

Analysis of paternity testing results by Identifiler™ system in Thailand

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ABSTRACT

One of the applications of Identifiler™ system is to use for paternity testing. This study aims to analyze the paternity testing results of trio and duo cases. A total of 500 paternity cases were divided into 120 trio and 380 duo cases. Combined paternity index (CPI) and power of exclusion (PE) were calculated for inclusion and exclusion results. Based on statistical analysis, the lowest CPI value obtained from trio cases was 1,340.89 with a probability of paternity more than 99.92%. In duo cases, the lowest value of CPI was 32.18 with a probability of paternity higher than 96.98%. Moreover, we noticed that approximately 50% of the CPI values in trio and duo cases were in a range of 10^6 – 10^8 and 10^4 – 10^6 , respectively. For exclusion results, D2S1338 had the highest PE value in trio cases (81.20%). In duo cases, D8S1179 showed the highest PE value (75.80%). TPOX had the lowest PE value in both trio and duo cases (29.10% and 24.60%). The minimal numbers of excluding loci were 7 and 3 in trio and duo cases, respectively. In addition, this study showed that a threshold value for CPI should be greater than or equal to 1,000.

Keywords: paternity testing; autosomal STR markers; combined paternity index; power of exclusion; Thailand

INTRODUCTION

A commercial DNA typing kit, AmpF/STR® Identifiler® PCR Amplification is a short tandem repeat (STR) multiplex assay that simultaneously amplifies 15 tetranucleotide repeat loci in a single tube (Butler, 2012). This kit is composed of 13 DNA markers recommended by the Combined DNA Index System (CODIS), which are CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11 and 2 additional DNA markers (D2S1338 and D19S433) (Miller *et al.*, 2003; Butler, 2006). In addition, the Amelogenin is used as a control marker for gender determination (Butler, 2005). The application of this kit is for paternity testing to determine the biological father of a child based on the variability in STR regions, which can be used to distinguish one's DNA profile from another's (Jacewicz *et al.*, 2004; Babol-Pokora *et al.*, 2006; El-Alfy and El-Hafez, 2012). There are two possible results of paternity

testing. One is a negative paternity result (exclusion), a mismatch in 3 or more gene regions between the alleged father and the child which means the alleged father is not the biological father (Ayadiet *al.*, 2007). The other, a positive paternity result (inclusion), a complete matching of DNA profile between the alleged father and the child which means the alleged father has greater than a 99.999% chance of being the biological father (Jacewiczet *al.*, 2004; Tug and Akduman, 2009). For statistical analysis, the combined paternity index (CPI) and power of exclusion (PE) are routinely reported for inclusion paternity cases and exclusion paternity cases, respectively. In paternity testing, mutation of STR alleles is an important issue because a complete matching between a child and alleged father cannot be made when a mutation is present. Mainly, mutation of STR alleles occurs as a result of a gain or loss of a single repeat unit (Butler, 2005). A high mutation rate for STR marker could result in a false exclusion at the particular locus. It was previously reported that D21S11, FGA, D7S820, D16S539, and D18S51 have the highest mutation rates with the most polymorphic genotypes and possess the highest number of observed alleles, which is useful in human identity investigation (Butler, 2005). The purpose of this study is to analyze and compare the paternity testing results between trio cases and duo cases.

MATERIALS AND METHODS

Population

In this study, all 500 paternity testing results determined by AmpFISTR® Identifier® PCR amplification kit with 15 DNA markers (Life Technology, USA) during 2012–2013 were collected. The data were obtained from Human Genetics Laboratory, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. The

paternity testing results were divided into 2 major groups which were 120 trio cases and 380 duo cases. For trio cases, there are 100 inclusion paternity cases and 20 exclusion cases, while duo cases include 300 inclusion paternity cases and 80 exclusion cases.

Statistical analysis

For all 15 autosomal STR loci, the combined paternity index (CPI) was determined for inclusion paternity cases by Rapid DNA program based on John Buckleton's recommendation. The power of exclusion (PE) was determined for exclusion paternity cases, which was calculated by a modified version of PowerStats software package Version 1.2.

Paternity Index (PI)

Paternity Index (PI) is the relative probability that the alleged father and not an unrelated, randomly selected male of the same ethnic background transmitted the obligate allele to the child. This is a likelihood ratio and is presented in the following formula, $PI = X/Y$, where X is probability that the tested man is the father. Y is probability that a random man is the father (Gjertson *et al.*, 2007). Therefore, the combined paternity index (CPI) is calculated by multiplying the individual PIs for each STR locus, $CPI = PI_1 * PI_2 * \dots * PI_N$, (Stephenson, 2010). CPI is ratio that indicates the likelihood of the alleged father being the biological father in comparison to the likelihood of a random, unrelated man in the population being the father.

The power of exclusion (PE)

The power of exclusion (PE) is the parameter evaluating the loci or system efficiency in excluding a non-related individual in paternity investigation (Oliveira *et al.*, 2006). The PE has been commonly presented by the formula, $PE = h^2 (1-$

$2 \cdot h \cdot H^2$), where h is the proportion of heterozygous individuals and H the proportion of homozygous individuals in the population sample (Brenner, 1990; Oliveira *et al.*, 2006; SWGDAM, 2010).

RESULTS AND DISCUSSION

The comparison of CPI values between 100 trio and 300 duo cases which have inclusion paternity results was shown in Figure 1. The lowest value of CPI obtained from trio cases was 1,340.89 with a probability of paternity greater than 99.93%. In duo cases, the lowest value of CPI was 32.18 with a probability of paternity greater than 96.99%. In addition, the highest value of CPI from trio cases was 524,455,803,620.27 with a probability of paternity greater than 99.999999998%. In duo cases, the highest value of CPI was 35,433,401,625.84 with a probability of paternity greater than 99.999999997%. The minimal CPI value in both trio and duo cases

from this study were lower than the results of Babol-Pokora *et al.*, (2006). Their results showed that 50% of CPI value in duo cases were lower than 99.999% which were not sufficient to ascertain fatherhood status according to Polish Forensic Genetic Commission (Babol-Pokora *et al.*, 2006). According to the legal threshold for the presumption of paternity through genetic testing, a minimum CPI of 1,000 or a probability of paternity of 99.9% of men in the population is required. Therefore, a threshold value for CPI in this study should be greater than or equal to 1,000 (Pankeet *et al.*, 2001; Barbaro, 2012). Moreover, from our study we noticed that approximately 50% of the CPI values in trio and duo cases were in a range of 10^6 - 10^8 and 10^4 - 10^6 , respectively. Practically, when the statistic value does not meet the requirement, additional laboratory testing is needed.

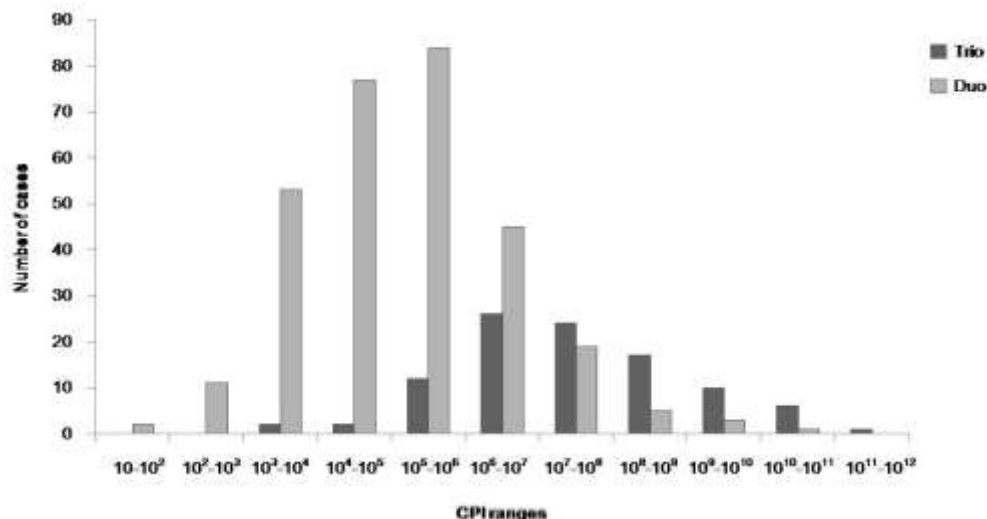


Figure 1 Comparison of combined paternity index (CPI) values of 100 trio and 300 duo cases with inclusion paternity results. The lowest value of CPI obtained from trio cases was 1,340.89 with a probability of paternity greater than 99.93%. In duo cases, the lowest value of CPI was 32.18 with a probability of paternity greater than 96.99%. In addition, the highest value of CPI from trio cases was 524,455,803,620.27 with a probability of paternity greater than 99.999999998%. In duo cases, the highest value of CPI was 35,433,401,625.84 with a probability of paternity greater than 99.999999997%.

Power of exclusion values for each STR locus were shown in Figure 2. In trio cases, D2S1338 had the highest PE value of 81.20%. In duo cases, D8S1179 had the highest PE value of 75.80%. On the other hand, D2S1338 was reported to have the highest PE value in both trio and duo cases in Central Poland population (Babol-Pokora *et al.*, 2006). For the lowest PE value, our results were consistent with the report from Babol-Pokora *et al.*, (2006), which showed that TPOX had the lowest PE value in both trio and duo cases at 29.10% and 24.60%, respectively. In Thai population, Chanpreechaya *et al.*, (2009) reported that CSF1PO and TPOX had the lowest PE values, whereas D13S317 and FGA had the highest PE values (Chanpreechaya and Taechowisan, 2009). In

addition, Shotivaranon *et al.* (2009) reported that FGA had the highest PE value at 73.60%, which was different from our study and their results showed TPOX with the lowest PE value of 25.70%, which was concordant with this study (Shotivaranon *et al.*, 2009). The discrepancy of these results may be due to the difference in sample size: n=500 (in this study), n=80 (Chanpreechaya *et al.*, 2009) and n=929 (Shotivaranon *et al.* 2009). Another study of PE value was reported from analysis of a population in the Middle - West region of Brazil, showing that the combination of 18 STR loci of 3 multiplex amplification systems gave the higher PE value and was efficient and enough for the analysis of paternity cases (Oliveira *et al.*, 2006).

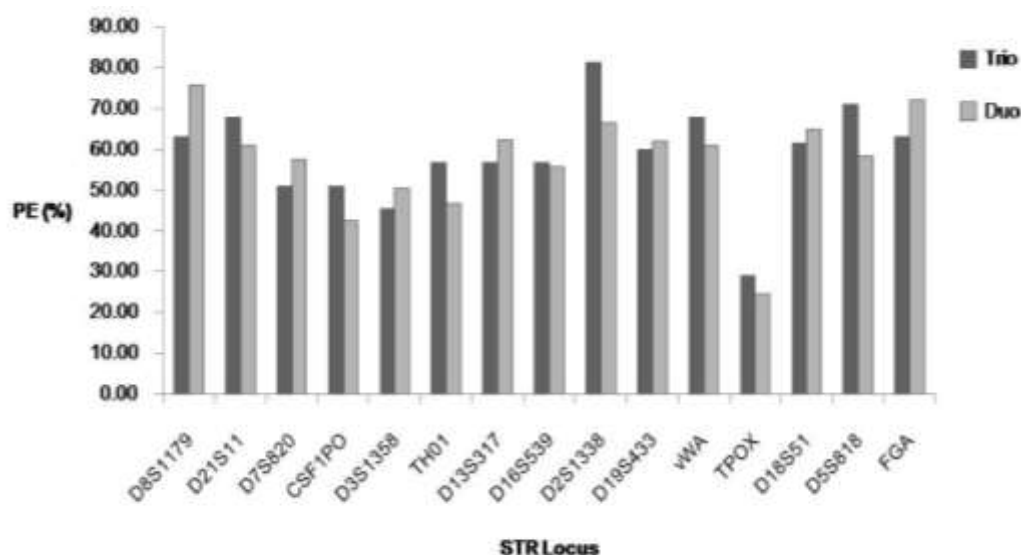


Figure 2 Power of Exclusion (PE) values for each DNA marker between 20 trio and 80 duo cases with exclusion paternity results. In trio cases, D2S1338 had the highest PE value (81.20%). In duo cases, D8S1179 had the highest PE value (75.80%). TPOX had the lowest PE value in both trio and duo cases, which are 29.10% and 24.60%, respectively.

The minimal numbers of excluding STR loci in trio and duo cases were 7 and 3, respectively, which was different from the data of Central Poland (Figure 3). There were 4 loci in trio cases and 1 or 2 of excluding loci in duo cases (Babol-Pokora *et al.*, 2006). The sample size in this study included only 80 exclusion duo cases and 20 exclusion trio cases but the report of Central Poland consisted of 150 exclusion cases in both duo and trio. In addition, the lowest number of excluding loci in Egypt population was 5 to 7 from 20 excluded paternity cases (El-Alfy and El-Hafez, 2012).

In summary, this study showed that D2S1338 and D8S1179 were the most effective genetic markers for determining individuals who are not real biological fathers in trio and duo cases, respectively. On the contrary, TPOX was the lowest powerful STR in both trio and duo cases. A threshold value for CPI should be greater than or equal to 1,000. Moreover, there was no mutation event detected and the lowest number of non-matching genetic markers in duo cases was 3 loci, which was enough for paternity conclusion. However, if one or two mutations occur, additional DNA tests are recommended to confirm the results.

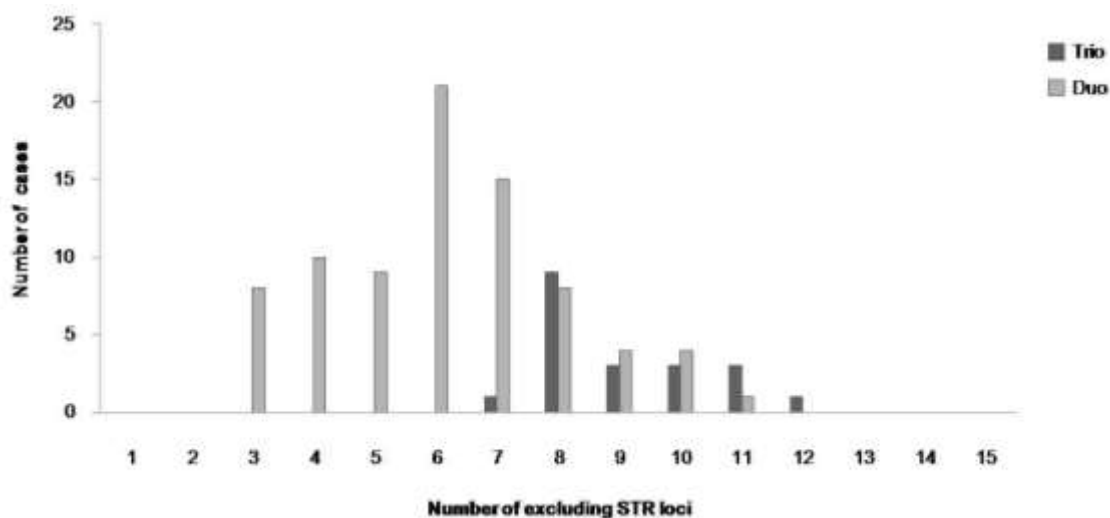


Figure 3 Comparison of the number of excluding STR loci between 20 trio and 80 duo cases with exclusion paternity results. The minimal numbers of excluding STR loci in trio and duo cases were 7 and 3, respectively.

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