Multiplex-STR panels comprehensive for a timely molecular diagnosis of ADPKD

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ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited renal disease caused by either *PKD1* or *PKD2* mutations that can lead to fatal end-stage renal disease (ESRD). Direct mutation analysis of ADPKD remains complicated and time consuming. Haplotype-based linkage analysis within-pedigree is a straightforward, alternative molecular method to diagnose ADPKD, especially in some urgent situations. This study aimed to develop a rapid and efficient short tandem repeat (STR)-haplotype analysis for Thai ADPKD families by investigating 100 unrelated Thai chromosomes for the informativeness of the 10 and 8 STRs located flanking or within the *PKD1* and *PKD2* genes, respectively. The method was based on multiplex fluorescent polymerase chain reaction (MF-PCR) coupled with detection by laser-induced fluorescent capillary electrophoresis (CE). Two highly informative, multiplex STR panels for rapid, inexpensive and comprehensive molecular diagnosis of both PKD1 ('PKD1A': 7 Plex STR) and PKD2 ('PKD2': 8 Plex STR) were validated and demonstrated the usefulness in ADPKD families with unknown causative mutations who require timely decision for kidney transplantation from the living-related kidney donors or planning for pre-implantation genetic diagnosis (PGD). This study would be beneficial for differential diagnosis among PKD1, PKD2 or related cystic diseases to timely give a genetic counseling, appropriate management and prognosis to the patients' families, not only Thais but also Asians and other populations.

Keywords: ADPKD; linkage analysis; short tandem repeat (STR); multiplex; Thai population

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited renal disease, occurring in 1:400–1,000 worldwide (Harris and Rossetti, 2010). The majority, 85% of cases with more severe phenotype is resulted from mutations in the *PKD1* gene (16p13.3) while 15%

being from those in the *PKD2* gene (4q21) (Harris and Rossetti, 2010). Mutations in both genes promote fluid-filled cysts that interrupt renal function and progress to end-stage renal disease (ESRD) (Harris and Rossetti, 2010). Albeit the availability of the next generation sequencing (NGS), direct mutation analysis in ADPKD is still limited because of the high cost of test, genetic complexity of the *PKD1* gene along with no hot-spot mutations as well as lots of variants of uncertain significance (VUS) in both genes that make genetic test report equivocal (Harris and Rossetti, 2010). Moreover, approximately 26% and 9% of ADPKD cases had non-definite and no mutations, respectively in either *PKD1* or *PKD2* genes. Therefore, linkage analysis remains a valuable test that requires lower costs and shorter time for reliably indicating disease status (Harris and Rossetti, 2010).

Timely molecular diagnosis is essential for particular situations in ADPKD such as 1) ESRD patients who have urgent needs of renal transplantation from their related-living kidney donors in whom no defective ADPKD genes can be found and 2) pre-implantation genetic diagnosis (PGD) in the couples who avoid transmitting the genetic defect to their children. Linkage analysis is used to track the gene within a single family with known disease status but without actually knowing the mutation, by identifying a marker that co-segregates with the gene of interest. Highly polymorphic DNA markers such as short tandem repeat (STR) and single nucleotide polymorphism (SNP) should locate either in or flanking such a gene. Accuracy of this test depends on the ability of DNA markers to distinguish between affected and unaffected members in a family (informativeness) which may vary among ethnic groups (Wang *et al*., 1995).

Since ADPKD is a life-threatening and highly-prevalent inherited renal disorder which frequently requests for timely molecular diagnosis and there is no other laboratory in Thailand offering linkage analysis for ADPKD, this study thus aimed to develop a rapid and efficient STR-haplotype analysis for ADPKD families by investigating the allele frequency and heterozygosity of the selected STRs for *PKD1* and *PKD2* genes in 100 unrelated Thai chromosomes to evaluate the informativeness. The STR-marker panels were then applied to test in known and unknown ADPKD families to demonstrate of the usefulness of the assays.

MATERIALS AND METHODS

This study recruited Thai families diagnosed to have ADPKD who attended the clinic at Siriraj Hospital, being approved by Siriraj Institutional Review Board (Si-IRB), COA number: Si 439/2008. After informed consent of the patients and normal subjects were obtained, blood samples were collected for genomic DNA extraction. A total of 10 and 8 STR loci flanking or within *PKD1* and *PKD2* genes, respectively were selected for validation (Figure 1), the primer sequences were obtained from the NCBI Probe Database (ProbeDB) (http://www.ncbi.nlm.nih.gov/probe/, January 2013) with CW4-forward primer being modified to be 5′-AGTGCTGGCATTACAGGCATGAACC-3′. Moreover, new markers were also included, i.e. PKD1-TG1 (Zeevi *et al*., 2013), MGPKD2-1 and MGPKD2-10 (Bae *et al*., 2004). Multiple STR loci were amplified as 3 different sets using optimal concentration of each fluorescent-labeled primer in the KAPA 2Gfast multiplex mix (Kapa Biosystems, USA) (Table 1). The multiplex STR-PCR products were detected with laser-induced fluorescent capillary electrophoresis (CE) using the ABI 3130xl Genetic Analyzer (Applied Biosystems, USA). Haplotypes for each family were assigned manually.

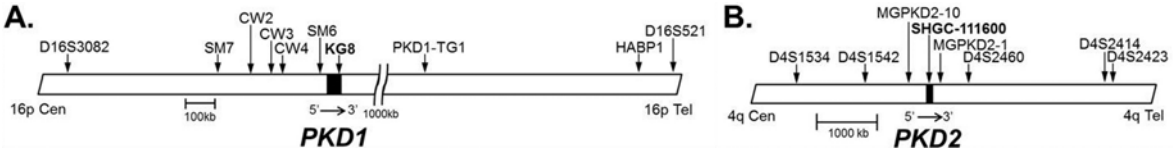

Figure 1 Schematic map showing the location of STR markers for *PKD1* (A) and *PKD2* genes (B).

Table 1 Concentration of primer mixtures and 5'-fluorescent dye labeled for 3 multiplex STR-PCR sets

STR Set	Primers with Fluorescent Dye Labeled at 5' End (Primer concentration in primer mixture)			
	PET (red)	**FAM (blue)**	**NED (yellow)**	**VIC (green)**
PKD1A	D16S3082 (3 μM)	SM7 (3.5 μM)	CW2 (7.5 μM)	CW4 (3.5 μM)
(7-Plex STR)	SM6 (0.5 μM)	PKD1-TG1 (5.25 μM)	D16S521 (1.5 μM)	
PKD1B	-	KG8 (0.2 μM)	CW3 (3.5 μM)	HBAP1 (5 μM)
(3-Plex STR)				
PKD2	MGPKD2-1	D4S414 (2.81 μM)	MGPKD2-10 (5.31 μM)	D4S1534 (2.81 μM)
(8-Plex STR)	(16.56 μM)		D4S2460 (2.81 μM)	D4S1542 (2.81 μM)
				SHGC-111600 (2.81 μM)

To determine the informativeness of these markers, heterozygosity (HET) and polymorphism information content (PIC) were calculated in accordance with the formulas below. The informative STR panels were validated and applied for linkage analysis of ADPKD families with known (Thongnoppakhun *et al*., 2004; Thongnoppakhun, 2012) and unknown causative mutations in either *PKD1* or *PKD2*.

Heterozygosity (HET)

$$HET = 1 - \sum_{i=0}^{n} (fi)^2$$

Polymorphism Information Content (PIC)

$$PIC = 1 - \sum_{i=0}^{n} (fi)^2 - \sum_{i=0}^{n-1} \sum_{j=i+1}^{n} 2fi^2 fj^2$$

Where n = number of alleles,

fi = frequency of each allele, $j = i+1$

RESULTS AND DISCUSSION

The informativeness of STR markers for ADPKD is summarized in Figure 2. Number of alleles is not associated with HET and PIC value. Almost all STR markers for *PKD1* have high informativeness values (HET>0.7, PIC>0.59) (Ott, 1992; Todhunter *et al*., 2003) while SM6 and D16S521 have acceptable values. Unfortunately, the only one STR located within the *PKD1* gene, KG8, is not informative enough in Thais, being contrary to other ethnic groups in Europe i.e. Hungarian, Scottish and Spanish of which HET/PIC values were 0.65/0.64 (Endreffy *et al*., 2009), 0.49/0.46 (Snarey *et al*., 1994) and 0.56/0.54 (Endreffy *et al*., 2009), respectively. This locus also showed in other Asian populations i.e. Iranian (0.80/0.77) (Radpour *et al*., 2005) and Japanese (HET=0.46) (Taiwan Polymorphic Marker Database (TPMD), April 2015). Nevertheless, the use of a single STR Set 'PKD1A' (7-Plex STR) is generally sufficient for informative linkage analysis of *PKD1*. The 'PKD1B' (3-Plex STR) set may be optional to enhance the accuracy in some families. For the *PKD2* gene, 5 out of 8 STRs (in 'PKD2': 8-Plex STR) have a high informativeness, while the one within the gene, SHGC-11600 is not polymorphic at all, thus could be ignored.

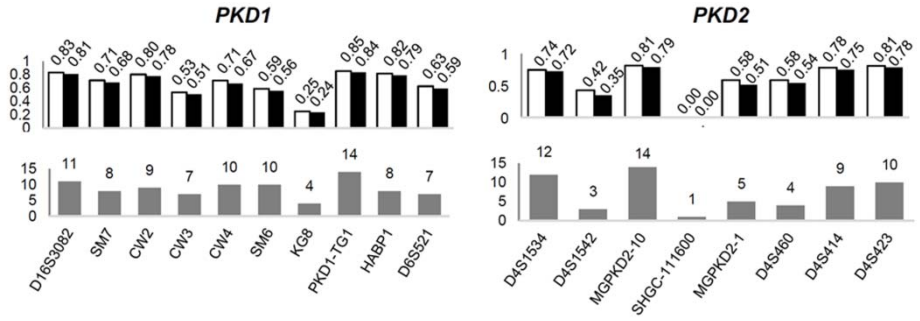

Figure 2 Characteristics of STR markers for *PKD1* (left) and *PKD2* (right) genes calculated from 100 unrelated Thai chromosomes.

Interestingly, there is a remarkable difference of the informativeness of MGPKD2-1 and D4S460 for *PKD2* in Thais compared to Korean of which the HET/PIC values were 0.37/0.32 (Bae *et al*., 2004) and 0.74/- (Lee *et al*., 2001), respectively. However, all of the STR markers for *PKD1* and *PKD2* are generally highly informative in Thais, similar to worldwide populations except the ones as indicated above, thus seemingly quite universal (as opposed to SNPs which are rather population-specific).

Validation of the informative STR-marker panels by linkage analysis in ADPKD families revealed complete concordance with known causative mutations in either *PKD1* or *PKD2* genes (Thongnoppakhun *et al*. 2004; Thongnoppakhun, 2012) (Figure 3). Furthermore, the new panels of STR markers were successfully applied for molecular diagnosis in ADPKD families requesting for decision of related-living kidney donor or PGD planning, for which informative haplotypes were obtained as exemplified in Figure 4.

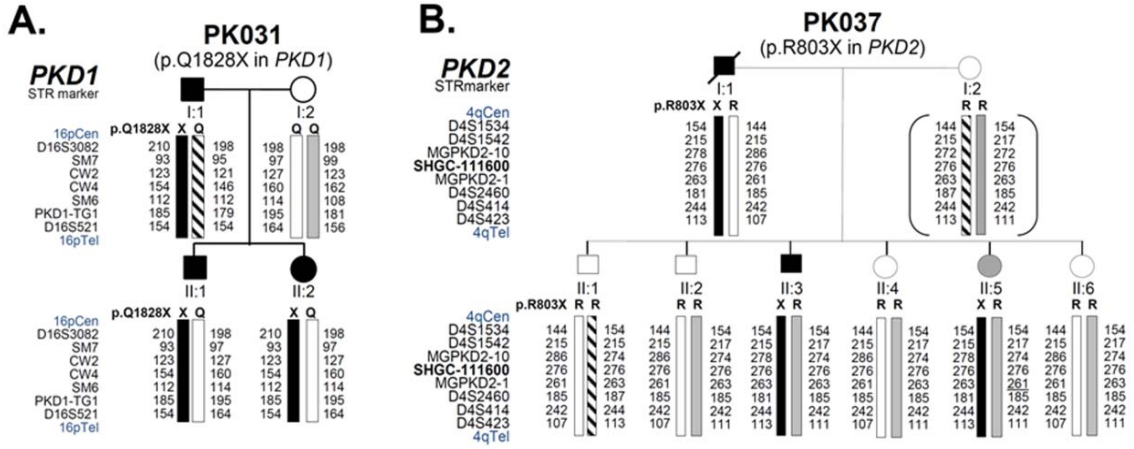

Figure 3 Validation of STR Set 'PKD1A' (7-Plex STR) and 'PKD2' (8-Plex STR) for linkage analysis of 2 representative ADPKD families known to be caused by a *PKD1* mutation, p.Q1828X (PK031, A) (Thongnoppakhun *et al*., 2004) and *PKD2* mutation, p.R803X (PK037, B) (Thongnoppakhun, 2012). Disease haplotypes (black bars) co-segregated with all of the affected individuals, indicating the *PKD1*- and *PKD2*-linked families, respectively, being completely concordant with the known causative mutations. Presymptomatic diagnosis of an individual II:5 in the PK037 family to have PKD2 was obtained from both direct and linkage analyses.

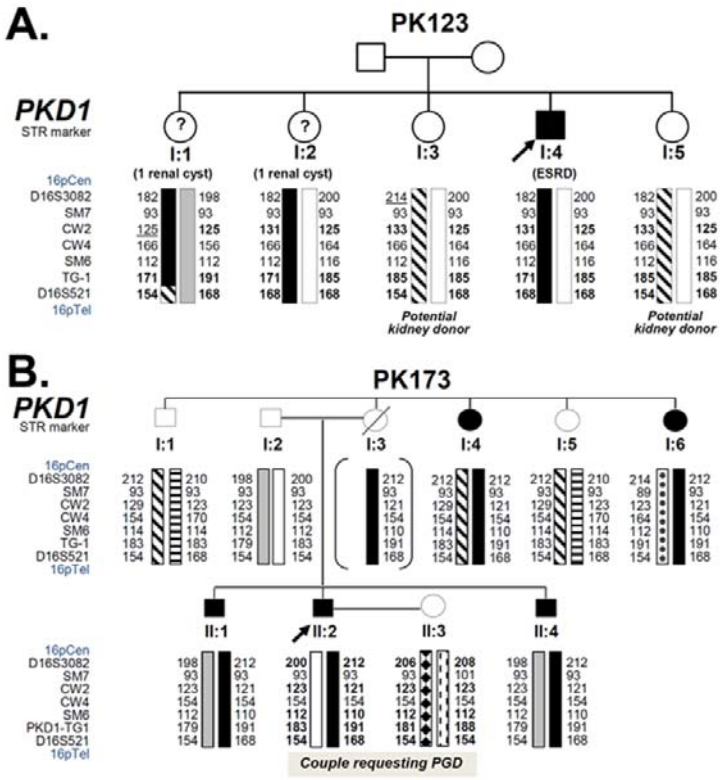

Figure 4 Application of the STR panel 'PKD1A' (7-Plex STR) for molecular diagnostics of ADPKD families with unknown mutation. Decision of related-living kidney donor for the patient I:4 (PK 123 family, A) can be successfully make to be two individuals, I:3 and I:5 as not having the disease haplotypes, even if in the case of a *de novo* mutation in the proband (since I:1 and I:2 were likely asymptomatic, having one renal cyst each). In the PK173 family (B), II:2 (PKD1 patient) and his wife (II:3) have planned to perform PGD. Linkage analysis can differentiate the affected haplotype from the other three normal ones by 5 STR markers used (as shown in bold texts), being useful for their future PGD. The STR Set 'PKD2' (8-Plex STR) was also tested in the two families and found to be unlinked to *PKD2* (data not shown).

Besides the informativeness of STR markers, the usefulness of STR-based linkage analysis for ADPKD depends on the distance of markers from the disease locus, the pedigree size and structure, sample number analyzed and diagnostic accuracy of phenotype. The method is useful in pedigrees with multiple affected members, as it allows finding rare variants that segregate through families. In probands with indeterminate mutations or VUS, linkage study could provide evidence for disease confirmation or exclusion. Typing of multiplex-STR panels in linkage analysis will also be helpful to monitor the allele dropout encountered in PGD. However, the linkage analysis should be used with cautions in cases of *de novo* mutations, mosaicism and other genetic complexity (Harris and Rossetti, 2010).

CONCLUSION

This study has developed highly informative, multiplex STR panels using MF-PCR and CE for straightforward, inexpensive and comprehensive molecular diagnosis for both PKD1 and PKD2 which could accurately infer the presence of mutation within a family and detect the possible recombination. To our knowledge, these

comprehensive panels have never been reported elsewhere. Their advantages were demonstrated in ADPKD families with unknown causative mutations who require either fast-track diagnostic/ pre-symptomatic testing, decision for kidney transplantation from the living-related donors or planning for PGD. It is also useful for clinicians to differential diagnose (whether PKD1, PKD2 or other related cystic diseases) to timely give a genetic counseling, appropriate management and prognosis to the patients' families, not only Thais but also Asians and other populations. Further improvement of this diagnostics can be achieved via extra sets of intragenic SNPs for each gene.

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