Population Genetic Data on Loci LDLR, GYPA, HBGG, D_7S_8 and GC in the Bangkok Population Compared with Rural Thais from Trat Province

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Abstract

Prior to the introduction of any DNA marker as a tool for person identification and paternity test in certain ethnic groups, a population genetic database should be constructed. Using multiplex primers in single tube polymerase chain amplification, 5 loci of unrelated genes in the PM Amplitype® kit (Perkin Elmer) were studied in two Thai population groups : 228 DNA samples were extracted from blood collected at the Borai rural area in Trat province; another 123 DNA samples were collected at the outpatient clinic, Department of Forensic Medicine, King Chulalongkorn Memorial Hospital, Bangkok. Analysis of alleles and genotypes was performed after reversed dot blot hybridization of PCR products to allelic sequence specific probes immobilized on the membrane strip followed by nonradioisotopic detection according to the manufacturer's protocol. Population genetic statistic parameters including discrimination power (DP), the probability of matching (PM), power of exclusion for trio (PE trio) and typical paternity index (PI typical) were computed. Both Thai population groups showed no significant deviation from the Hardy Weinberg Expectation (HWE). The combined DP of all 5 loci in the PM Amplitype® markers was 0.993636 for rural Thais and 0.994409 for Thais from Bangkok. The combined PM for rural Thais and those living in Bangkok was 0.006364 and 0.005591, respectively. The combined PE trio was 0.696825 and 0.698875 in both Thai population groups and the combined PI typical values were <1.0.

In conclusion, person identification using PM Amplitype DNA markers was efficient and satisfactory within certain limits. Hence, the application of PM Amplitype® DNA markers for paternity tests should be cautiously considered and applied in combination with other parameters.

Key word : PM Markers, Population Genetic Database, DNA Markers

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Single and multiple loci probes recognizing highly polymorphic DNA sequences throughout the genome have become powerful tools for both person identification in forensic casework and paternity testing[1-3]. However, DNA probe technology requires substantial amounts of DNA for which the extraction procedures are rather tedious. The polymerase chain reaction (PCR) *in vitro* amplifies specific loci and subsequent detection of polymorphic alleles contained within the PCR product offers a relatively simple analytical technique. Several genetic loci amenable to PCR can now be analyzed using commercially available kits. These loci are: low density lipoprotein receptor, LDLR[4]; glycophorin A, GYPA[5]; hemoglobin G gamma-globin, HBGG[6]; D_7S_8[7] and Group specific component, GC[8] which were all included in the Amplitype® PM PCR Amplification and Typing kit from Perkin Elmer Corporation. The kit includes amplification reagents that allow the simultaneous amplification of the five genetic loci mentioned together with one controlled locus (HLA DQα). The genotype for each locus is determined from the pattern of blue dots on the strip after reverse dot blot hybridization followed by biotinylated enzyme linked color detection.

It is now known that evidence of DNA has a higher discrimination power compared with conventional genetic markers. But prior to the introduction of a new test for person identification in forensic casework or paternity testing, population data of that particular marker including allele frequencies should be established[9,10]. In this paper, we compared population genetic data of PM markers in partially related Thai families living in Bangkok to those of unrelated subjects living at Trat Province which is located near the Thai-Cambodian border and calculated the population genetic indices.

MATERIAL AND METHOD

Blood samples

Unrelated blood samples: Heparinized blood of 228 unrelated Thais was collected from the Borai rural area at Trat province and kept at -20°C since 1992. These samples were taken from people attending the Borai Malaria Clinic for malarial parasite checking.

Bangkok blood samples: The 123 heparinized blood samples were collected from people attending the outpatient clinic of the Department of Forensic Medicine at Chulalongkorn Hospital, Thai Red Cross Society from 1996 to 1997. These samples were taken for blood group and paternity testing.

DNA extraction and amplification

The unrelated blood samples were thawed, then 200 µl each were washed twice with phosphate-buffered saline (PBS). The pellet was resuspended in lysis buffer and digested with Proteinase K (Boringer Manheim) at 55°C for 2 hours or more[11]. Aliquots of 4 µl of the extract were used for amplification.

Blood samples of 50 µl from the Bangkok group were used for DNA extraction using Chelex - 100[12] and 8 µl of the DNA extract were used for amplification.

The Amplitype® PM amplification kit was used. Samples were amplified in a Perkin Elmer DNA Thermal Cycler 480 using parameters recommended by the manufacturer[13]. Verification of amplification at all six loci was determined by electrophoresis of the product on a 2 per cent agarose gel (Nusieve) in 1x TBE[14] buffer containing ethidium bromide at 80 V for 25-30 min.

DNA typing

The types of the five loci in the PM kit were determined according to the manufacturer's recommendations[13,15]. The PCR product was heat denatured at 95°C for 9 min. This step should not exceed 10 min otherwise the signal might be diminished. After hybridization and stringent washing, the strips should be rinsed once with 1x Citrate buffer before adding 5 ml 1x Citrate buffer (0.1 M Sodium citrate, pH 5.0) and shaking at room temperature for 5 min. Omitting this step will cause low signal intensity after colour development.

Statistical analysis

The frequency of each allele for each locus was calculated separately from the number of each genotype in both the rural Trat group and the Bangkok group. Possible divergence from the Hardy-Weinberg expectations (HWE) was determined for both groups by a standard χ^2 analysis of the observed and expected genotypes.

The unbiased estimate of the expected heterozygosity was computed from the formula:[16]

$$h = n(1-\Sigma x^2) / (n-1)$$

Where x = allele frequencies and n = total number of alleles observed.

In addition, each locus of both groups was calculated for statistical values of: the polymorphism information content, PIC[17,18]; discrimination power, DP[19,20]; probability of matching, PM[21]; power of exclusion[21], PE trio (alleged father, mother and child) and PE duo (single parent/child) from the formula

PE trio $= \sum Pi\,(1 - Pi)^2 + \sum (PiPj)^2\,(3Pi + 3\,Pj\text{-}4)$

PE duo $= \sum Pi^2\,(1 - Pi)^2 + \sum 2PiPj\,(1 - Pi - Pj)^2$

Where Pi = most common allele, Pj = next most common allele; probability of inclusion[21], Pi trio and Pi duo; and typical paternity index, PI typical [22]. The combined statistical values calculated for all five loci of PM marker® were DP, PM, PE trio, PE duo, PI typical, Pi trio and Pi duo.

RESULTS

At the LDLR locus, only 166 out of 228 samples from Trat province could be clearly typed. The negative result at this locus might be due to the degradation of the DNA template since the blood samples had been kept at -20°C since 1992. The allele and genotype frequencies found among rural Thais at Trat province are shown in Table 1. Comparison of allele frequencies and genotype frequencies of all five loci in the Bangkok samples (Table 2) to rural Thais yielded similar results. It should be noticed that there was no C allele found at the HBGG locus in both groups (Table 1 and 2).

A standard χ^2 analysis of the observed and expected genotypes showed no significant deviation ($P>0.05$) from the Hardy-Weinberg equilibrium (Table 1 and 2) in both Thai groups for all the loci studied. Table 3 presents the statistical parameters necessary for a population genetic database. The combined discrimination power (DP) of all 5 loci in the PM® marker kit was 0.9936 for rural Thais and 0.9944 for Bangkok Thais. The combined probability of matching (PM) which signified the chance that two unrelated individuals might match

Table 1. Allele frequencies and genotype frequencies of PM marker in rural Trat group. (n = 228).

Alleles	LDLR[a]	GYPA[b]	HBGG[c]	D_7S_8[d]	GC[e]
A	0.286	0.759	0.351	0.612	0.217
B	0.714	0.241	0.649	0.388	0.417
C	NA*	NA	0.000	NA	0.366
Genotypes					
AA	0.096	0.548	0.088	0.377	0.031
AB	0.380	0.421	0.526	0.474	0.236
BB	0.524	0.031	0.386	0.149	0.158
AC	NA	NA	0.000	NA	0.136
BC	NA	NA	0.000	NA	0.281
CC	NA	NA	0.000	NA	0.158

* = No allele C in the Amplitype® PM PCR Amplification and Typing Kit (Perkin – Elmer Corporation)

a = LDLR, n = 166, Observed Heterozygosity = 0.380; Expected Heterozygosity (unbiased) = 0.41; HWE test by comparing X^2 between observed and expected genotype not significantly different, $P > 0.05$

b = GYPA, Observed Heterozygosity = o.421, Expected Heterozygosity (unbiased) = 0.367; HWE test not significant, $P > 0.05$

c = HBGG, Observed Heterozygosity = 0.526, Expected Heterozygosity (unbiased) = 0.457; HWE test not significant, $P > 0.05$

d = D_7S_8, Observed Heterozygosity = 0.474, Expected Heterozygosity (unbiased) = 0.476; HWE test not significant, $P > 0.05$

e = GC, Observed Heterozygosity = 0.654, Expected Heterozygosity (unbiased) = 0.647; HWE test not significant, $P > 0.05$

Table 2. Allele frequencies and genotype frequencies of PM markers in Bangkok Thai group (n = 123).

Alleles	LDLR[a]	GYPA[b]	HBGG[c]	D_7S_8[d]	GC[e]
A	0.313	0.667	0.366	0.545	0.171
B	0.687	0.333	0.634	0.455	0.362
C	NA*	NA	0.000	NA	0.467
Genotypes					
AA	0.130	0.447	0.106	0.260	0.049
AB	0.358	0.439	0.528	0.553	0.122
BB	0.512	0.114	0.366	0.187	0.146
AC	NA	NA	0.000	NA	0.122
BC	NA	NA	0.000	NA	0.309
CC	NA	NA	0.000	NA	0.252

* = No allele C in the Amplitype® PM PCR Amplification and Typing Kit (Perkin – Elmer Corporation)

a = LDLR, Observed Heterozygosity = 0.358; Expected Heterozygosity (unbiased) = 0.432; HWE test by comparing X^2 between observed and expected genotype not significantly different, $P > 0.05$

b = GYPA, Observed Heterozygosity = 0.439, Expected Heterozygosity (unbiased) = 0.446; HWE test not significant, $P > 0.05$

c = HBGG, Observed Heterozygosity = 0.528, Expected Heterozygosity (unbiased) = 0.466; HWE test not significant, $P > 0.05$

d = D_7S_8, Observed Heterozygosity = 0.553, Expected Heterozygosity (unbiased) = 0.498; HWE test not significant, $P > 0.05$

e = GC, Observed Heterozygosity = 0.553, Expected Heterozygosity (unbiased) = 0.624; HWE test not significant, $P > 0.05$

at all 5 loci was 0.006364 and 0.005591 in rural and Bangkok Thais, respectively. The statistical parameters for paternity testing were computed in both Thai groups as shown in Table 3. The combined power of exclusion for trio (alleged father, mother and child) of 5 loci in the PM kit was 0.696825 for rural Thais and 0.698875 for the Bangkok Thais group. The combined PI typical of these 5 loci in both groups was < 1.0.

DISCUSSION

In vitro amplification of DNA using multiplex primers from the Amplitype® PM kit yielded 6 bands of PCR products on ethidium bromide stained agarose gel electrophoresis. The band sizes were 138 bp, 151 bp, 172 bp, 190 bp, 214 bp and 239-242 bp[13].

In both Thai population groups, the total number of 351 DNA samples did not test positive for the C allele of the HBGG locus. Neither did the control DNA from Amplitype® kit yield the C allele on the HBGG locus. The database from different American ethnic groups[23] showed the C allele frequencies of the HBGG to be very low in Caucasian, South Eastern Hispanic and South Western Hispanic Americans (Table 4). The African American group exhibited 0.297 and 0.317, 0.097, 0.090 for C allele frequencies and AC, BC, CC genotype frequencies, respectively, at the HBGG locus. The Swiss population database[24] gave no C allele and no genotype frequencies associated with the C allele at this locus. This implies that the C allele of the HBGG locus is a rare allele with very low distribution frequencies in the Thai population.

The statistic parameters taken into consideration when searching for a marker suitable for person identification were the power of discrimination (DP) and the probability of matching (PM). The

Table 3. Statistical data related to person identification and paternity testing of PM® marker in both rural Thais and Bangkok Thais.

Statistical Parameters / Locus	RURAL THAI GROUP (n = 228)							
	PIC	DP	Pm	PE_{TRIO}	PE_{DUO}	Pi_{TRIO}	Pi_{DUO}	$PI_{TYPICAL}$
- LDLR*	0.325	0.5667	0.4333	0.1625	0.0834	0.8375	0.9166	0.847
- GYPA	0.299	0.5311	0.4689	0.1495	0.067	0.8505	0.933	0.79
- HBGG	0.352	0.5998	0.4002	0.1759	0.1037	0.8241	0.8963	0.92
- D_7S_8	0.362	0.6116	0.3884	0.1811	0.1128	0.8189	0.8872	0.954
- GC	0.586	0.7985	0.2015	0.3693	0.1838	0.6307	0.8162	1.415
COMBINED 5 LOCI	-	0.993636	0.006364	0.696825	0.444949	0.3031745	0.55505	0.83100
Statistical Parameters / Locus	BANGKOK THAIS (n = 123)							
- LDLR	0.338	0.5827	0.4173	0.1688	0.0925	0.8312	0.9075	0.88
- GYPA	0.346	0.5926	0.4074	0.1728	0.0988	0.8272	0.9012	0.903
- HBGG	0.356	0.6051	0.3949	0.1782	0.1077	0.8218	0.8923	0.936
- D_7S_8	0.373	0.623	0.377	0.1865	0.123	0.8135	0.877	0.996
- GC	0.557	0.7791	0.2209	0.3449	0.1722	0.6551	0.8278	1.33
COMBINED 5 LOCI	-	0.9944	0.005591	0.698875	0.47021	0.301125	0.529789	0.98527

*n = 166

higher value of DP or a figure approaching 1.000000 and the lowest PM are desirable. The DP for the PM marker of 0.993636 and 0.9944 in both rural Thais and Bangkok Thais indicated that, by using this PM marker, you can differentiate definitely 9936 to 9944 men from 10,000. The remaining 64 to 56 men needed additional markers to discriminate each individual from the others. Although the efficiency of PM marker in person identification is quite good, the paternity index and probability of exclusion, PE trio are not high enough. Our study in some families has shown that each individual member of the family can be identified and discriminated from another, but two alleged men could not be sufficiently discriminated with regards to the possibility of being the true father.

The data of population genetic statistics for 5 loci of the PM Amplitype® kit of rural Thais living near the Thai Cambodian border and the Bangkok Thais yielded almost the same values. There were no significant deviations from the Hardy-Weinberg equilibrium for all 5 loci in both groups. This indicated that person identification within a Thai population could be achieved to a certain level using the PM Amplitype® markers.

SUMMARY

A database of 228 rural Thais and 123 Bangkok Thais was established for all 5 loci of the PM Amplitype® marker, Perkin Elmer. Population genetic statistical parameters important for assessing the efficiency of these markers in terms of person identification and paternity testing were analysed and compared between these two Thai population groups. Deviation from Hardy-Weinberg was also computed.

Table 4. Comparison of allele frequencies and genotype frequencies of PM® amplitype loci in different American ethic group[23], Swiss[24] and Thai.

Allele		AFRICAN AMERICAN N = 145	CAUCASIAN AMERICAN N = 148	SOUTH EASTERN HISPANIC N = 94	SOUTH WESTERN HISPANIC N = 96	SWISS N = 100	RURAL THAI N = 228	BANGKOK THAI N = 123
[a]LDLR	A	0.224	0.453	0.415	0.562	0.435	0.350	0.313
	B	0.776	0.547	0.585	0.438	0.565	0.650	0.687
GYPA	A	0.479	0.584	0.532	0.656	0.525	0.760	0.667
	B	0.521	0.416	0.468	0.344	0.475	0.240	0.333
HBGG	A	0.507	0.470	0.426	0.344	0.475	0.351	0.366
	B	0.197	0.524	0.548	0.609	0.525	0.649	0.634
	C	0.297	0.007	0.027	0.047	0.000	0.000	0.000
D_7S_8	A	0.614	0.615	0.585	0.682	0.585	0.612	0.545
	B	0.386	0.385	0.415	0.318	0.415	0.388	0.455
GC	A	0.103	0.257	0.277	0.271	0.280	0.217	0.171
	B	0.707	0.172	0.223	0.208	0.175	0.417	0.362
	C	0.190	0.517	0.500	0.521	0.545	0.366	0.467
Genotype		AFRICAN AMERICAN N = 145	CAUCASIAN AMERICAN N = 148	SOUTH EASTERN HISPANIC N = 94	SOUTH WESTERN HISPANIC N = 96	SWISS N = 100	RURAL THAI N = 228	BANGKOK THAI N = 123
[a]LDLR	AA	0.048	0.176	0.191	0.313	0.210	0.188	0.130
	AB	0.352	0.554	0.447	0.500	0.450	0.324	0.358
	BB	0.600	0.270	0.362	0.188	0.340	0.488	0.512
GYPA	AA	0.228	0.351	0.330	0.448	0.240	0.548	0.448
	AB	0.503	0.466	0.404	0.417	0.570	0.422	0.440
	BB	0.269	0.182	0.266	0.135	0.190	0.030	0.112
HBGG	AA	0.262	0.223	0.160	0.135	0.240	0.088	0.106
	AB	0.172	0.493	0.521	0.365	0.470	0.526	0.528
	BB	0.062	0.277	0.266	0.406	0.290	0.386	0.366
	AC	0.317	0.000	0.011	0.052	0.000	0.000	0.000
	BC	0.097	0.000	0.043	0.042	0.000	0.000	0.000
	CC	0.090	0.007	0.000	0.000	0.000	0.000	0.000
D_7S_8	AA	0.338	0.358	0.340	0.458	0.330	0.376	0.260
	AB	0.552	0.514	0.489	0.448	0.510	0.474	0.552
	BB	0.110	0.128	0.170	0.094	0.160	0.150	0.188
GC	AA	0.014	0.054	0.053	0.083	0.070	0.030	0.048
	AB	0.131	0.074	0.181	0.083	0.130	0.236	0.123
	BB	0.517	0.047	0.043	0.063	0.000	0.159	0.146
	AC	0.048	0.331	0.266	0.292	0.290	0.136	0.123
	BC	0.248	0.176	0.181	0.208	0.220	0.280	0.308
	CC	0.041	0.318	0.277	0.271	0.290	0.159	0.252

a = N = 166 FOR RURAL THAI GROUP

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ข้อมูลพันธุศาสตร์ประชากรของตำแหน่ง LDLR, GYPA, HBGG, D_7S_8 และ GC ในคนกรุงเทพฯ เทียบกับคนชนบทที่จังหวัดตราด†

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วิไล อโนมะศิริ, ปร.ด., อัญชลี ก่องศรีสุข, วท.ม.*, ธนศักดิ์ สืบหลินวงศ์, พ.บ.****

ก่อนที่จะนำวิธีวิเคราะห์ ดี เอน เอ ใดมาใช้เพื่อพิสูจน์บุคคลและพิสูจน์ความเป็นพ่อในประชากรใด จะต้องทำการศึกษาข้อมูลพันธุศาสตร์ประชากรในกลุ่มชนนั้น ได้นำการตรวจยีน 5 ตำแหน่งโดยใช้ชุด PM Amplitype® (Perkin Elmer) มาวิเคราะห์ ดี เอน เอ 228 ตัวอย่าง จากคนไทยชนบทที่อำเภอบ่อไร่ จังหวัดตราด และ 123 ตัวอย่างจากผู้ป่วยนอกของฝ่ายนิติเวชศาสตร์ โรงพยาบาลจุฬาลงกรณ์ กรุงเทพ ฯ วิเคราะห์ชนิดอัลลีย์และจีโนไทพ์ของตัวอย่างเหล่านี้หลังจากนำ PCR product ไปทำปฏิกิริยาคู่สม (hybridization) กับดีเอนเอตรวจสอบจำเพาะตามวิธีของผู้ผลิตชุดน้ำยา คำนวณค่าสถิติพันธุศาสตร์ประชากร เช่น DP (Discrimination power), Pm (Probability of matching), PE trio (Probability of exclusion, trio), PI typical (Paternity index, typical) อีกทั้งความเบี่ยงเบนจาก Hardy–Weinberg (HWE) จากการศึกษาไม่พบความเบี่ยงเบนจาก HWE ในประชากรไทยทั้งสองกลุ่ม ($P > 0.05$) ค่า DP combined เท่ากับ 0.993636 ในกลุ่มคนไทยชนบท และ 0.9944 ในคนกรุงเทพ ฯ ค่า Pm combined สำหรับคนไทยชนบทและคนกรุงเทพ ฯ เป็น 0.006364 และ 0.005591 ตามลำดับ ส่วนค่า combined PE trio ในทั้งสองกลุ่มเป็น 0.696825 และ 0.698875 และ combined PI typical น้อยกว่า 1

สรุปการพิสูจน์บุคคลโดยตรวจวิเคราะห์ ดี เอน เอ 5 ตำแหน่งของชุด PM Amplitype® DNA markers ได้ผลเป็นที่พอใจในระดับหนึ่ง ส่วนการที่จะนำวิธีดังกล่าวมาใช้พิสูจน์ พ่อลูก ควรต้องพิจารณาอย่างระมัดระวังและควรใช้ร่วมกับการตรวจอื่น

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