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Original Article

Mutational analysis of *PKD1* gene in a Chinese family with autosomal dominant polycystic kidney disease

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Abstract: Autosomal dominant polycystic kidney disease (ADPKD) is a hereditary disease and common renal disease. Mutations of PKD genes are responsible for this disease. We analyzed a large Chinese family with ADPKD using Sanger sequencing to identify the mutation responsible for this disease. The family comprised 27 individuals including 10 ADPKD patients. These ADPKD patients had severe renal disease and most of them died very young. We analyzed 6 survival patients gene and found they all had C10529T mutation in exon 35 of *PKD1* gene. We did not found gene mutation in any unaffected relatives or 300 unrelated controls. These findings suggested that the C10529T mutation in PKD1 gene might be the pathogenic mutation responsible for the disease in this family.

Keywords: Autosomal dominant polycystic kidney disease, PKD1, mutational analysis, Chinese family

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is a hereditary renal disorder, characterized by gradually formation and enlargement of fluid-filled epithelial cysts in bilateral kidneys and accounts for up to 10% of end-stage renal disease [1]. *PKD1* and *PKD2* are two mapped and proven disease-causing genes. The *PKD1* gene is located in 16p13.3 [2]. The *PKD2* gene is located at 4q13-23 [3, 4]. *PKD1* encodes polycystin-1. The polycystin-1 is a cell-cell/matrix interactions receptor protein. It could regulate cell proliferation and apoptosis. PKD2 encodes polycystin-2, a transient receptor potential (TRP) ion channel. It could regulate the intracellular Ca^{2+} concentration. More than 80 mutations were identified in the PKD1 gene. Mutations of the PKD1 gene account for approximately 85% of all ADPKD cases and are responsible for more serious form of the disease [5]. These mutations cause various amino acids alterations such as substitution, deletion, or insertion of nucleotides. PKD1_/_ may be caused defective migration of endothelial cells to form the mature glomerulus [6]. Mutations of PKD1 and PKD2 are highly diversified. The method to analysis the PKD gene mutation was RFLP, gene linkage analysis, SSCP, HPLC *et al.*

Materials and methods

Subjects and ethics statement

We recruited a large Chinese family from Linyi people's hospital (**Figure 1**). The study was conducted in accordance with the principles of the declaration of Helsinki, and informed consent was obtained from all the PKD families and 300 control individuals prior to their participation in the study. 10 members were diagnosed as patients in the pedigree. They were diagnosed by ultrasound examination according to Ravine's criteria [7] or by the descriptions from the proband and other family living members.

Mutational analysis of PKD genes

The mutational analysis of PKD genes was performed in the family and normal controls. Total genomic DNA of all available family members and 300 unrelated healthy controls was extracted from peripheral blood leukocytes using a standard phenol-chloroform procedure. All exons with intronic flanking sequences of the PKD1 and PKD2 genes in the probands were amplified by PCR and subsequently detected directly by Sanger sequencing.

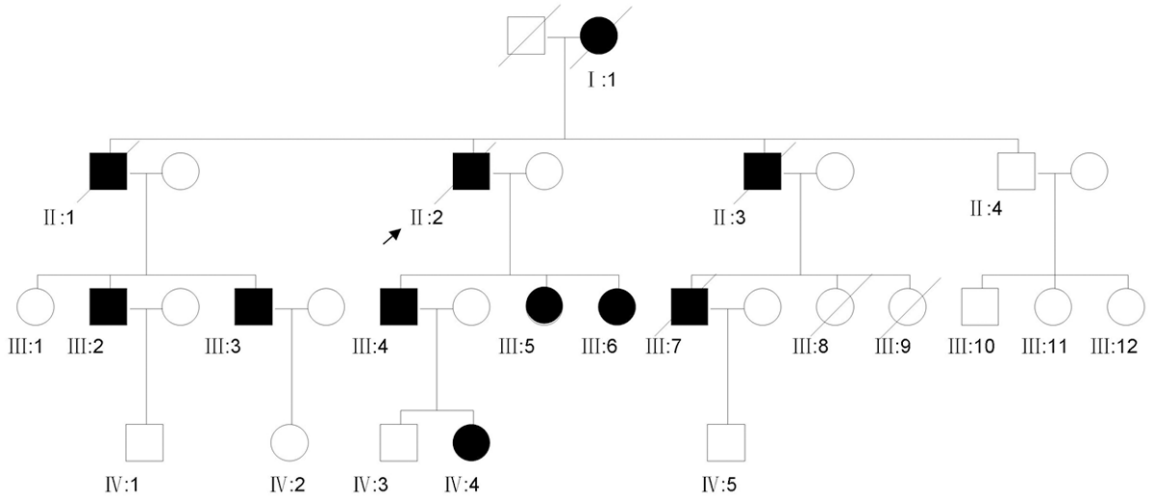

Figure 1. Pedigree of the Chinese autosomal dominant polycystic kidney disease family. The proband II:2 is shown by the arrow.

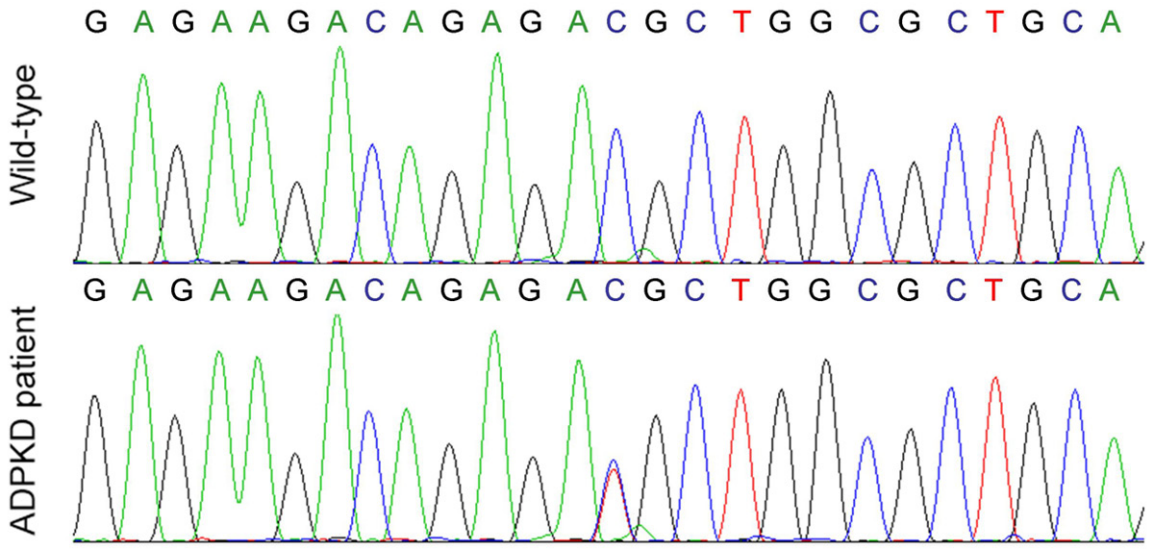

Figure 2. Identification of the c.10529C>T (p.Thr3510Met) mutation in *PKD1* gene.

Results

Clinical manifestations of ADPKD patients in a Chinese family

The PKD families' disease onset early and progressed rapidly, many of them died from ESRD (end stage renal disease). The proband II 2, who was severely affected by the disease, onset hypertension and renal disease at 38 years old, he died from ESRD at the age of 43. The I 1 woman died at the age of 53. The II 3 diagnosed ADPKD because of hypertension and ESRD at 63, he died from cerebral hemorrhage at the age of 64. The III 4 was diagnosed ADPKD because of mild hypertension since the age of 30. His blood pressure was well compensated and had no symptoms for 15 years. The most severely affected by the disease was III 7, he was diagnosed ADPKD because of cerebral hemorrhage and severe hypertension at the age of 33. He combined pancreatitis and with ESRD reached at the age of 36. He died at the 40 years. III 2 diagnosed ADPKD at 43 years old and he now is a ESRD patient need dialysis treatment. The III 5, III 6 and III 3 were diag-

nosed ADPKD by renal ultrasound, but they all had no renal disease symptoms.

All affected members in the family had cysts in bilateral kidneys and hypertension. Many of the patients combined with cerebral hemorrhage. The patients with ESRD died young, the average age of die was 50 years old. The male patients in the pedigree seemingly had more severe clinical symptoms and had ADPKD onset earlier than those female patients.

Identification of PKD1 mutation

All exons with intronic flanking sequences of the PKD1 genes in the patients were amplified and subsequently sequenced. We found a mutation in PKD1 Exon35 c.10529C>T (p. Thr3510Met) (**Figure 2**). We revealed an ACG → ATG substitution converting Thr 3510 to Met at exon 35 in all the patients. We did not find any seeming mutational hot spots in PKD1 and PKD2. The mutation of PKD1 had been reported in these documents [8-13] (http://pkdb.mayo.edu/cgi-bin/reference.cgi?germ=germline&gene=PKD1&designation=T3510M&clinical=Likely%20Neutral&score=0). Because the mutation only found in discrete people, so PKD Foundation (http://www.pkdcure.org/) sort the mutation's clinical significance is likely neutral. We found the mutation only detected in patients, it was not detected in their unaffected relatives and 300 unrelated normal controls. So we considered the PKD1 Exon35 c.10529C>T (P.Thr3510Met) gene mutation was the pathogenic factor in this ADPKD pedigree.

Discussion

The PKD1 gene is divided into 46 exons and it had about 52 kb of genomic DNA. The mutations in PKD1 Exon 35 c.147413>T had been reported in Japanese [12, 14], Chinese [15]. Mutation in PKD1 Exon 35 c.10529C>T had been found in Finnish [10], Japanese [11, 12], Han Chinese [16]. The mutation only been detected in single patient but not in pedigree. This alteration caused a missense mutation from threonine to methionine at codon 3510. In this mutation, threonine has a polar OH group but methionine does not. Therefore it is possible that this alteration causes the gene to produce a different structured protein. In this study we found a large ADPKD pedigree in east China. In our study, we found the pedigree had PKD1 mutation. All the patients in the pedigree carried PKD1 Exon35 c.10529C>T gene mutation. We didn't found the gene mutation in the healthy relatives and healthy controls. So we consider the PKD1 Exon35 c.10529C>T gene mutation was the virulence gene of the pedigree. Its function need further study.

ADPKD is the most common genetic cause of renal failure, and it also combined with heart and cerebral vessels disease. The ADPKD poor prognostic factors include PKD1 mutation (particularly truncating mutation), men, and early onset of hypertension [17]. PKD1 mutation carriers had poorer renal prognosis than those carried PKD2 patients. PKD1 mutation carriers were diagnosed of hypertension 10 years earlier than PKD2 mutation carriers. Truncating PKD1 mutation carrier was 2.74 times more likely to develop ESRD than those carrying a no truncating PKD1 mutation [18]. Other study also found that mutations of the PKD1 gene are responsible for approximately 85% of all ADPKD cases and account for more serious form of the disease [19]. In this pedigree, the patients had PKD1 gene mutation and the disease onset early and severely. The patients' chiefly symptoms were hypertension and cerebrovascular disease. The departed saints were died from ESRD.

The symptoms of male patients in the pedigree were severer than that of female patients, and they died from ERSD younger. We consider the PKD1 gene mutation was related to the disease severity in the pedigree.

ADPKD is an autosomal dominant inherent disease, the morbidity of offspring is 50%. The incidence of ADPKD in live births is 1:400 to 1:1000. Individuals with PKD gene mutation may develop ADPKD in the mid-life. Detect the fetal PKD hereditary gene may guide eugenics. In this family, discovery the fetal PKD1 gene mutation will directly be applied for prenatal diagnosis of ADPKD gene carry.

In conclusion, in this study, we reported a *PKD1* c.10529C>T mutation in a large Chinese family with ADPKD. It is the first observation of this mutation in a large family. Direct mutation diagnosis and prenatal diagnosis in clinical practice would be helpful for this family.

Disclosure of conflict of interest

None.

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