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Screening of Three Exons of PKD1 gene In Five Patients with Autosomal Polycystic Kidney Disease

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common genetic kidney disorders with the incidence of 1 in 1,000 births. ADPKD is genetically heterogeneous with two genes identified: PKD1 (16p13.3, 46 exons) and PKD2 (4q21, 15 exons). Eighty five percent of the patients with ADPKD have at least one mutation in the PKD1 gene and fifteen percent of the patients have one mutation in PKD2 gene. Direct sequencing of one patient and his sequence of PKD1 gene demonstrated a missense mutation GCC----CCC substitution in exon 13 with cause change amino acid of Alanine to Proline at codon 1029. Three brothers have deletion mutation in exon 15, one patient missense mutation GGC---GCC in exon 19 which cause change amino acid of Glycine to Alanine at codon 2530. Molecular diagnostics of ADPKD relies on mutation screening of PKD1 and PKD2, which is complicated by extensive allelic heterogeneity and the presence of six highly homologous sequences of PKD1. PCR strategy was used to screen sequence variants with heteroduplex analysis and several affected individuals were discovered to have clusters of base pair substitutions in exons 13 and 19 with del 20 pb (3601-3620) in exon15.

Keywords: ADPKD; PKD1; PKD2; Libyan patients; Kidney failure.

المستخلص

يعتبر مرض الكلى المتعدد الكيسات الوراثي السائد (ADPKD) **واحدًا من أكثر وأهم اضطرابات الكلى الوراثية شيوعًا مع حدوث حالة واحدة من** 1000 **ولادة. ويعتبر** ADPKD **من الامراض غير المتجانسة وراثيا . وتم تحديد اثنين من الجينات المرضية وهى** PKD1 (16p13.3 46 exons), PKD2 (4q21 ,15, 46exon) **لدى** 85٪ **من المرضى الذين يعانون من مرض الكلى المتعدد الاكياس الوراثى** ADPKD **طفرة واحدة على الأقل في جين** PKD1 **و** 15٪ **الاخرين من المرضى لديهم طفرة في جين** PKD2. **و يعتمد التشخيص الجزيئي لمرض الكلى المتعدد الكيسات الوراثي السائد** (ADPKD) **على تحري طفرات** PKD1 **و** PKD2 **، وهو أمر معقد بسبب عدم التجانس الأليلي الشامل ووجود ست متواليات متجانسة للغاية من** PKD1.

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لقد استخدمنا فى هذا البحث استراتيجية طريقة عمل البلمرة التسلسلية PCR **للبدء في فحص المتغيرات المتسلسلة مع تحليل الهترودوبلكس** hetroduplex **وتم اكتشاف عدد خمسة من الطفرات منهم طفرة حدف فى 20 قاعدة في أكسون** exon 15 **وطفرة استبدال قاعدة بقاعدة فى أكسون** 13 **و** 19. **وكانت نتيجة البحث للتسلسل المباشر لمريض واحد وتسلسله من الجين** PKD1 **أظهر طفرة استبدال** CCC **في** exon 13 **نتج عنه تغيير فى حمض أميني من الألنين** Alanine **إلى البرولين عند الكودون** 1029. **كما وجدنا من ضمن البحث لدى ثلاثة أخوة طفرة الحذف في 20 قاعدة في** exon 15 **أما الحالة الاخيرة فوجدنا فيها طفرة واحدة أخرى لوحظت عند تغيير** GGC --- GCC **في** exon 19 **التي تسبب فى تغيير الحمض الأمينى من الجلايسين إلى ألانين في كودون** 2530. **يعتمد التشخيص الجزيئي لمرض الكلى المتعدد الكيسات الوراثي السائد** (ADPKD) **على تحري طفرات** PKD1 **و** PKD2 **، وهو أمر معقد بسبب عدم التجانس الأليلي الشامل ووجود ست متواليات متجانسة للغاية من** PKD1.

Introduction

Polycystic kidney disease (PKD) is a common genetic disorder. PKD can cause renal failure in adults and children. PKD is characterized by the accumulation of fluid-filled cysts, ovarian cysts, prostatic cysts and cardiac valve disease (Harris and Toores, 2009).

The renal cysts originate from the epithelia of the nephrons and renal collecting system which are lined by a single layer of cells that have higher rates of cellular cells (Nadasdy et al,1995).

PKD can be inherited as an autosomal dominant polycystic kidney disease (ADPKD) which is a common disease that occurs in both children and adults. Autosomal recessive polycystic kidney disease (ARPKD), however, is uncommon and occurs primarily in neonates and children (Kim and Park, 2016). The ADPKD is one of the most common genetic diseases in humans affecting all ethnic groups worldwide with an incidence of 1 in 500 to one in 1000 (Gabow and Grantham, 1997).

The ADPKD accounts for more than 10% of all cases of end-stage renal disease (ESRD) (Willson, 2004). Two causal genes (PKD1 and PKD2) have been identified for ADPKD. PKD1 is located on chromosome 16 (16p13.3) gene and PKD2 is located on chromosome 4(4q21) (Mochizuki et al., 1996; Torres and Harris, 2007). PKD1 gene has 46 exons, 45 introns, encodes transcript with 14.2kb in length. It extends to 50kb of genomic DNA (Harris and Toores, 2009) and does code polycystin 1 protein (4302 AA) (Takiar and Caplan, 2011). On the other hand, PKD2 encodes 3-kb, has 15 exons, 14 introns, extends 70 kb in genomic DNA and produces polycystin-2 protein (968AA) (Kim and Park, 2016). The PKD1 gene is mutated in about 15% of ADPKD cases (Harris and Torres, 2009).

The exact function of polycystin, however, remains unknown. The mechanism of pathogenesis of ADPKD is also still unclear (Ma et al., 2009). Polycystin 1 is a receptor protein for cell–cell matrix interactions and play roles in the regulation of cell proliferation and apoptosis (Goilan, 2011).

About 1920 mutations have been identified in the PKD1 gene, most of which are point mutations (missense/nonsense) or deletion/insertion mutation that lead to frame shifts and stop codon, leading to premature termination or, most likely, to loss of polycystin 1 function completely (Jamshidi et al.,2016).

Materials and Methods

Blood Samples Collection

Blood samples were collected from five-patients with polycystic kidney disease (PKD) who are attending the Kidney Transplant Center, Central Hospital, Tripoli, Libya. Five milliliter of blood was drawn from each patient on October 22nd, 2015. Blood were collected in tubes containing anticoagulant (EDTA) and kept in a freezer at -20°C until use.

Genomic DNA Extraction

DNA was extracted from blood samples by QIA amp DNA blood mini Kit from Kiagen.

Agarose Gel Electrophoresis

Agarose Gel Electrophoresis was done by QIAquick.

Polymerase Chain Reaction (PCR)

Oligonucleotide primers were used to amplify exons (13,15 and 19) of PKD1 gene (Table 1). Amplification of PDK1 gene was carried out by thermocycler apparatus. The PCR was prepared in a final volume of 50**µl** containing 100 ng of genomic DNA, 125 **µ**M each of dATP, dCTP, dGTP and dTTPs 200 PM of forward and reverse primers for each of exons 13.15 and 19, 2% DMSO ,1X TAE buffer and 1 **µl** of DNA polymerase.

Steps of PCR were as follows: 94°C for 5 min for one cycle and denaturation at 94°C for 30 sec, annealing at 63°C for one minute and extention at 72°C at one minute for 30 cycles. The final step cycle were carried out at 72°C for 10 minutes. Two percent agarose gel was used to visualize the DNA by UV light (Saiki et al., 1988).

Oligonucleotide primers were used to amplify exons (13,15 and 19) of PKD1 gene. Amplification of PDK1 gene was done by thermocycler apparatus.

Table 1. The Oligonucleotide primers used to amplify exons (13,15 and 19) of PKD1 gene. Amplification of PDK1 gene was done by thermocycler apparatus.

Oligo name		**Sequence**
Pkd1._13_F		**ACC-CTC-CCC-TCC-TCA-CAG**
Pkd1._13_R		**GGG-AAC-GGA-GAA-GAG-GAA-CT**
Pkd1._15_F		**CTG-ACC-TTC-CAG-AAC-GTG-GT**
Pkd1._15_R		**AAG-CAG-AGC-AGA-AGG-CAG-AG**
Pkd1._19_F		**GAT-CCC-CCG-ACT-CTG-TGA-C**
Pkd1._19_R		**GAG-GCT-GCC-CTT-GTA-GAC-AC**

DNA Sequencing Method

PCR products were sequenced by a technique derived from the Sanger method and analyzed by electrophoresis on an ABIprim 373 sequencer and a 313XL plate sequencer (Applia`s 16 capillaray sequencer).(Tan et al.,2009).

Results

Genomics DNA was extracted from five patients by a method of QiagenKit and the quality of extracted DNA was analyzed by agarose gel electrophoresis (Fig. 1).

PKD1 gene specific PCR primers were synthesized for exons 13, 15 and 19 and PCR was conducted and products were checked by 2.0% agarose gel electrohpresis (Fig. 2).

Direct sequencing of one patient and his sequence of PKD1 gene demonstrated a missense mutation GCC----CCC substitution in exon 13 which caused amino acid of alanine to Proline at codon 1029. Moreover, three brothers frame shift in CD3601-3620 del20bp in exon 15, one patient exhibit missense mutation GGC -----GCC in exon 19 which caused change of amino acid of Glycine to Alanine at codon 2530 (Table 2).

The results of direct sequencing of PKD1 exons 13, 15 and 19 in 5 patients demonstrated GCC-----CCC in one person, CD 3601-3620 del 20bp in three persons and GGC-----GCC in one person.

The results of direct show sequencing of PKD1 exon 15 del 20 pb (GCG GCC CAG GCG GAT GTG CG).

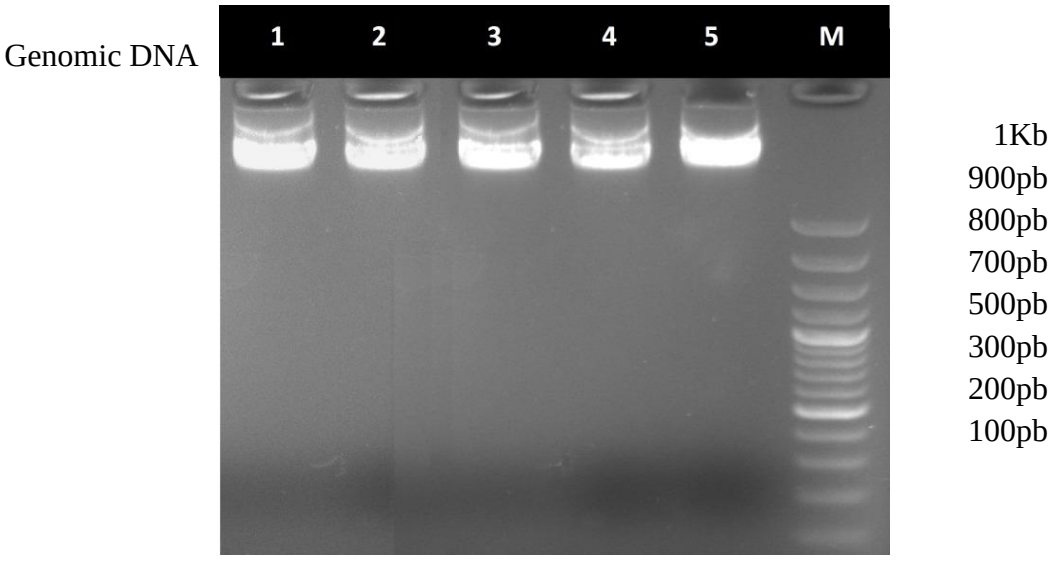

Fig. 1. M (1kb marker) and Genomic DNA, patients (1-5), were visualized by 2% agarose gel electrophoresis.

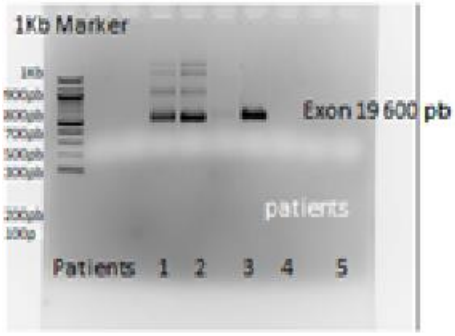

Fig. 2. Gel electrophoresis of the amplicons of exon 19 For PKD1 gene for five patients (1,2,3,4,5) size of PCR Products 600 bp.

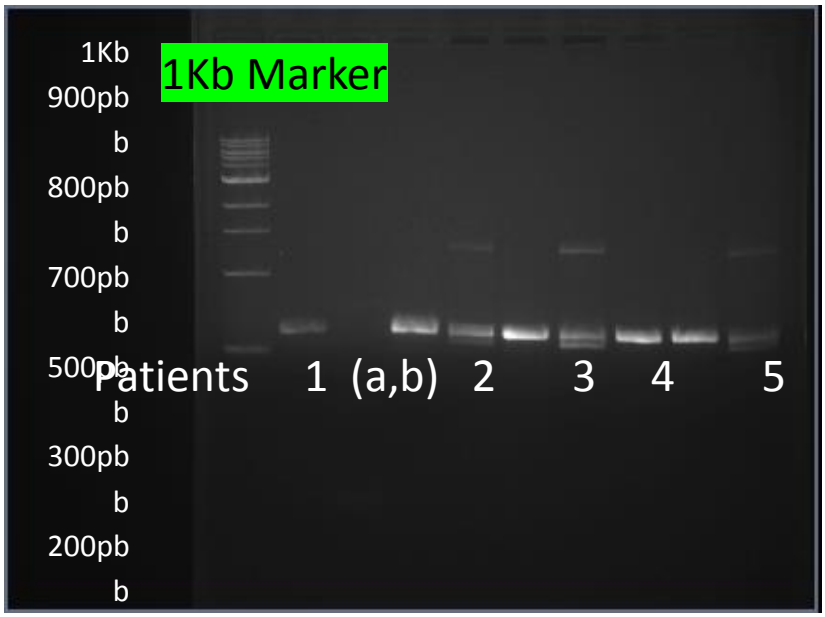

Fig. 3. Gel electrophoresis of the amplicons of exon 15 for PKD1 gene for five patients (1 a-b,2,3,4,5). Size of PCR Products 138 bp.

The results of direct sequencing of PKD1 exons 13, 15 and 19 in 5 patients demonstrated GCC-----CCC in one person, CD 3601-3620 del 20bp in three persons and GGC-----GCC in one person.

The results of direct sequencing of PKD1 exon 15 show del 20 pb (GCG GCC CAG GCG GAT GTG CG).

Discussion

APDK is the most common inherited kidney disease in humans affecting all ethnic group worldwide with an incidence of 1 in 500 to 1 in 1000 by an important allelic variety with many described variants (Gabow and Granthan, 1997). A number of methods have been used to screen for mutation in ADPKD for clinical and research aims but the sequencing method is still the best method of choice to screen for ADPKD mutations (Garcia-Conzales et al, 2007 and Hoefele et al, 2011).

Table 2. Pathogenic sequence changes of the PKD1 gene identified by direct sequencing in a set of patients from Central Hospital, Tripoli, Libya.

Patients	Age / years	Exon	Gender	Nucleotide change	Amino Acid change	Mutation
1	44	15	Male	3601-3620 del 20bp	----------	deletion
2	45	15	Male	3601-3620 del 20bp	----------	deletion
3	50	15	Male	3601-3620 del 20bp	----------	deletion
4	39	13	Male	GCC-------CCC	Ala1029Pro	Missense
5	47	19	Female	GGC-------GCC	Gly253oAla	Missense

The most frequently common types of mutations with pathogenic significance are missense, insertions, deletions and nonsense mutations (Seck et al, 2013). PKD1 gene mutations are considered to be an important cause of ADPKD. About 85% of patients have mutations which occur in PKD1 gene and about 15% of cases have PKD2 mutations (Torres et al, 2007).

Genetic testing is not always required for all ADPKD patients but preliminary diagnosis is often made by CTS scan, Ultrasound and MRI. Young people with positive family history are recommended to take genetic testing (Torres et al, 2007).

Our results identified a genetic variant of PKD1 in ADPKD that affected three brothers and two persons. It was (3601-3620) del20 bpb in Exon 15 of the PKD1 gene. In addition we found two other missense mutations, one of them was GCC---CCC, Ala 1029 Pro located in exon 13 of PKD1 gene and the other missense mutation GCC---CCC Gly 2530 Ala located in exon 19 of PKD1 gene.

Since all the genetic mutation in ADPKD are from patients with end stage renal diseases on hemodialysis treatment, the mutations are considered as pathogenic and affected the cystic formation in their kidney seriously resulting in kidney failure. Our results support findings from similar studies with the same missense mutation that has been found in Iranian, Chinese and Taiwanese patients (Chang et al, 2013; Lia et al, 2014).

We also found that three brothers had 20bp deletion in exon 15, but in Thai families there was deletion of 20 bp observed in intron 43 (Rungroj et al, 2001). In another study there was 74bp deletion in exon 14 (Thongnoppakhun et al, 2000).

We conclude that there are two missense mutations in two patients and 20 bp deletion mutations in other three patients. This result leads us to further persue future studies for better understanding of genetic variants in Libyan ADPKD patients.

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