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**CASE REPORT**

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# Identification of DYS385 Allele Variants by Using Shorter Amplicons and Northern Thai Haplotype Data

**ABSTRACT:** Primers currently used for amplification of locus DYS385 on the Y chromosome give quite long amplicons that are difficult to resolve on native polyacrylamide gels. We therefore designed new primers that give much shorter products for easy separation. With this system, two allele variants that were characterized by sequencing as 14.1 (GAAA → GAAAA) and 14.2 (GA insertion), were identified in two unrelated Northern Thai men due to their decreased mobility. Interestingly, initial capillary electrophoresis mistyped the 14.1 variant amplified with the traditional primers as allele 14. Forty different haplotypes were found in 147 Northern Thais with a haplotype diversity of 0.9430. Haplotype 13–18 (frequency 0.136) is the most frequent one; for comparison; haplotype 14–18 (frequency 0.074) was found to be the most common in a population from Bangkok ($n = 95$) in an earlier study. This striking difference within Thailand's population probably results from genetic differences in the founding populations.

**KEYWORDS:** forensic science, DNA typing, population genetics, Y-chromosome, DYS385, short tandem repeat, polymerase chain reaction, polyacrylamide gel electrophoresis, capillary electrophoresis, Thailand

Short tandem repeats (STRs) on the Y-chromosome proved helpful in father-son paternity testing and forensic case works, e.g., sexual offense (1). These Y-STRs however, vary substantially in their degree of polymorphism, naturally the most interesting ones are those with a highly variable number of repeat units. The bilocal marker DYS385 (repeat sequence is GAAA) belongs to this group and it has been investigated in many different populations. Two alleles of variable size obtained by the polymerase chain reaction (PCR) using a single primer pair are treated as one single haplotype. Published primers (2,3) give quite long amplicons, up to 404 bp (allele 20), and their separation by native polyacrylamide gel electrophoresis (PAGE) is difficult. In this paper, we describe new primers which give much shorter amplicons that are easily separated by PAGE. With this system we could identify two DYS385 allele variants and collect population data from the Northern Thais.

## Materials and Methods

### *Materials and Methods Used in Chiang Mai, Thailand*

DNA was extracted from blood of 149 unrelated Northern Thai men in the Chiang Mai province by the Chelex method (4). For amplification, 1.5 μL DNA extract was used in manual hot start PCR, total volume 15 μL, standard buffer conditions: 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mg/mL bovine serum albumin, 0.05% Tween-20, 100 μM of each dNTP. The primer concentration was 0.25 μM. Sequence of the newly designed primers, forward primer: 5′ GAG AAA GAG GAA AGA GAA AGA AAG G 3′; reverse primer: 5′ ATC TAT TCC AAT TAC ATA GTC CTC C 3′. PCR conditions: initial denaturation 2:30 min at 94°C, hold at 85°C for addition of primers, then 30 cycles of 45 s 94°C, 30 s 55°C, 30 s 72°C. Five μL of the PCR products were run on native polyacrylamide gels, gel dimensions: 16 cm wide, 20 cm long, 1 mm thick; polyacrylamide concentration: %T = 8.5, %C = 4.8; gels were silver stained and dried for record. An allelic ladder was constructed from cut out and eluted known reference allele bands, the alleles were reamplified and combined to give a ladder consisting of allele 10–20. Haplotype diversity was estimated according to Perez-Lezaun et al. (5) as $D = 1 - [(N/N - 1)(\Sigma f_i^2)]$, where $N$ is sample size and $f_i$ is allele frequency. Allele variants were sequenced using the ABI Prism dRhodamine terminator cycle sequencing ready reaction kit according to the instructions of the manufacturer. The forward primer F2 (see Fig. 1) was used as sequencing primer. Sequencing was repeated to confirm the previous sequencing result.

### *Methods Used in Muenster, Germany*

PCR amplification with the traditional DYS385 primers (3) using a GeneAmp PCR System 9600 thermal cycler was carried out in a final volume of 25 μL containing 1 μL of Chelex extract, 1.5 mM of $MgCl_2$, 0.4 mg/mL of BSA, 200 μM of each dNTP, 0.25 μM of each primer (forward primer labeled with 6-FAM), and 2.5

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agcatgggtg acagagctag acaccatgcc aaacaacaac aaagaaaaga aatgaaattc

agaaaggaag gaaggaagga gaaagaaagt aaaaaagaaa gaaagagaaa aagagaaaaa

                                  F2
gaaagaaaga gaagaaaGAG AAAGAGGAAA GAGAAAGAAA GGaaggaagg aaggaaggaa

gg (10 repeats: GAAA GAAA GAAA GAAA GAAA GAAA GAAA GAAA GAAA GAAA)

                          R1;  R2
gagaaaaa    gaaaGGAGGA CTATGTAATT GGAATAGATa gattattttt taaaatattt

ttattacctt tacagttttt ttaaatgccg ccatttcaga aagaaatctg gtcagcagcc

                  R3; R4
cttaccagct ttacctagca tccct
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FIG. 1—*Sequence of the DYS385 locus with the positions of primers. Sequences in italics: positions of forward primer F1 and reverse primer R4 according to Kayser et al. (3); boxed sequences: positions of forward primer F1, reverse primer R1 and reverse primer R3 described by Schneider et al. (2); sequences in bold capitals: positions of forward primer F2 and reverse primer R2 newly designed in this study. The primers flank 10 GAAA repeat units. Note: Kayser et al. and Schneider et al. use an identical forward primer F1; the reverse primer R3 used by Schneider et al. is shorter by a single A (very last T of the shown sequence) than reverse primer R4 used by Kayser et al.; reverse primer R3 was not used in this study.*

units of AmpliTaq Gold DNA polymerase (ABI). The cycling protocol was: initial denaturation for 10 min at 94°C followed by 31 cycles of 1 min 94°C, 1 min 60°C, 1 min 72°C and a final elongation at 72°C for 30 min. PCRs were analyzed using denaturing capillary gel electrophoresis (CE) with laser-induced fluorescence detection on ABI PRISM 310 Genetic Analyzer using the GeneScan 2.1 software for determination of fragment lengths. Alleles were typed against a ladder consisting of the sequenced alleles 8, 10–18, 20.

## Results and Discussion

At the beginning of the investigation of the DYS385 locus in the Chiang Mai lab, we used previously published primers (2). Large sized amplicons 264–300 bp (allele 11–20) were obtained as expected; separation by PAGE either required an increase in voltage or much longer run times for satisfactory resolution of bands, which interfered with the electrophoretic analysis of all other small sized STR systems in our laboratory. For these reasons, we designed a new primer pair that gives shorter products (allele 11:126 bp; allele 20:162 bp) compatible with the electrophoresis of the other STR systems (Fig. 1). We were also aware of the fact that degraded DNA from forensic casework can be more successfully amplified as short PCR products (6).

Twenty Northern Thai men were typed in Chiang Mai, Thailand, using this new primer pair and PAGE; clearly separated allele bands were obtained. The same men were typed in Muenster, Germany, using the primers described by Kayser et al. (3) and CE. Both experiments resulted in identical allele typing for 19 men. Another man was typed as haplotype 13–14 by CE. But when the alleles of that particular man were amplified in Chiang Mai with the new primers and analyzed by PAGE, we noticed that the allele "14" band was located a little bit above the normal allele 14 position. It appeared that we found an allele 14 variant (later identified as allele 14.1) that had a somewhat decreased mobility in polyacrylamide gels under non-denaturing conditions (Fig. 2*a*). We eluted the variant allele from a polyacrylamide gel and sequenced it. An otherwise normal sequence was found except that the third GAAA repeat had changed to a GAAAA by insertion of an A. Since DNA sequencing and initial CE gave conflicting results CE was repeated with newly extracted and amplified DNA using the Kayser primers (3) and our newly designed primers. This time the result confirmed the size of the allele variant as being a 14.1 allele (Fig. 2*b*).

Later, we detected a second allele length variant by PAGE that had a mobility similar to allele 14.1. DNA sequencing showed that it was a 14.2 allele with an insertion of GA after repeat 9 and CE confirmed this result. This allele variant probably is the result of an AA deletion in a former repeat ten. Fueredi et al. (8) detected a similar 17.2 allele variant by CE in a Hungarian population. Both allele variants could be specific markers in the respective Thai male lineages, on the other hand, it cannot be excluded that they resulted from *de novo* mutations. To clear that point, relevant male members of those lineages have to be tested.

In an extended concordance study with samples from several worldwide populations, we have typed in Muenster by means of CE and the new primers more than 100 men that had already been typed with the conventional primers (3). No discrepancy could be observed except for one case: the haplotype 14–18 appeared as 14–17.3 when using the conventional primers. Sequencing on both strands revealed that in the 3′ flanking region (FR) a deletion had occurred (data not shown; GenBank/EMBL/DDBJ accession no. AJ505747). This part of the 3′ FR is not amplified by the novel primers.

In a Turkish male with both primer pairs, an allele 12.1 was identified with repeat eight having changed from GAAA to GAAAA by adenine insertion (data not shown; GenBank/EMBL/DDBJ accession no. AJ505746). Such an adenine insertion has recently been described by Cali et al. for DXYS156-Y (9).

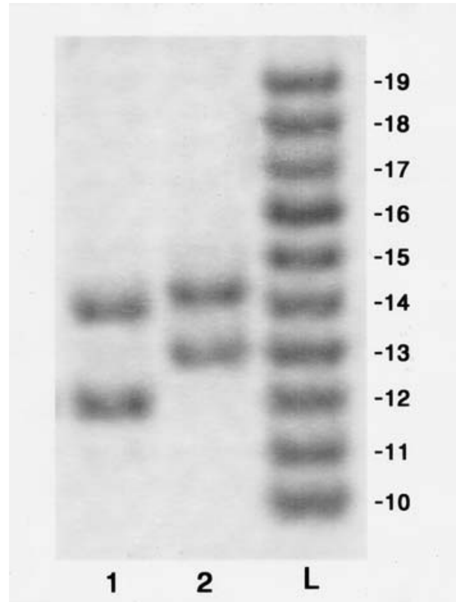
Schneider et al. (2) sequenced DYS385 alleles and reported three sequence variants: repeat No. 7 was changed to GCAA in allele 13, repeat No. 11 was changed to GACA in allele 19, and repeat No. 4 to GAAG in allele 12. Sequence variants might exist in the Thai population as well. But it would take a systematic sequencing approach to search for any, that, however, was beyond the scope of this study.

We analyzed in total 149 Thai men from Northern Thailand with the new primers in Chiang Mai. In two of the 149 cases, no PCR results for DYS385 were obtained with either the Schneider or our primers whereas other STR loci could be amplified normally. For several reasons, amplification of DYS385 could have failed, for example, when a larger deletion removed both DYS385 sites from the chromosome. The inactivation of a primer binding site by a smaller deletion or point mutation is another possibility. These events probably should have happened early before the duplication of the DYS385 sites because two independent mutations in both sites are an unlikely event.

Haplotype frequency data of 147 men are shown in Table 1. Forty different haplotypes were found, the haplotype diversity is 0.9430. The most frequent haplotype is 13–18 (0.136) followed by 13–17 and 13–19 (0.102 each), these three haplotypes together (combined frequency 0.340) count for more than one-third of the cases and resemble a striking peak in the haplotype distribution.

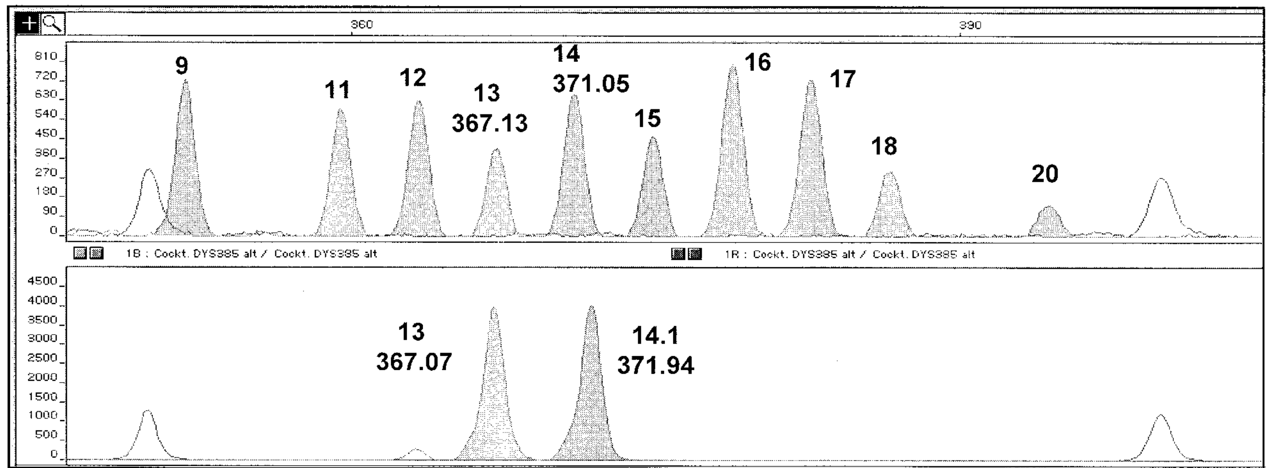
We compared the most frequent DYS385 haplotypes in nine populations (Table 2). Distinct frequencies were observed in each population. Allele 13 appears to be distributed at a high frequency among the East-Asian populations, but there is no particular haplotype that could be used as marker for East-Asian origin. Surprisingly, a pronounced difference in haplotype frequency exists

a)




b)

A




B

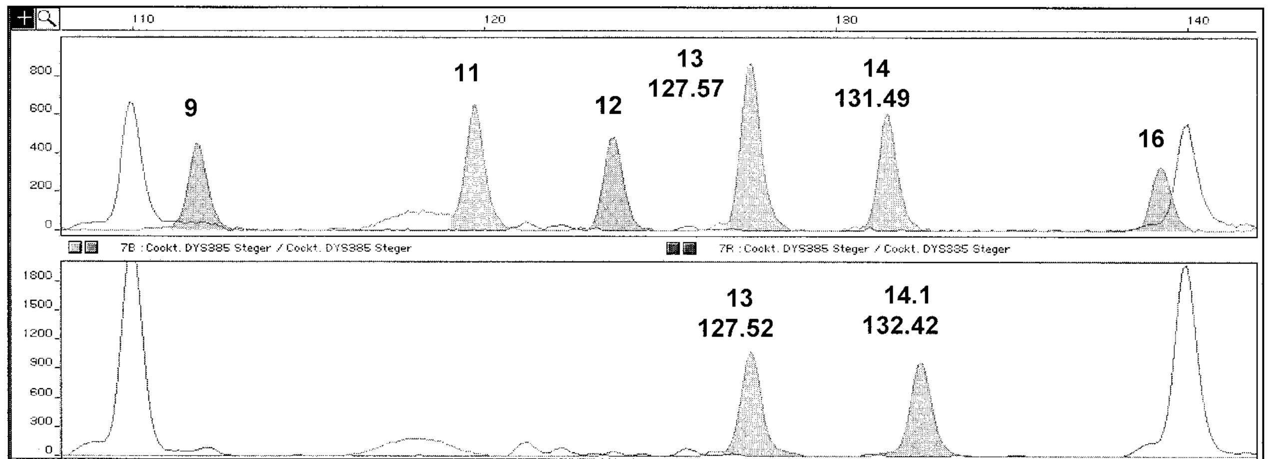



FIG. 2—a) *Identification of allele variant 14.1 by polyacrylamide gel electrophoresis. Lane 1: haplotype 12–14; Lane 2: haplotype 13–14.1; L: allelic ladder with alleles 10-19. Allele 14.1 is clearly shifted upward when compared to a "normal" allele 14, see allele 14 in lane 1 and in the allelic ladder.* b) *Identification of allele variant 14.1 by capillary electrophoresis. Panel A, amplicons with conventional primers (2); upper row: DYS385 allelic ladder (alleles indicated); lower row: individual with allele variant 14.1. Panel B, amplicons with novel primers (this study); upper row: DYS385 allelic ladder (alleles indicated); lower row: individual with allele variant 14.1. Filled peaks: DYS385 alleles; open peaks: internal lane standard (genRES LS500 ROX, Serac, Bad Homburg, Germany). Fragment lengths of alleles 13 and 14/14.1 are given in bp.*

TABLE 1—*DYS385 haplotype frequencies in Northern Thais.*

| Haplotype | *n* | Frequency | Haplotype | *n* | Frequency |
|---|---|---|---|---|---|
| 11–11 | 1 | 0.007 | 13–20 | 6 | 0.041 |
| 11–12 | 3 | 0.020 | 14–16 | 2 | 0.014 |
| 11–13 | 2 | 0.014 | 14–17 | 2 | 0.014 |
| 11–14 | 4 | 0.027 | 14–18 | 1 | 0.007 |
| 11–17 | 4 | 0.027 | 14–19 | 5 | 0.034 |
| 11–21 | 1 | 0.007 | 14.2–19 | 1 | 0.007 |
| 12–12 | 1 | 0.007 | 15–15 | 1 | 0.007 |
| 12–14 | 1 | 0.007 | 15–16 | 5 | 0.034 |
| 12–16 | 3 | 0.020 | 15–17 | 1 | 0.007 |
| 12–17 | 6 | 0.041 | 15–18 | 4 | 0.027 |
| 12–18 | 5 | 0.034 | 15–19 | 4 | 0.027 |
| 12–19 | 2 | 0.014 | 15–20 | 4 | 0.027 |
| 13–13 | 3 | 0.020 | 15–21 | 2 | 0.014 |
| 13–14 | 7 | 0.048 | 16–16 | 1 | 0.007 |
| 13–14.1 | 1 | 0.007 | 16–17 | 1 | 0.007 |
| 13–15 | 2 | 0.014 | 16–18 | 2 | 0.014 |
| 13–16 | 5 | 0.034 | 16–21 | 1 | 0.007 |
| 13–17 | 15 | 0.102 | 17–18 | 1 | 0.007 |
| 13–18 | 20 | 0.136 | 17–21 | 1 | 0.007 |
| 13–19 | 15 | 0.102 | 18–20 | 1 | 0.007 |

Haplotype diversity = 0.9430.

TABLE 2—*Comparison of the most frequent DYS385 haplotypes in various populations.*

| | Black (West Africa)* *n* = 119 | Moroccans* *n* = 30 | Germans† *n* = 250 | Filipinos‡ *n* = 106 | Japanese§ *n* = 100 | Koreans‖ *n* = 316 | Chinese (Chengdu)§ *n* = 100 | Northern Thai¶ *n* = 145 | Bangkok Thai§ *n* = 95 |
|---|---|---|---|---|---|---|---|---|---|
| Haplotype (frequency) | 15–16 (0.151) | 13–14 (0.600) | 10–14 (0.068) | 12–16 (0.132) | 12–16 (0.050) | 10–18 (0.098) | 13–13 (0.090) | 13–17 (0.102) | 14–18 (0.074) |
| Haplotype (frequency) | 16–16 (0.143) | 13–15 (0.133) | 11–14 (0.412) | 13–14 (0.160) | 12–17 (0.080) | 10–19 (0.092) | 13–17 (0.070) | 13–18 (0.136) | 13–13 (0.063) |
| Haplotype (frequency) | 16–17 (0.092) | | 11–15 (0.068) | | 13–17 (0.140) | 13–19 (0.067) | 13–19 (0.080) | 13–19 (0.102) | 13–18 (0.063) |
| Haplotype Diversity | 0.9200 | 0.6100 | 0.8080 | 0.9400 | 0.9300 | 0.9606 | 0.9500 | 0.9430 | 0.9600 |

* according to Gamero et al. (12).
† according to Lessig and Edelmann (13).
‡ according to Miranda et al. (14).
§ according to Schneider et al. (2)
‖ according to Shin et al. (15).
¶ data from this study.

between the Northern Thais and a Thai population sample from Bangkok (2) (95 blood donors; Note: Bangkok experiences a large amount of gene flow from various parts of the country). Whereas for the latter ones, haplotype 14–18 was the most frequent one (0.074 or 7 men in 95), there was only one man from the North with this type. How can this striking difference within Thailand's population be explained? From historical records, we know that Thailand's population is far from being homogenous, e.g., the people of the North speak a different dialect than the people of the Bangkok area and they have their own distinct culture. Our data suggest that in addition to the cultural differences, there are also genetic differences in the founding populations. Marked frequency differences of thalassemia mutations in Thailand support this notion (10,11).

In conclusion, our experience suggests that whenever the nature of the target sequence allows it, primers that give relatively short products are preferable. Long amplicons may be difficult to separate by CE and in some cases also by PAGE, thereby valuable genetic information of allele variants might be missed. PAGE, a long used tool in biochemistry still proves quite useful in STR analysis. Automated CE offers fast and accurate analysis under carefully adjusted conditions. Unfortunately, for developing countries like Thailand, the higher operating costs still limit the use of this new technology. Finally, the striking difference in the population data for the DYS385 locus in two Thai populations suggests once again that it is advisable to establish specific STR databases for particular populations.

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