozygous for both mutations developed ESRD at age 24 years old. By contrast, all other affected subjects carrying only one hypomorphic *PKD1* mutation were mildly affected.

There are various genetic testing methods like DNA linkage analysis which should be used with caution keeping in mind the *de novo* mutations, mosaicisms, and hypomorphic alleles present in APKD. Gene based mutation screening can be done however, it is difficult to differentiate mis sense mutations from benign variants; mutations detected in approximately 65%-75% of subjects; approximately 8% of patients have no confirmed pathogenic mutation. Recent availability of protocols for long-range (LR-PCRLR-PCR) and locus specific amplification of PKD1 has enabled complete mutation screening of this complex gene [49]. NGS offers sensitivity of 99.2% and specificity of 99.9% in identifying mutations in PKD1 and PKD2 [50] therefore, the recommended mode of genetic testing should be NGS however, cost is the limiting factor.

Risks of genetic testing

During genetic counseling both ethical and emotional considerations should be addressed there are many other risks to the patient and their family who are considering genetic testing or receiving a genetic diagnosis. In the developed nations genetic testing is usually paid for by the National Health Service, there are health insurance policies which often cover the cost of genetic testing performed at the request of a doctor. However, adverse genetic diagnosis results into the fear of discrimination and the associated cost of enhanced insurance premiums represent a significant emotional and financial burden on the families. This situation is further worsened in developing countries. As part of the genetic counselling process, these issues should be discussed with affected families and informed consent should be obtained prior to genetic testing. All centers involved in the genetic counseling must keep in mind that a negative result does not exclude genetic disease as mutations may be missed, with sensitivity for genes covered by the test depending on methodology and analysis used. Alternatively, a mutation may be present in a gene not covered by the chosen test, for example a novel genetic association. This combined with the profound clinical and pathological heterogeneity of genetic and idiopathic ADPKD highlights that universal genetic testing in ADPKD is inappropriate and unlikely to be cost-effective. Rather, mutational screening should be directed towards those in whom a genetic etiology is likely and should therefore be reserved for patients presenting with primary ADPKD. It is important to create nationwide data basis so that proper diagnostic guidelines are devised and practiced. Genetic testing should be performed in a timely manner, early confirmation of a genetic diagnosis in childhood-onset ADPKD would minimize the adverse effects of current therapies on the growing child. It is very crucial to know the mutational status among live related renal transplants in countries like India.

To summarize, it may be suggested that genetic testing should be considered when important clinical decisions need to be made regarding the need for renal biopsy, the intensity and duration of immunosuppression and pre-transplantation therapy

Genetic Testing

Traditionally, genetic testing in diagnostic laboratories has used Sanger sequencing, frequently in association with exon copy number analysis, to assess specific disease-related genes individually. In genetically heterogeneous disorders, with multiple causal genes, such as PKD, this method can be expensive and time-consuming owing to the cost of screening multiple individual genes. The advent of high-throughput massively parallel sequencing (NGS methods) allows for a higher diagnostic

yield, time savings and a reduction in cost. Typically, diagnostic laboratories utilize a targeted capture of a 'panel' of genes of interest followed by sequencing on an NGS platform. Sanger sequencing still plays an important role for the confirmation of genetic variants identified via NGS. The limitations of Sanger sequencing include the need to ensure both adequate coverage of regions of interest and adequate analysis to detect copy number variants such as exonic deletions. As with most of the Sanger sequencing approaches, this method will miss deep intronic or regulatory region variants unless specifically targeted.

Whole-exome sequencing (WES) or whole-genome sequencing (WGS) employ NGS methods to attempt to sequence the coding portion of the genome (the exome) or the entire genome, respectively. This approach is not limited to known candidate genes and therefore could identify mutations in novel genes, thereby expanding the heterogeneity of ADPKD and enhancing our understanding of the pathogenesis and molecular mechanisms. WES is increasingly being implemented in the clinical setting, but its widespread application is limited by the amounts of data generated and the requirements for robust bioinformatics support and assessments of the pathogenicity of larger numbers of variants.

WES and WGS are hampered by the fact that large numbers of genetic variants are identified; including variants of unknown significance and incidental or secondary findings may raise several ethical and practical issues relating to consent, data storage and analysis.

Predicting deleterious missense mutations

Presently large numbers of bioinformatics tools are being used to evaluate the functional impact of several unclassified missense variants that alter a single aminoacid residue. PolyPhen is used for this purpose we need prior knowledge of protein id, wild type and variant amino acid. Alignment of these homologous protein sequences, PolyPhen computes profile scores for both the allelic variants. Profile scores are logarithmic ratios of the likelihood of a given amino acidoccurring at a site to the likelihood of this amino acid at any site (background frequency). A variant is predicted to be damaging if the absolute difference between the profile scores of two amino-acid variants is >1.7. After the prediction the functional assays are carried out to know the exact role of mutation that may be further helpful in genetic testing and counseling.

Future Directions

Most of the routine diagnostics and prognostics in ADPKD are based upon imaging which is more frequently used. Identification of more sophisticated molecular techniques has revolutionized the molecular diagnostics, by reducing the costs. The genetic and clinical information can play a central role in the management of patients with ADPKD [51]. It is important to identify the ADPKD patientsto provide more personalized patient care. Full genetic landscaping of ADPKD will help in the genetic counseling and the specific targeted therapies.

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