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Statistic Value for Determination of Parents-child Relationship using Single Nucleotide Polymorphisms (SNPs) 44 Loci in Thai Individuals

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Abstract

The current report determined the the parent-child relationship based upon 44 SNPs markers by iPLEX® Pro Sample ID Panel using statistic values of paternity index (PI). The sample study had two groups: True parent-child (n= 38 families) and unrelated groups (n=38 families). The results showed that the true parent-child group shared 44 loci (38 families: 100%), and the unrelated groups had loci mismatch between 2 to 13 loci. The four mismatch loci was the highest number of families (10 families: 26.32%). The maximum and minimum of PI values were 1.0120×10^7 and 83.9371 were among the true parent-child groups. The unrelated group's maximum and minimum PI values were 5.0065×10^4 and 7.0750, respectively. The lower limit of the distribution of the true parent-child intersected with the upper limit of the distribution of the unrelated between the values of 83.9371 to 5.0065×10^4. In this range of values, the distribution of the PI values could not be used to determine the relationship and therefore was recognized as a "gray zone". However, if eliminated mismatch for calculating relatedness of PI, it made the gray zone not allow for clear identification. This research showed that the difference between true parent-child and unrelated groups using 44 SNPs loci typing could help predict human relationships and be applied in other relationships in the future.

Keywords: SNPs, Parents-child, Paternity index

Introduction

DNA fingerprint in forensic casework is a crucial part of the Criminal Justice Process due to its reliability and accuracy.Currently, the DNA fingerprint determined the familial relationship based on a likelihood ratio (LR) of short tandem repeat (STR) markers. LR is the ratio of the probability of DNA profiles under two alternative hypotheses and is useful for identifying two persons' relationships, especially a parent/child and sibling relationship.(1-3) However, previous studies have looked at another DNA fingerprint, including single nucleotide polymorphisms (SNPs).

SNPs have lower mutation rates and can be genotyped after PCR with shorter amplicons than STRs, which are thought to be potentially helpful markers in forensic DNA analysis(4). Børsting et. al. studied the performance of 52 SNPs developed

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for human identification on 124 parent-child trios, showing that the PI values of true parent-child groups were much higher than unrelated groups(5). Before using SNPs to calculate human relationships, SNPs studies were found for human identification tests. The result shows that the 52-plex SNPs set can be readily adapted to a range of other genotyping methods offering the possibility of high-throughput solutions(6). Kim et. al. studied 30 SNPs in a total 8,842 unrelated Korean populations. They found the probability of individual humans duplicating SNPs was 4.83×10^{-13}, and the probability of duplicating SNPs was reduced by 10 times for every added 5 SNPs(7). The current study reports pairwise kinship testing with a combination of STRs and SNPs loci found that the 40 STRs with 91 SNPs could also distinguish most full-sibling relationships from half-sibling but cannot distinguish relatives who belong to the same autosomal other kinship such as the first cousin(8).

However, the SNPs study is currently being applied to calculate the human relationship, including parent-child, full-sibling, half-sibling, and other relationships in many populations except the Thai population. Therefore, the present study analyzed the PI value from 44 SNPs loci of true parent-child in comparison with unrelated groups. The analysis of PI distribution was conducted using manual calculation with the formulae from Peter M. Lee(2,9).

Materials and Methods

Study protocols were approved by the Human Research Ethics Committee of Thammasat University (Science), Thailand in accordance with the compliance to the Declaration of Helsinki, the Belmont report, CIOMS guidelines and the international practice (ICH-GCP) (COA No. 066/2019). The buccal swab was collected from 38 families in Thai individuals. We rub inside the cheek bulge for 15s per each by a cotton swab. The cotton swabs were extracted with QIAamp DNA Mini Kit (Qiagen, USA) and identified 44 SNPs markers (Table 1) using iPLEX® Pro Sample ID Panel (Agena Bioscience, USA). SNPs genotyping was performed on a MassARRAY® System (Agena Bioscience, USA).

The data were divided into two groups. The first group was a true parent-child group 38 families, including father, mother, and child. The second group was an unrelated group 38 families that shuffled from true parent-child group consisting of father, mother, and children who are not related to each other.

The results were determined by the Paternity index (PI), which was calculated using manual calculation (with Microsoft excel software) with formulas published by Peter M. Lee in 1997(2,9)and used allele frequency of Asian from dbSNP on National Center for Biotechnology Information (NCBI) (Table 1).

Table 1 Asian's allele frequency of 44 SNPs makers list

SNPs maker	Chro.(10)	Allele frequency				Study
		A	T	C	G	
rs10495407	1	0.2390			0.7610	The PAGE Study (11)
rs1413212	1		0.4940	0.5060		The PAGE Study (12)
rs1982986	1		0.5350	0.4650		HapMap (13)
rs891700	1	0.5005			0.4995	The PAGE Study (14)
rs876724	2		0.4625	0.5375		The PAGE Study (15)
rs1357617	3	0.2200	0.7800			HapMap (16)
rs3819854	3		0.6060	0.3940		HapMap (17)
rs2046361	4	0.3300	0.6700			HapMap (18)
rs251934	5	0.8415			0.1585	The PAGE Study (19)
rs717302	5	0.8851			0.1149	The PAGE Study (20)
rs214054	6	0.5160			0.4840	HapMap (21)
rs727811	6		0.7289		0.2711	The PAGE Study (22)
rs2714854	7		0.5080	0.4920		HapMap (23)

SNPs maker	Chro.[10]	Allele frequency				Study
		A	T	C	G	
rs11781516	8		0.5739	0.4261		ExAC [24]
rs2056277	8		0.1430	0.8570		The PAGE Study [25]
rs1360288	9		0.3693	0.6307		The PAGE Study [26]
rs1463729	9		0.3922	0.6078		The PAGE Study [27]
rs826472	10		0.2349	0.7651		The PAGE Study [28]
rs964681	10		0.7079	0.2921		The PAGE Study [29]
rs2076848	11	0.5900	0.4100			HapMap [30]
rs901398	11		0.6730	0.3270		HapMap [31]
rs10771010	12		0.5097	0.4903		The PAGE Study [32]
rs1994997	12	0.6540			0.3460	HapMap [33]
rs1335873	13	0.6886	0.3114			The PAGE Study [34]
rs354439	13	0.5980	0.4020			HapMap [35]
rs9583190	13		0.5280	0.4720		HapMap [36]
rs1454361	14	0.5230	0.4770			The PAGE Study [37]
rs2518968	15			0.6220	0.3780	HapMap [38]
rs8037429	15		0.4984	0.5016		The PAGE Study [39]
rs1382387	16	0.6127		0.3873		The PAGE Study [40]
rs729172	16		0.1594		0.8406	The PAGE Study [41]
rs1468118	17		0.6520	0.3480		HapMap [42]
rs2010253	17		0.5750	0.4250		HapMap [43]
rs740910	17	0.9024			0.0976	The PAGE Study [44]
rs1024116	18		0.1006	0.8994		The PAGE Study [45]
rs1493232	18	0.3580		0.6420		The PAGE Study [46]
rs2247221	18		0.4840	0.5160		HapMap [47]
rs1005533	20	0.2929			0.7071	The PAGE Study [48]
rs13050660	21		0.5650	0.4350		HapMap [49]
rs2831700	21	0.5400			0.4600	The PAGE Study [50]
rs914165	21	0.3334			0.6666	The PAGE Study [51]
rs1028528	22	0.6510			0.3490	The PAGE Study [52]
rs136337	22	0.3944		0.6056		The PAGE Study [53]
rs2040411	22	0.3447			0.6553	The PAGE Study [54]

Results and Discussion

1. Parentage allele sharing

Thirty-eight true parent-child groups and thirty-eight unrelated groups were tested with the 44 SNPs multiplex typing method and determined parentage based on loci shared between the child and mother and father. Thus, the outcome of parentage testing is inclusion or exclusion. In addition, this study showed the result of allele shared. The result showed that the true parent-child group shared 44 loci (100%), and the unrelated groups had loci mismatch between 2 to 13 loci (Table 2). The four mismatch loci were the highest families (10 families: 26.32%).

Table 2: Summary of the results of 38 true parent-child groups and 38 unrelated groups with loci shared

Relationship	SNPs result					Total
	All inclusion	Single mismatch	Two mismatches	Three mismatches	>Three mismatches	
True parent-child group	38	0	0	0	0	38
Unrelated group	0	0	1	4	33	38

2. Paternity Index (PI)

PI values produced from two groups were summarized in Table 3. Among the true parent-child groups, the median PI was 6.9982×10^4 (log 4.8450) with maximum and minimum values of 1.0120×10^7 and 83.9371 (log 7.0052 and 1.9240), respectively. The unrelated group's PI was calculated with alleles shared without alleles mismatch. The median PI was 454.8812 (2.6579) with maximum and minimum values of 5.0065×10^4 and 7.0750 (log 4.6995 and 0.8497).

Table 3: Statistic value of true parent-child and unrelated groups

Relationship	N	Mean	SD	Min	Max	Median
True parent-child group	38	6.1286×10^5	1.7162×10^6	83.9371	1.0120×10^7	6.9982×10^4
Unrelated group	38	3.4292×10^3	8.9449×10^3	7.0750	5.0065×10^4	454.8812

The distributions of PI values in two groups were compared. In the true parent-child groups, it was found that the lower limit of the distribution of the true parent-child intersected with the upper limit of the distribution of the unrelated between the values of 83.9371 to 5.0065×10^4(log 1.9240 to 4.6995). In this range of values, the distribution of the LR values cannot be used to determine the relationship and therefore recognized as a "gray zone" (Figure 1)

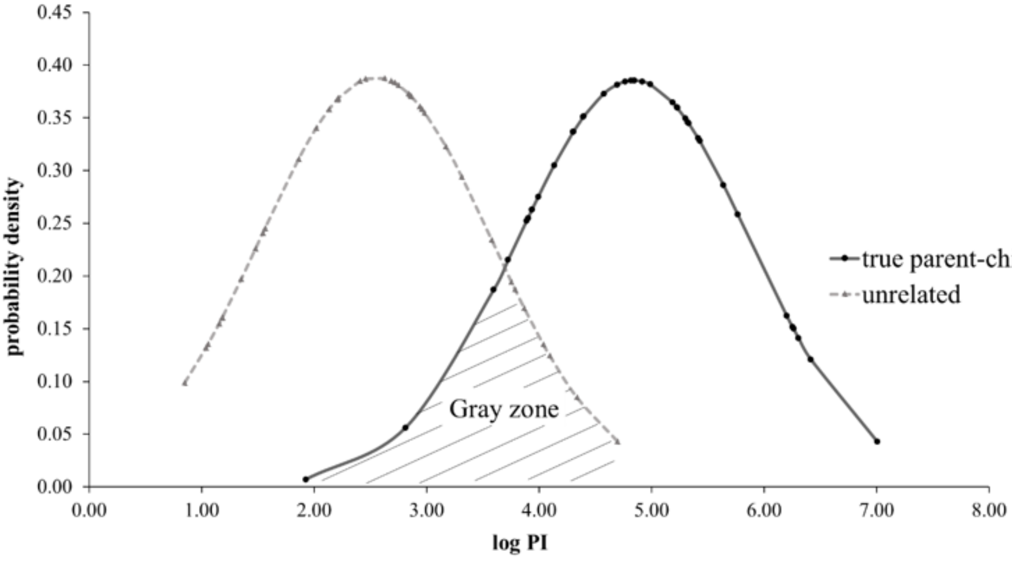

Figure 1: Distributions of PI values (expressed as log values) in the true parent-child groups and unrelated groups

The strength of PI thresholds in terms of supporting the prosecution's position was suggested by Butler JM in 4 positions (numbers of PI thresholds in parentheses); limited support (1 to 10), moderate support(10 to 100), strong support(100 to 1000), and very strong support(1000 and greater)(1). The true parent-child groups found 3 supports were moderate support, strong support and very strong support with 1 (2.63%), 1 (2.63%), and 36 (94.74%) families, respectively. The unrelated groups found 4 supports (families : percentage in parentheses) were limited support (1 : 2.63%), moderate support (10 : 26.32%), strong support (17 : 44.73%) and very strong support (10 : 26.32%).

The threshold for PI values of the true parent-child was 5.0065x10^4 or higher (log: 4.6995) and unrelated groups was 83.9371 or lower (log 1.9240). Cho et. al. compared the distribution of PI tested tested among 1209 true parent-child groups and 16 unrelated groups. The $\log_{10}$PI thresholds were analyzed from 50 in 136 SNPs loci. PI thresholds of true parent-child groups was among log 1.25 to 6.39, whereas unrelated groups log 0.63 to 3.57(55). This study found the overlaps between PI threshold from true parent-child and unrelated groups, similar to our current study.Moreover, this study showed the overlap of PI distribution between true parent-child and unrelated groups called "gray zone" similar to the previous study (6, 54-55). However, if eliminated mismatch for calculating relatedness of PI, it made the gray zone not allow for clear identification.

SNPs can be substituted for correlation calculations using STR because the correlation judgment model is similar to the STR analysis. The decision consists of all shared loci shared by parents and children. The relationship was calculated using the same principle of STRs analysis can differentiate between true parent-child and unrelated groups.To increase the reliability of the relationship prediction, additional STR, SNP and other genetic markers can be incorporated(56-58).

Conclusion

The scope of this research showed that the difference between true parent-child groups and unrelated groups using 44 SNPs multiplex typing could help predict human relationships. The results show the highlight of potential problems from eliminated mismatch for calculating the PI value. However, the accuracy of the interpretation should be concerned. Finally, the SNPs study is applied to calculate the human relationship, including full-sibling, half-sibling, and other relationships in the future.

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