Identification of Novel Nonsense Mutations in Iranian patients suffering from autosomal dominant polycystic kidney disease

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BMC Nephrology  ▶ BMC Series

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DOI: 10.21203/rs.2.18544/v1

SUBJECT AREAS   Urology & Nephrology

KEYWORDS
Autosomal dominant polycystic kidney disease, PKD1, mutational analysis, Iranian
Abstract

Background: Autosomal dominant polycystic kidney disease (ADPKD) is the predominant type of inherited kidney disorder, which occurs due to PKD1 and PKD2 gene mutations. ADPKD diagnosis is made primarily by kidney imaging; however, molecular genetic analysis needs to be implicated to confirm the diagnosis. It is critical to perform a molecular genetic analysis when the diagnosis is uncertain, particularly in simplex cases (i.e., a single occurrence in a family), in people with remarkably mild symptoms, or in individuals with atypical presentations. The main aim of this study is to determine the likelihood of PKD1 gene mutations in Iranian patients with ADPKD diagnosis.

Methods: Genomic DNA was isolated from blood samples from 26 ADPKD patients, who were referred to the Qaem Hospital in Mashhad, Iran. By using suitable primers, 16 end exons of PKD1 gene that are regional hotspots, were replicated with PCR. Then, PCR products were subjected to DNA directional Sanger sequencing.

Results: The results of DNA sequencing in the patients showed that exons 35, 36 and 37 were non-polymorphic, while most mutations had occurred in exons 44 and 45. Only in two patients, exon-intron boundary mutation had occurred in intron 44. Most of the variants were missense and non-synonymous types.

Conclusion: In this study, we present nine novel mutations/polymorphisms in PKD1. These data will contribute to an improved diagnostic and genetic counseling in clinical settings. Keywords: Autosomal dominant polycystic kidney disease; PKD1; mutational analysis; Iranian
One of the most prevalent inherited kidney disorders that involves both kidneys is Autosomal dominant polycystic kidney disease (ADPKD). It leads to a progressive loss of kidney function and possible kidney failure (1). About one to two infants in 1000 live at birth are affected by this disorder and approximately 10% of people who undergo dialysis; suffer from this disease (2, 3). ADPKD occurs in two types: type I and type II caused by PKD1 and PKD2 mutations, respectively (4, 5).

PKD2 mutations lead to end-stage kidney or renal disease at age 74 and occur in 10-15% of cases; on the other hand, PKD1 mutations lead in the average to end-stage kidney or renal disease at age 54.3 and occur in 80-90 % of total cases. The latter is the more severe form of the disease (1, 3, 5). Patients having end-stage kidney or renal disease should receive renal replacement therapy (RRT) support including dialysis and renal transplantation. Dialysis may be the only available modality in these patients. After all, dialysis has its limitations, including sometimes lack of vascular access, risks of vascular thrombosis and infections, diminished quality of life, and loss of biosynthetic functions of the kidney (6). ADPKD patients who have diagnosed before age 30 and hypertension and hematuria before age 35 have a worse renal outcome than those who do not have (7). ADPKD diagnosis is done typically by kidney ultrasound imaging, computed tomography scan or magnetic resonance imaging; however, considering the ADPKD similarity to other cystic kidney disorders, conventional imaging methods do not often lead to a definite and accurate diagnosis (1, 2). In this condition, genetic methods can be effective in an accurate and careful diagnosis. Besides, molecular diagnosis methods have an important role in the confirmation of definite diagnosis, especially in young kidney donors, patients with negative family history, people who presented ADPKD with unusual symptoms in childhood and patients who have relatives
suffering from this disorder (8, 9).

ADPKD is the most frequent genetic kidney pathology (frequency of about 0.1%), which results in 5%-8% of end-stage renal diseases (ESRDs). The ailment is progressive, ending in polycystic enlarged kidneys. This typically results in ESRD in late middle age (5). Polycystin-1, large multidomain protein, is the protein encoded by \textit{PKD1}. It has domains and regions that are homologous with a number of different proteins (10). International Polycystic Kidney Disease Consortium 1995 (11, 12). Polycystin-1 may have a function in cell-cell/matrix interactions (13) instead, polycystin-2 (the PKD2 protein) is homologous to an ion-channel subunit (14, 15).

Most cases of ADPKD leading to ESRD, are caused by \textit{PKD1} mutations (16).

Nevertheless, the genetic determination of the locus mutation has advanced slowly, due to the fact that (1) \textit{PKD1} contains a 12,906-bp coding sequence divided into 46 exons (2) and, the 5′ region of the gene, from upstream of exon 1 to exon 33, is inserted in a complex genomic area and repeated more than 4 times, on the same chromosome (European Polycystic Kidney Disease Consortium 1994). These \textit{PKD1}-like homologous genes (HG) have revealed a number of specific deletions and a low level of substitutions (about 2%), in comparison with \textit{PKD1} (17). HG loci have made the analysis of \textit{PKD1} highly difficult. Thus, the quantity of identified \textit{PKD1} mutations is still incomplete, with 82 modifications described in the Online Human Gene Mutation Database (HGMD) (18). A number of methods have been used to screen the repeated region (19-23), however, the 3′ area has received insufficient attention, with 57.3% of all mutations found in the single-copy area covering 20% of the coding region. \textit{PKD2} (a less-complex gene) has revealed 41 mutations with potential effects of truncating and possibly inactivating the translated protein (24). A discrete number of missense changes have also been described (19, 23-26).
Since numerous somatic mutations are needed to explain the formation of multiple cysts and the notion of a significant rate of formation of novel germline mutations (19), it has been proposed that infrequent mechanisms promote a high rate of \textit{PKD1} mutations. First, a long polypyrnidine region in IVS21, which could theoretically form triplex DNA structures (27, 28), has been considered as a possible cause for creating mutations in downstream exons (22). Later on, these multiple substitutions and other modifications were described to match HG sequences, possibly indicating a gene conversion with the remotely located HG loci (21, 29).

\textit{PKD1} gene (OMIM 601313) is located in the 16p13.3 chromosome region and consists of 46 exons. Exons 1-33 of \textit{PKD1} replicate around 6 times in homologous genes, HG which has defied \textit{PKD1} genetic analysis. Until January 2015, approximately 2322 \textit{PKD1} sequence variants and 278 \textit{PKD2} sequence variants were reported in ADPKD mutation databases, 1177 \textit{PKD1} sequence, and 211 \textit{PKD2} sequences in human gene mutation (16, 17). Although mutation data for \textit{PKD} genes of different populations are available, there are few reports for \textit{PKD} mutations in Iranian population.

The main goal of this study was to establish the probability of mutations in \textit{PKD1} gene in Iranian patients with ADPKD diagnosis obtained by PCR (Polymerase Chain Reaction) and DNA Sanger sequencing (30).

\textbf{Methods}

\textit{Patient selection}

Twenty-two ADPKD patients were collected from regional city hospital; Ghaem hospital (Mashhad, Iran) between April 2012 to March 2013; they were included at diagnosis and disease characteristics of the ADPKD. The present study was
approved by Mashhad University of Medical Sciences ethics committee, and before the blood sample was collected, all patients provided their informed consent.

We excluded clinically patients suffering from Von Hippel-Lindau disease and Tuberous Sclerosis. In addition, patients without symptoms of polycystic kidney disease or those who had other syndromes were also excluded in this study.

Amplification assay

Genomic DNA was isolated from 22 whole-blood samples using the standard salting-out method according to the manufacturer’s instructions and it was quantified by NanoDrop 1000 (Thermo Fisher Scientific, Waltham, MA, USA). Eight-specific primers within the area of the exon 31-46 were designed (Table1). using the Primer 3 software. and all sequences were checked for self- or inter-molecular annealing with nucleic-acid-folding software (OligoAnalyzer 3.1). We performed local-alignment analyses with the BLAST program to confirm the specificity of the designed primers (http://www-ncbi-nlm-nih-gov.acces.bibl.ulaval.ca/tools/primer-blast). Bidirectional sequence analysis was conducted for all PCR amplicons.

Isolated DNA was stored at -20 °C. For polymerase chain reaction (PCR), 150 ng of genomic DNA was amplified in a reaction tube containing 5 pmol of each primer, 1X PCR buffer, 2 mM MgCl2, 1 Unit Taq DNA polymerase (Genet Bio; South Korea), 0.2 mM dNTP mix, and 4 pmol of each primer. Cycling parameters were as follows: an initial denaturation at 95°C for 5 minutes, 35 cycles at 95°C for 30 s, annealing for 30 s at 52°C, 57°C, 69°C, 67°C, 54°C, 61°C and 62°C for primer#1 to#8 respectively, and a final extension step at 72°C for 35 s, ended by a last extension at 72°C for 5 minutes.

PCR products were analyzed by electrophoresis in a 1.5% agarose gel stained with ethidium bromide for identifying the PKD-specific products for use as a template in
sequencing reactions.

*Sanger sequencing*

Sequencing products were run on an ABI 3130XL Genetic Analyzer (Macrogen Company South Korea), according to the manufacturer’s guidelines.

Data analysis was performed with Chromas software version 2.6.5 (Technelysium, South Brisbane, Australia).

**Results**

Twenty-two patients with an average age of 36.69 ±7.30 years, who suffered from ADPKD, were studied. The sequencing results of the patients were reported in Table 1 and Figure 1. In patient 45.1, variations in rs10960 polymorphism in exon 44 led to the conversion of amino acid isoleucine to valine. This type of variation was considered "missense" that is recorded in the PKDB database as minor allele frequency (MAF:0.239). Moreover, a single nucleotide variant, which has also been reported in the PKDB database as almost neutral, was found as rs10960 in exon 45 of Patient 410.2. The intronic mutation was observed in intron 44 of patients 45.2 and 412.1. In four patients, including 45.3, 410.1, 417.1, and 419.1, exon 45 had a synonymous mutation (Ala4092Ala) and was reported as rs3087632 with MAF: 0.262 in the database PKDB.

**Novel variants**

The first variant was observed in exon 44 of Patient 45.5. This variant led to a synonymous variant in exon 44 (Gly4068Gly). rs200796474 variation was synonymous in Patient 47.1, by which serine amino acid is converted into serine (TCC / TCT). Missense mutation of rs142768096, by which glycine amino acid to valine was observed in exon 44 of Patient 48.1 (GCT / GTC). In Patient 49.4, the
missense mutation converting serine to alanin (GCT/TCT) was found in exon 45. Patient 49.2 also had the missense change converting arginine amino acid to glycine in exon 45(GGG/AGG). In Patient 411.2, the missense converting arginine amino acid to leucine was observed in exon 45 and the missense variation converting arginine amino acid to glutamine was found in the same exon of the same patient (CGT/CTT). The missense change converting valine to isoleucine occurred in exon 44 of Patient 418.2 (GTG/ATG). Moreover, the nonsense mutation converting glutamine amino acid to arginine had occurred in exon 44 of the Patient 421 and was recorded as definitely pathogenic in the PKDB database. In addition, the missense variation converting threonine amino acid to alanine was found in some part of the exon 45 in the Patient 422 (ACC/GCC).

In silico functional analysis

The detected sequence variations found in our study were checked with the currently published list of PKD gene variants in the Human Gene Mutation Database (HGMD) (31) and Autosomal Dominant Polycystic Kidney Disease Mutation Database (PKDB).

The effects of novel variations were analyzed by a web-based computational pathogenicity prediction tool, which included Mutation Taster (32) and expasy translate tools. By using expasy tool, we checked related protein products for sequence and length alteration by altered CDS (NM_001009944). For easy communication of information about mutations, all of them were named based on CDS according to standard mutation nomenclature for molecular diagnostic aims.

The following databases have been used to retrieve polymorphism data: The UniProt database (http://www.uniprot.org) (UniProtKB ID Q8IYM9), the NCBI dbSNP database (https://www.ncbi.nlm.nih.gov/SNP/), Catalogue of Somatic Mutations in Cancer
(COSMIC http://cancer.sanger.ac.uk/cosmic) and 1000 Genomes (http://www.1000genomes.org/). Variants presented in dbSNP, 1000 Genomes or COSMIC were marked as “non-novel variants”. Functional effects of nsSNPs were predicted using Polyphen-2 (http://genetics.bwh.harvard.edu/pp2).

Discussion

To date, 2,322 pathogenic mutations for PKD1 and 278 for PKD2 have been reported in the PKDB (32) although the relative frequency of such mutations at each gene locus is unknown. In addition to these two related genes for ADPKD, Daoust et al. in the French-Canadian population, identified a family in which a classical clinical presentation of ADPKD resulted from a mutation at a locus genetically distinct from either of the previously described loci for this disease. This suggests the existence of a third genetic locus for ADPKD (5).

In our study, 16 end exons of PKD1 gene were studied and results of sequencing in the patients showed that exons 35, 36 and 36 were non-polymorphic, without any mutations and most mutations occurred in the exons 44 and 45. In most of the patients, variants were mostly missense and same-sense types. Our results showed that there is no definite hot spot in PKD1 and therefore, complete PKD1 mutation analysis is needed for ADPKD Iranian patients who need a genetic diagnosis. Our new detected mutations in the Iranian population have made PKD mutation database richer, having high importance in the genetic consultation of ADPKD patients.

Regarding large genes involved in the ADPKD disease, screening all gene regions is expensive and time-consuming; hence, to overcome this issue a database could be
generated for mutations of polycystic kidney disease among the Iranian population to determine the common mutations and characterize the mutation hotspots in Iranian population. Also, considering the clinical similarity of ADPKD with other kidney cystic diseases causing faulty clinical diagnosis in the absence of familial history, molecular study for PKD1 and PKD2 genes in suspected patients is recommended. Identified pathogenic mutations in this study could be confirmed in future studies with more ADPKD families. Besides, genotype-phenotype correlation studies could be performed to determine the severity of each variant and the outcome of patients associated with a specific variant.

Conclusion

In this study, we present nine novel mutations/polymorphisms in PKD1. These data will contribute to an improved diagnostic and genetic counseling in clinical settings.

Abbreviations

Autosomal dominant polycystic kidney disease (ADPKD)
End-stage renal diseases (ESRDs)
Polymerase chain reaction (PCR)
Polycystic Kidney Disease Mutation Database (PKDB)
Human Gene Mutation Database (HGMD)

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Mashhad University of Medical Sciences. An informed consent was obtained from each individual participating in this study.
Consent for publication

Our study is not a case report, and identifying images or other personal or clinical detail of participants that compromise anonymity are not included. Consent to publish from the patients, or in case of minors, the patients' guardians is “Not Applicable”.

Availability of data and materials

All data are included in this published article. Any additional information related to this study is available from the author for correspondence upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was financially supported by grants from Vice-Chancellor of Research, Mashhad University of Medical Sciences, Mashhad, Iran, (Grant# 901006 ). The funders of this study do not have any roles in study design, data collection, analysis, result interpretation, writing and the decision to submit the manuscript for publication.

Authors’ contributions

FK performed the experiments. MAK, AT, participated in the study design and scientific discussion of the data. MAK supervised the study. All authors contribute to the writing, read and approved the final manuscript.

Acknowledgments

We thank Dr. Hassan Boskabadi, from Mashhad University of Medical Sciences for providing human blood samples. Special thanks go to Mr. Mohammad Bagher Eskandari at Ghaem Genetic Diagnostic Laboratory Mashhad, for technical assistance. We thank all the family members for their participation and their
physicians for the clinical evaluation of the patients. Our sincere thanks also go to Dr. Ali Javadmanesh support in research programs.

References

1. Torres VE, Harris PC, Pirson Y. Autosomal dominant polycystic kidney disease. Lancet. 2007;369(9569):1287-301.

2. Grantham JJ. Clinical practice. Autosomal dominant polycystic kidney disease. N Engl J Med. 2008;359(14):1477-85.

3. Torres VE, Harris PC. Autosomal dominant polycystic kidney disease. Nefrologia. 2003;23 Suppl 1:14-22.

4. Srivastava A, Patel N. Autosomal dominant polycystic kidney disease. Am Fam Physician. 2014;90(5):303-7.

5. Daoust MC, Reynolds DM, Bichet DG, Somlo S. Evidence for a third genetic locus for autosomal dominant polycystic kidney disease. Genomics. 1995;25(3):733-6.

6. Yeo WS, Ng QX. Disruptive technological advances in vascular access for dialysis: an overview. Pediatric Nephrology. 2017; 29:1-6.

7. Johnson AM, Gabow PA. Identification of patients with autosomal dominant polycystic kidney disease at highest risk for end-stage renal disease. J Am Soc Nephrol. 1997; 8(10):1560-7.

8. Gabow PA. Autosomal dominant polycystic kidney disease—more than a renal disease. American Journal of Kidney Diseases. 1990;16(5):403-13.

9. Fick GM, Johnson 7.AM, Hammond WS, Gabow PA. Causes of death in autosomal dominant polycystic kidney disease. Journal of the American Society of Nephrology. 1995;5(12):2048-56.
10. Hughes J, Ward CJ, Peral B, Aspinwall R, Clark K, San Millan JL, et al. The polycystic kidney disease 1 (PKD1) gene encodes a novel protein with multiple cell recognition domains. Nat Genet. 1995;10(2):151-60.

11. Moy GW, Mendoza LM, Schulz JR, Swanson WJ, Glabe CG, Vacquier VD. The sea urchin sperm receptor for egg jelly is a modular protein with extensive homology to the human polycystic kidney disease protein, PKD1. J Cell Biol. 1996;133(4):809-17.

12. Sandford R, Sgotto B, Aparicio S, Brenner S, Vaudin M, Wilson RK, et al. Comparative analysis of the polycystic kidney disease 1 (PKD1) gene reveals an integral membrane glycoprotein with multiple evolutionary conserved domains. Hum Mol Genet. 1997;6(9):1483-9.

13. Huan Y, van Adelsberg J. Polycystin-1, the PKD1 gene product, is in a complex containing E-cadherin and the catenins. J Clin Invest. 68-1459:10(104;1999).

14. Mochizuki N, Cho G, Wen B, Insel PA. Identification and cDNA cloning of a novel human mosaic protein, LGN, based on interaction with G alpha i2. Gene. 1996;181(1-2):39-43.

15. Chen ML, Hoshi T, Wu CF. Heteromultimeric interactions among K+ channel subunits from Shaker and eag families in Xenopus oocytes. Neuron. 1996;17(3):535-42.

16. Hateboer N, Lazarou LP, Williams AJ, Holmans P, Ravine D. Familial phenotype differences in PKD11. Kidney Int. 1999;56(1):34-40.

17. Loftus BJ, Kim 15.UJ, Sneddon VP, Kalush F, Brandon R, Fuhrmann J, et al. Genome duplications and other features in 12 Mb of DNA sequence from human chromosome 16p and 16q. Genomics. 1999;60(3):295-308.

18. Krawczak M, Cooper DN. The human gene mutation database. Trends Genet.
1997;13(3):121-2.

19. Peral B, Gamble V, Strong C, Ong AC, Sloane-Stanley J, Zerres K, et al. Identification of mutations in the duplicated region of the polycystic kidney disease 1 gene (PKD1) by a novel approach. Am J Hum Genet. 1997;60(6):1399-41.

20. Watnick T, Phakdeekitcharoen B, Johnson A, Gandolph M, Wang M, Briefel G, et al. Mutation detection of PKD1 identifies a novel mutation common to three families with aneurysms and/or very-early-onset disease. Am J Hum Genet. 1999;65(6):1561-71.

21. Watnick TJ, Gandolph MA, Weber H, Neumann HP, Germino GG. Gene conversion is a likely cause of mutation in PKD1. Hum Mol Genet. 1998;7(8):1239-43.

22. Watnick TJ, Piontek KB, Cordal TM, Weber H, Gandolph MA, Qian F, et al. An unusual pattern of mutation in the duplicated portion of PKD1 is revealed by use of a novel strategy for mutation detection. Hum Mol Genet. 1997;6(9):1473-81.

23. Thomas R, McConnell R, Whittacker J, Kirkpatrick P, Bradley J, Sandford R. Identification of mutations in the repeated part of the autosomal dominant polycystic kidney disease type 1 gene, PKD1, by long-range PCR. Am J Hum Genet. 1999;65(1):39-49.

24. Veldhuisen B, Saris JJ, de Haij S, Hayashi T, Reynolds DM, Mochizuki T, et al. A spectrum of mutations in the second gene for autosomal dominant polycystic kidney disease (PKD2). Am J Hum Genet. 1997;61(3):547-55.

25. Daniells C, Maheshwar M, Lazarou L, Davies F, Coles G, Ravine D. Human gene mutations. Gene symbol: PKD1. Disease: Polycystic kidney disease. Hum
26. Perrichot RA, Mercier B, Simon PM, Whebe B, Cledes J, Ferec C. DGGE screening of PKD1 gene reveals novel mutations in a large cohort of 146 unrelated patients. Hum Genet. 1999;105(3):231-9.

27. Van Raay TJ, Burn TC, Connors TD, Petry LR, Germino GG, Klinger KW, et al. A 2.5 kb polypyrimidine tract in the PKD1 gene contains at least 23 H-DNA-forming sequences. Microb Comp Genomics. 1996;1(4):317-27.

28. Blaszak RT, Potaman V, Sinden RR, Bissler JJ. DNA structural transitions within the PKD1 gene. Nucleic Acids Res. 1999;27(13):2610-7.

29. Phakdeekitcharoen B, Watnick TJ, Ahn C, Whang DY, Burkhart B, Germino GG. Thirteen novel mutations of the replicated region of PKD1 in an Asian population. Kidney Int. 2000;58(4):1400-12.

30. Rossetti S, Strmecki L, Gamble V, Burton S, Sneddon V, Peral B, et al. Mutation analysis of the entire PKD1 gene: genetic and diagnostic implications. Am J Hum Genet. 2001;68(1):46-63.

31. Stenson PD, Mort M, Ball EV, Howells K, Phillips AD, Thomas NS, et al. The human gene mutation database: 2008 update. Genome medicine. 2009;1(1):13.

32. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nature methods. 2014;11(4):361-2.

33. Liu B, Chen S-C, Yang Y-M, Yan K, Qian Y-Q, Zhang J-Y, et al. Identification of novel PKD1 and PKD2 mutations in a Chinese population with autosomal dominant polycystic kidney disease. Scientific reports. 2015;5:17

Tables
Table 1. Covering area of the primers

| Primer                | Covering exon and intron                      |
|-----------------------|-----------------------------------------------|
| Primer 1              | Int44-exo45-int45-exo46-int46                |
| PKD1ex45-46           | Int41-exo42-int42-exo43-int43                |
| Primer 2              | Int37-exo38-int38-exo39-int39                |
| PKD1ex42-43           | Int43-exo44-int44-exo45-int45                |
| Primer 3              | Int30-exo31-int31-exo32-exo33-int33-exo34-int34 |
| PKD1ex38-39           | Int 34-exo35-int35-exo36-int36-exo37-int37   |
| Primer 4              | Int39-exo40-int40-exo41-int41                |
| PKD1ex44-45           | Int40-exo41-int41-exo42-int42-exo43          |

Table 2. Results of DNA sequencing of Iranian patients with ADPKD

| Patient | Location | Variation | Rs#       | Type of variation | PKDB/HGMD database |
|---------|----------|-----------|-----------|-------------------|---------------------|
| 45.1    | Exon44   | Ilu4045val| 10960     | Missense          | ATC/GTG             |
|         |          |           |           | Missense ATC/GTG  |                     |
| 45.2    | Intron44.45 | -      | -        | -                 | -                   |
| 45.3    | Exon44   | Ala4092Ala| 3087632   | Synonymous         | GCA/GCG             |
|         |          |           |           | Likely neutral    |                     |
| 45.4    | Exon44   | Gly4068Gly| -        | Synonymous         | GGG/GGT             |
|         |          |           |           | Novel             |                     |
| 47.1    | Exon45   | Ser4013ser| 2007964474| Synonymous        |                     |
|         |          |           |           | Novel             |                     |
| 48.1    | Exon44   | Gly4032Val| 142768096| Missense          | GCT/GTC             |
|         |          |           |           | Novel             |                     |
| 49.4    | Exon45   | Ala4103 ser| -     | Missense         | GCT/TCT             |
|         |          |           |           | Novel             |                     |
| 49.2    | Exon45   | Gly4102Arg| -        | Missense         | GGG/AGG             |
|         |          |           |           | Novel             |                     |
| 410.1   | Exon45   | Ala4092Ala| rs3087632 | synonymous       |                     |
|         |          |           |           | Likely neutral    |                     |
| 410.2   | Exon45   | ilu4045val| rs10960   | Missense         | ATC/GTC             |
|         |          |           |           | Likely neutral    |                     |
|   | Exon   |       |       |                  |                      |
|---|--------|-------|-------|------------------|----------------------|
| 411.2 | Exon45 | Arg4114leu | - | Missense CGT/CTT | Novel               |
| 412.1 | Intron44.45 | - | - | - | -                   |
| 412.2 | Exon45 | Arg4091Gln | - | Missense CGG/CAG | Novel               |
| 417.1 | Exon45 | Ala4092Ala | rs3087632 | Synonymous GCA/GCG | Likely neutral      |
| 418.2 | Exon44 | Val4035Ile | - | Missense GTG/ATG | Novel               |
| 419.1 | Exon45 | Ala4092Ala | rs3087632 | synonymous | Likely neutral      |
| 421   | Exon44 | Gln4005Arg | - | Nonsense CAG/CGG | Definitely Pathogenic |
| 422   | Exon45 | Ther4073Ala | - | Missense ACC/GCC | Novel               |

**Figures**
Figure 1
The sequencing results of the patients