STR TRONG SÀNG LỌC DI TRUYỀN TIỀN LÀM TỔ BỆNH THẬN ĐA NANG NHIỄM SẮC THỂ THƯỜNG TRỘI DO CÁC BIẾN THỂ GÂY BỆNH CỦA GEN PKD1 TẠI VIỆT NAM

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TÓM TẮT

ADPKD là bệnh thận di truyền phổ biến nhất, đặc trưng bởi sự sản sinh và tăng sinh nhiều u nang trong thận và tiến triển dần dần dẫn đến suy thận giai đoạn cuối. Do kích thước rất lớn của gen PKD1, nhiều vị trí của các biến thể gây bệnh trên gen và sự hiện diện tới sáu vùng gen giả với 97,7% đối với PKD1, các phương pháp chẩn đoán trực tiếp đối với biến thể gây bệnh của gen PKD1 đang gặp nhiều thách thức. Sử dụng phân tích liên kết di truyền để kiểm tra sự di truyền của STR với gen PKD1 trong chẩn đoán tiền làm tổ là một cách tuyệt vời để chẩn đoán bệnh thận đa nang di truyền và xác định sự hiện diện của nhiễm DNA ngoại lai và hiện tượng ADO. Bảy STR liên quan đến PKD1 đã được chọn để khuếch đại và phân tích trên 16 mẫu máu của bệnh nhân ADPKD. Sản phẩm PCR của STR được thực hiện bằng phương pháp điện di mao quản để đánh giá kích thước, từ đó xác định các alen di truyền có biến thể gây bệnh và chẩn đoán di truyền bằng phôi trước khi chuyển phôi. Sinh thiết được thực hiện trên 13 mẫu phôi vào ngày thứ năm cho thấy sáu mẫu phôi chứa biến thể PKD1 gây bệnh và bảy mẫu phôi không có biến thể PKD1 gây bệnh thích hợp cho việc chuyển phôi.

Nghiên cứu này đã thực hiện thành công chẩn đoán di truyền tiền làm tổ bệnh ADPKD ở người Việt Nam.

Từ khóa: Phân tích liên kết di truyền, STR, PKD1, bệnh thận đa nang nhiễm sắc thể thường trội, PGT

SUMMARY

SHORT TANDEM REPEATS IN PREIMPLANTATION GENETIC DIAGNOSIS OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE DUE TO PATHOGENIC VARIANTS OF THE PKD1 GENE IN VIETNAM

ADPKD is the most prevalent inherited kidney disease, characterized by the production and proliferation of numerous cysts in the kidney and progressive progression that results in end-stage renal failure. Due to the enormous size of the PKD1 gene, the numerous positions of pathogenic variants on the gene, and the presence

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of up to six pseudogene regions with 97.7% to PKD1, direct diagnostic approaches for the pathogenic variation of the PKD1 gene are challenging. Using genetic linkage analysis to examine the inheritance of STRs with the PKD1 gene in preimplantation diagnosis is an excellent way to diagnose hereditary polycystic kidney disease and determine the presence of foreign infection and the ADO phenomenon. Seven PKD1-associated STRs were selected for amplification and analysis on 16 ADPKD patients blood samples. PCR products of STRs are carried out by capillary electrophoresis to assess the size, thereby identifying genetic alleles with pathogenic variants and making genetic diagnoses with embryos before transplanting. Biopsies performed on 13 embryo samples on day five revealed six embryo samples harbouring the pathogenic PKD1 variant and seven embryo samples without the pathogenic PKD1 variant suitable for embryo transfer.

This study successfully performed the preimplantation genetic diagnosis of ADPKD in Vietnamese.

Keywords: Linked genetic analysis, STR, PKD1, Autosomal-dominant polycystic kidney disease, PGT

I. INTRODUCTION

Polycystic Kidney Disease (PKD) is a prevalent inherited kidney disease, with an estimated prevalence of 1/500-1/1 000 in Western countries [17], caused by a pathogenic variant appearance of the PKD1 (MIM #601313) and PKD2 (MIM #173910) genes [1], with 85% of cases caused by a pathogenic variant on the PKD1 gene. The PKD1 gene is situated on chromosome 16 and has a size of 47.2 kb [8] with 46 exons (16p13.3). The polycystin-1 transmembrane protein is encoded by the PKD1 gene (PC1). Any pathogenic mutation of the PKD1 gene will affect the PC1 protein, disrupt several cellular signalling pathways [16] and lead to anomalies in ion transport, polarity, reproduction, and cyclic cell death in renal epithelial cells, resulting in renal cyst formation. Cysts in patients with polycystic kidney disease enlarge the kidneys and lead to the progressive loss of typical structures, resulting in a decline in kidney function and progression to renal failure [2].

Current methods to directly discover pathogenic variations in the PKD1 gene are based on the same fundamental premise of multiplying the sequence sections of the PKD1 gene with techniques such as LR-PCR and Nested-PCR. Then, pathogenic variants are identified using sequencing techniques such as NGS and Sanger sequencing on the amplified products and comparing the results to the standard human genome sequence data. [13,12,5]. In addition, some investigations employ additional techniques, such as MLPA, QFM-PCR, Q-PCR, and Array-CGH. [1,6].

However, direct genetic analysis methods still face some difficulties due to the large size of the PKD1 gene and the many pathogenic variant sites on the gene. In addition, there are up to 6 regions of pseudogene structure (high similarity (97.7%) with sequences from 5'UTR to exon 32 on chromosome 16 [1,8]), causing many

difficulties in gene amplification. Gene sequencing techniques take a long time to conduct the technique, and the cost of testing is relatively high. In addition, when diagnosing biopsied embryo samples, it is necessary to evaluate the phenomenon of ADO and DNA contamination.

In addition to approaches for directly detecting the PKD1 variation, some writers worldwide have diagnosed ADPKD by genetic linkage analysis [4,11,10]. This method is based on the co-inheritance of the causal gene and its associated short tandem repeats (STRs) in affected families. The presence or lack of STRs linked to the disease gene makes it feasible to determine a carrier of the disease gene. Due to the small size of STR fragments, their amplification is more straightforward and can be performed on embryos that have been biopsied and genomically propagated. In addition, the STR analysis method can assess the ADO phenomenon and DNA contamination during embryo biopsy and whole genome amplification of day five embryos.

II. MATERIAL AND METHODS

2.1. Patient description

The study was performed on 16 peripheral blood samples from members of 3 families of ADPKD patients, and 13 embryos were biopsied on day 5 of these families. ADPKD patients have correctly identified the pathogenic variant of the PKD1 gene by next-generation sequencing. Family PKD1.01: ADPKD patient carries the pathogenic variant on exon 29 of PKD1: c.9859_9861delCTC (NM_001009944); family PKD1.02: ADPKD patient carries a pathogenic variant on exon 5 of PKD1: p.P253Q (NC_000016.10/NT_187607.1); Family PKD1.03: ADPKD patient carries a pathogenic variant on exon 29 of PKD1: p.Phe3257fs (NM_001009944). Families collected information about ADPKD and created genetic pedigrees.

All people described in this research were signed written informed consent for the publication of the case details, and the protocol was approved by the Ethical Review Committee of Vietnam Military Medical University (No.1068/2019/VMMU-IRB). This study was also conducted using good clinical practice following the Declaration of Helsinki and its later amendments or comparable ethical standards.

2.2. Short tandem repeat

The haplotypes of microsatellite markers within and around the PKD1 gene were determined for each subject. The markers used were D16S475, D16S3082, D16S283 (SM7), D16S663 (CW2), D16S291 (CA2.5) located in the upstream gene, 1 in the gene: D16S3252 (KG8) and 1 in the downstream gene: D16S52 (Table 1). They have polymorphism information content (PIC) values that range from 0.25 to 0.83 in the local Thai population [9]. The locations of the markers relative to PKD1 are shown in Fig 1.

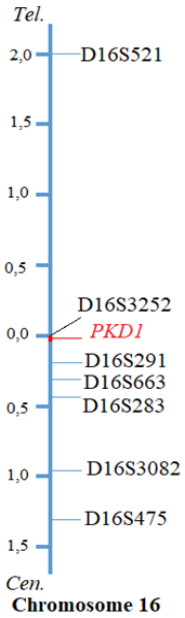

Figure 1: Location map of the STRs and the PKD1 gene on chromosome 16 (based on the position parameters of the STRs on https://genecards.weizmann.ac.il/geneloc_ncbi37)

Table 1: Characteristics of selected microsatellite markers including sequence of primers, polymerase chain reaction products size [4,15,18].

Marker	Primer sequence (5'-3')		Product size (bp)	Reference
D16S475	Forward*	TGAACTGAGGTCCTACCACTG	207-243	[4]
	Reverse	AGAAACTACTGGCAGGAACAGA		
D16S3082	Forward*	CCATGTGTCACCTTAACCTTTCC	143-175	[18]
	Reverse	TGGCCGGTCTTTCCAGG		
D16S283	Forward*	CCATGTGTCACCTTAACCTTTCC	81-107	[18]
	Reverse	TGGCCGGTCTTTCCAGG		
D16S663	Forward*	TGTAAAACGACGGCCAGTGTCTTTCTAGGAA TGAAATCAT	123-147	[18]**
	Reverse	ATTGCAGCAAGACTCCATCT		
D16S291	Forward*	AAGGCTGGCAGAGGAGGTG	109-142	[15]
	Reverse	CAGTTGTGTTTCCTAATCGGCG		
D16S3252	Forward*	TGTAAAACGACGGCCAGTGTACACAGAAGC AGGCACAG	201-217	[4] **
	Reverse	GGCAAGTAGCAGGACTAGGC		
D16S521	Forward*	TGTAAAACGACGGCCAGTGAGCGAGACTCC GTCTAAA	174-190	[18]**
	Reverse	CAGCAGCCTCAGGGTT		

** Primer fluorescently labeled with FAM*

*** The primers used for the reaction were previously with some modifications*

DNA Extraction

As directed by the G-spinTM Total DNA Extraction Kit, peripheral blood samples from relatives of ADPKD patients were retrieved. Following extraction, the DNA solution was examined by SpectraMax QuickDrop for concentration and purity.

Whole genome Amplification for Embryos'genome

After performing IVF, embryo samples from 3 families were cultured until day five and biopsied to obtain 3-5 cells at the Military Institute of Clinical Embryology and Histology (MICEH). DNA from biopsied embryos was amplified using the REPLI-g® Single Cell Kit (QIAGEN, Germany), diluted with nuclease-free water to a concentration of 10-20 ng/µL and stored at -20^{0}C.

PCR amplification

Single-primer PCR was performed to amplify STRs for blood and embryo samples. Each PCR reaction tube for amplifying the individual STR fragment has a volume of 25 µL, which contains 12.5 µL of GoTaq Green Mastermix 2X; 0.5 µL each forward and reverse primer and 2.5 µL DNA template (concentration: 10-20 ng/µL), water sufficient.

The thermal cycle for amplifying the STR segments is as follows: 95℃-5 min, 35 cycles include denaturation at 94℃ for 30s, annealing at 56℃ for 30s, extension at 72℃ for 45s and a final extension at 72℃ for 10 minutes. After running the PCR reaction, the amplified product is electrophoresed on 3% agarose gel with GeneRuler 100bp DNA Ladder (Thermo Fisher Scientific) to check, then conducting capillary electrophoresis.

Capillary electrophoresis

One µL of fluorescent PCR product was combined with 24.5 µL of Hi-Di Formamide (Thermo Fisher Scientific, USA) and 0.5 µL of WEN ILS 500 size standard before being denatured at 95°C for 5 minutes, cooled to 4°C, and resolved in SeqStudio Genetic Analyzer (Thermo Fisher Scientific, USA). Using GeneMapper 6.0 software, a post-electrophoresis analysis was done.

Based on the results of analyzing the allele sizes and their inheritance to conclude the group of alleles associated with the pathogenic variant of the PKD1 gene, thereby giving the results of pre-implantation genetic diagnosis for this disease with embryo samples of the ADPKD families.

III. RESULTS

The DNA template for the PCR reaction amplified the STRs to be studied in each family's DNA sample. Then, 3% agarose gel electrophoresis was used to evaluate the PCR results (Fig 2).

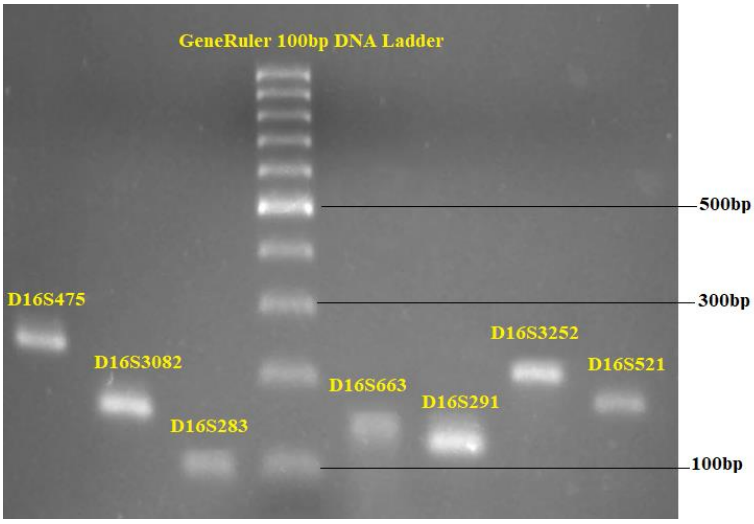

Figure 2: Electrophoresis results of PCR products of one DNA sample in this study and GeneRuler 100bp DNA Ladder (Thermo Fisher Scientific) on 3% agarose gel

It is possible to determine the group of inherited alleles linked with illness genes and the group of inherited alleles with normal genes based on the study findings of allele size in each marker in combination with the clinical characteristics of pedigree members. It is then possible to diagnose embryos with disease-causing genes and embryos without disease-causing genes.

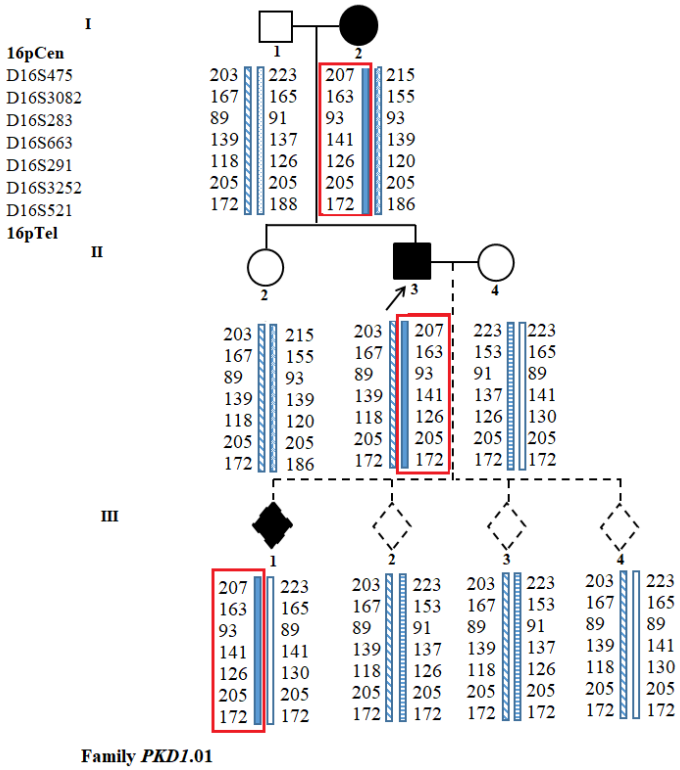

Figure 3: Pedigrees of Family PKD1.01 with haplotypes for the PKD1 associated polymorphic markers in this study

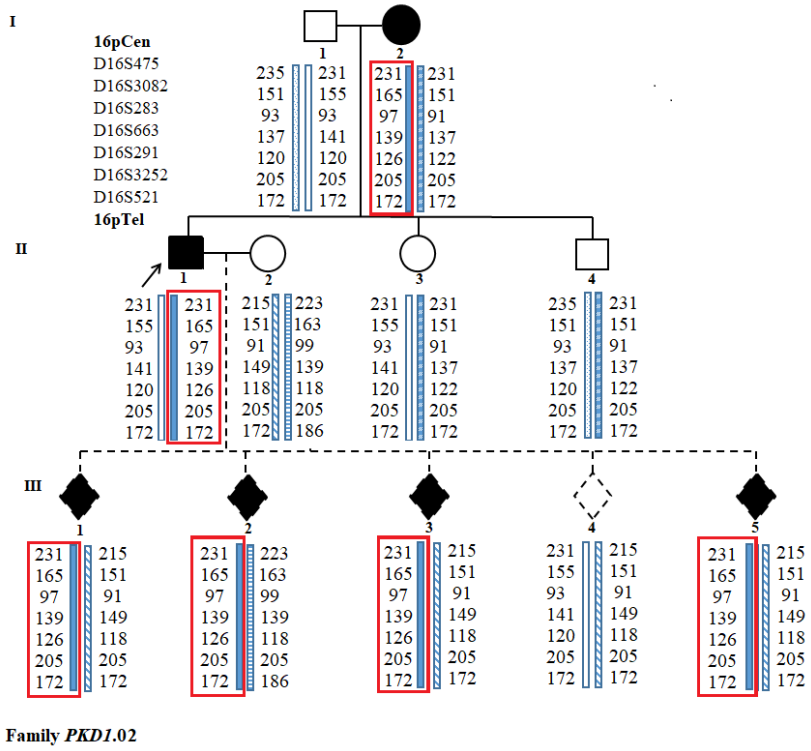

Figure 4: Pedigrees of Family PKD1.02 with haplotypes for the PKD1 associated polymorphic markers in this study

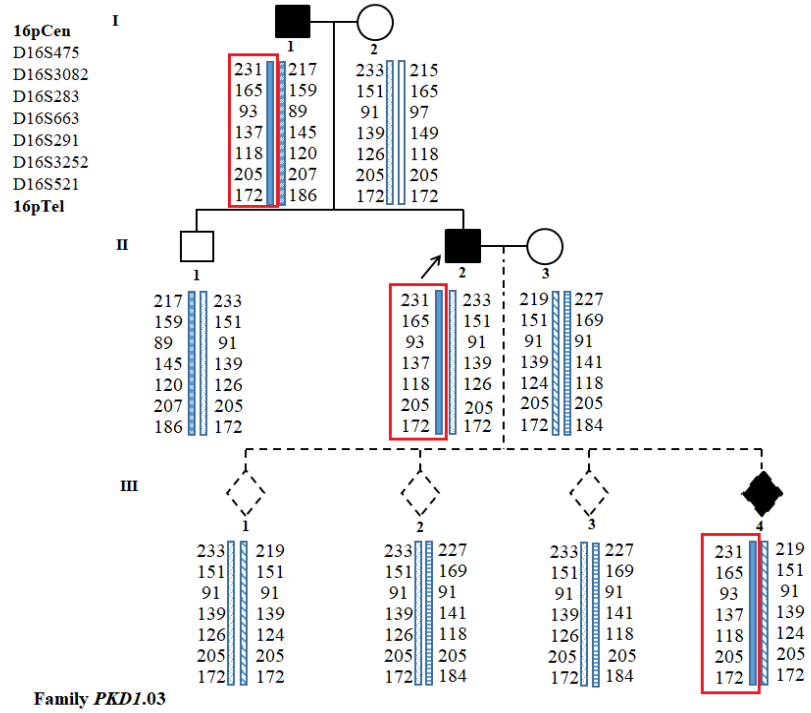

Figure 5: Pedigrees of Family PKD1.03 with haplotypes for the PKD1 associated polymorphic markers in this study

Names of the PKD1 associated STRs are located on the left of the pedigrees. Numbers indicate lengths of PCR products (bp) for different alleles of STRs. The alleles located in the red box are genetically linked to the pathogenic variant of PKD1.

With the analysis results of all three families (Fig 3, fig 4, fig5), it can be seen that the alleles are successfully multiplied, and the size is consistent with the design primer characteristics. With embryo samples, the results of STR analysis showed that embryos carrying disease-causing variants inherited from the father or mother could be distinguished from embryos that did not carry these variants. In addition, the absence of ADO and foreign contamination during biopsies and whole-genome multiplication ensures that the diagnostic results on embryos are entirely reliable and help to make informed decisions regarding genetic information for families during the selection of embryos for implantation in the mother's uterus.

IV. DISCUSSION

No studies provide information on the heterozygosity index and genetic polymorphism index of STRs related to the PKD1 gene in the Vietnamese population at this time. Based on the genetic data of Asian populations [11,7], the STR markers utilized in this investigation have a high frequency of heterozygosity and polymorphism. As proposed by the European Society of Human Reproduction and Embryology in 2020 [3], markers keep within a 2 Mb (2 cM) distance from PKD1, reducing their capacity to cross-exchange during meiosis in germ cells and facilitating genetic analysis of the marker's relationship with PKD1.

In this study, after studying the genetic results of markers in all three patient families, it was determined that the selected STR loci were totally related to the PKD1 gene in the reported samples, ensuring the accuracy of genetic diagnosis of the PKD1 gene in embryo samples. In contrast, the study discovered that markers D16S3252 (KG8) and D16S521 had a high rate of homology, and that each locus included just two alleles. In families when both parents are homozygous for the same allele, these STRs did not add information to the genetic research method. In order to propose a STRs panel for PGT-M of Vietnamese ADPKD patients, further research is required to examine the heterozygosity and polymorphism index for STRs related with the PKD1 gene in the Vietnamese population.

The method of genetic analysis linked to the analysis of STRs associated with disease genes has many advantages when it is possible to identify carriers of disease genes more easily than direct genetic evaluation methods (especially for genes with large size and complex variants such as PKD1), can be applied on families carrying different pathogenic variants, and saves the patient's family time and money. In addition, the evaluation of ADO and contamination during

biopsies and whole genome amplification of embryo samples is aided by linked genetic analysis. This improves the accuracy of embryo sample diagnostic results, preventing false negatives. Currently, according to the guidelines of the European Society for Human Reproduction and Embryology for 2020 [3], the use of whole genome amplification followed by linkage genetic analysis for the detection of genetic illnesses is becoming one of the PGT-M approaches. Nonetheless, the linkage genetic analysis method has limitations when examining de-novo and mosaic variations.

V. CONCLUSION

The initial study provided preimplantation genetic diagnostic information for 14 embryos from 3 families of ADPKD patients due to a pathogenic variant on the PKD1 gene based on genetic analysis of the association of 7 STRs with the PKD1 gene in Vietnamese. This diagnostic method combined with screening for chromosomal abnormalities on embryo samples, helps families of ADPKD patients to select normal embryos that do not carry disease genes before transplanting into the mother's uterus and helping to have healthy children. The method also can diagnose early (when they are asymptomatic) ADPKD family members.

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