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A Dutch population study of the STR loci HUMTHO1, HUMFES/FPS, HUMVWA31/1 and HUMF13A1, conducted for forensic purposes

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Abstract We report on a Dutch population study of the STR loci HUMTHO1, HUMFES/FPS, HUMVWA31/1, and HUMF13A1, in which we used multiplex amplification and automated fragment detection. Genotype and allele frequencies showed no deviation from Hardy-Weinberg and linkage equilibrium. The improved Bonferroni procedure was used to combine the results of several tests. The power of discrimination of a complete profile exceeded 0.9998. We compared the allele frequencies in the Dutch sample to the frequencies in other populations using a biplot to visualize alleles and populations simultaneously. The Dutch sample was similar to most other Caucasian samples. The data demonstrate that the genetic systems in this report are a valuable tool for forensic identity testing in The Netherlands.

Key words DNA · Forensic · STR · HumTHO1 · HumFES/FPS · HumVWA31/1 · HumF13A1 · Multiplex PCR · Population study

Introduction

The use of the PCR technique and automated allele detection of short tandem repeat (STR) loci offers many advantages in forensic casework, such as a short analysis time, relatively low costs, high precision [17] and reliability even with small or degraded samples [21, 25, 35]. Several typing techniques have been compared in a collaborative experiment conducted under the aegis of the European DNA Profiling Group (EDNAP) [21]. One of the conclusions was that the multiplex PCR technique with automated fluorescent detection allows consistent and correct typing of the STR loci HUMTHO1, HUMFES/FPS, HUMVWA31/1 and HUMF13A1. Population data on the loci HUMTHO1 and HUMVWA31/1 have been reported by many groups [3, 6, 7, 16–18, 22–24, 26, 28, 29, 31], but fewer data are available on the loci HUMFES/FPS and HUMF13A1 [3, 16, 30, 34].

In this paper we report on a study of the four STR loci HUMTHO1, HUMFES/FPS, HUMVWA31/1, and HUMF13A1 in The Netherlands. The profiles were obtained using a fluorescence-based quadruplex PCR system reported by Kimpton et al. [20] and Lygo et al. [25]. We present the genotype and allele frequencies found in a sample of 195 unrelated Caucasian individuals. Furthermore, the observed heterozygote frequency and the expected frequency assuming Hardy-Weinberg equilibrium (HWE) were calculated, as were the precision and the power of discrimination. To check whether the frequency of a profile consisting of all four loci can be estimated by multiplying within and across loci, we tested for both HWE and linkage equilibrium.

In the forensic DNA literature, reports of performing more than one test for HWE or linkage equilibrium and combining the dependent test results with the Bonferroni procedure are common (e.g. [2, 15, 16]). We discuss some consequences of this procedure for the ability to detect deviations.

The HUMTHO1 and HUMVWA31/1 allele frequencies in the Dutch sample were compared with those in other population samples, including some non-Caucasian samples. The populations and alleles are visualized in a single plot: a biplot [10]. This type of plot is related to principal components analysis and has many variants, which are increasingly used in anthropometric and genetic analyses [4]. It allows a quick overview of the differences between populations and deviant allele frequencies. The analysis is restricted to HUMTHO1 and HUMVWA31/1, since few data are so far available on the loci HUMFES/FPS and HUMF13A1.

Materials and methods

Population sample

The sample consisted of 195 unrelated Caucasian donors (employees and students of the Dutch Forensic Science Laboratory).

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Multiplex amplification conditions and detection system

The coamplification of HUMTHO1, HUMVWFA/31, HUMFES/FPS and HUMF13A1 was performed using the procedure of Kimpton et al. [20]. The quadruplex PCR reaction mixture was according to Kimpton et al. [20] and was obtained from the Forensic Science Service (Birmingham, UK). Typing of the amplified DNA-samples was according to the method of Kimpton et al. [20], using the automated fluorescent detection system on an ABI 373A DNA sequencer. Electrophoresis was carried out in standard 6% polyacrylamide denaturing sequencing gels (12 cm well-to-read). Allelic ladders for HUMTHO1, HUMVWFA/31, HUMFES/FPS and HUMF13A1 were provided by Kimpton and Gill (Forensic Science Service, Birmingham, UK). The length of the amplified DNA fragments was determined from the internal lane standard Genescan-350 ROX (Perkin Elmer). Fragment sizes were automatically estimated using Genescan PCR Analysis software (Genescan 1.2.2–1, ABI) by the Southern local method [8]. Allele designations were automatically performed using Genotyper 1.0 DNA Fragment Analysis Software (ABI) and were assigned based on the number of repeat units. In order to adjust the window (size range) for each allele we estimated the fragment sizes of the allelic ladders which were run on 35 different gels. The window for each allele was set to its average size ± 3 standard deviations.

Statistical methods

We estimated the population frequency of allele i at locus j as the sample frequency x_{ij}, and its standard error as $\sqrt{x_{ij}(1-x_{ij})/2n}$, where n is the number of individuals in the database [7]. Assuming HWE, the expected frequency of heterozygotes f_j and its standard error were calculated as $(1-\Sigma x_{ij}^2)2n/(2n-1)$ and $\sqrt{f_j(1-f_j)/n}$ respectively [27].

The power of discrimination of locus j is calculated as $1-\Sigma y_{kj}^2$ [9], where y_{kj} is the sample frequency of genotype k at locus j. Its standard error can be derived from [27] by applying the formulas to genotypes instead of alleles:

$$\sqrt{2(n-1)\left[(3-2n)\,Q_j^{\,2} + 2(n-2)\sum_k (y_{kj}^{\,3}) + Q_j\right]/n3} \qquad (1)$$

where $Q_j = \Sigma y_{kj}^2$. The combined power of discrimination is calculated as $1-\prod Q_j$, and its standard error as $\prod Q_j \sqrt{\prod(1+(SE_j/Q_j)^2)-1}$, where SE stands for the standard error calculated in Equation (1).

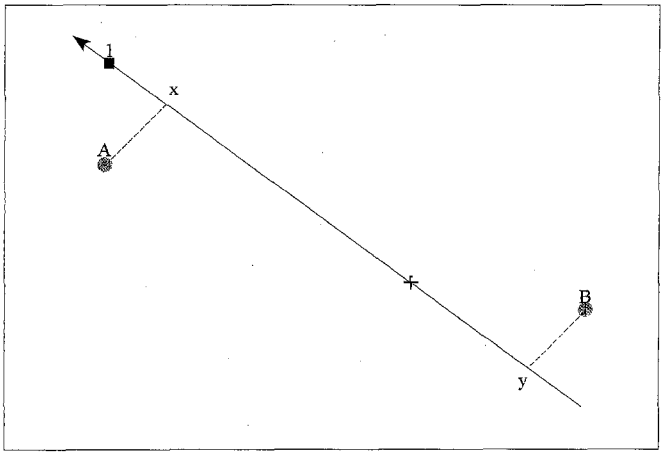

Fig. 1 Perpendicular projection of populations A and B on the axis drawn through allele 1 and the origin (+) of a biplot yields the values $x > 0$ and $y < 0$. These values approximate the deviation of the allele 1 frequency in populations A and B, respectively, from the mean allele 1 frequency. Hence, population A has a relatively high allele 1 frequency, whereas population B has a relatively low allele 1 frequency

We performed the exact test for HWE (Markov Chain method, [11]), and the match-matrix test [32] for linkage equilibrium. The allele frequencies in other populations were compared with those in the Dutch population using a likelihood ratio test adjusted for low-frequency cells.

A biplot [10] was used to visualize the allele frequency data table which consists of rows (populations) and columns (alleles). The type of plot we used is obtained from relative allele frequencies by subtracting column means and scaling of row factors with the singular values [10]. The data analysis was performed using the software Spectramap (Smit Consult, Drunen, The Netherlands). The biplots are interpreted in the following way: alleles are represented by squares, and populations by circles. Populations that are close together in the plot have similar allelic compositions, in contrast to populations that are far apart. The population frequencies of any allele a can be compared by perpendicular projection of the populations on a line drawn through allele a and the origin (indicated by +) (Fig. 1). The horizontal and vertical directions in the biplot each account for a certain fraction of the total information (i.e. the total variance of the allele frequencies). These fractions are reported in the legend to Fig. 3.

Results

Sample statistics

The sample frequency of genotypes and alleles are presented in Table 1. Standard deviations of the fragment size estimates are also indicated. The locus HUMF13A1 had the largest standard deviation (0.38 bp at allele 16). Furthermore, the powers of discrimination are shown. Note that the combined power of discrimination exceeded 0.9998. In addition, we compared all 195 profiles in the database pairwise (18,195 pairs of profiles), finding one pair of individuals that matched at all 4 loci, and 82 pairs that matched at 3 loci.

Hardy Weinberg and linkage equilibrium

The exact test [11] did not indicate any deviations from HWE (Table 2). The match-matrix test [32] indicated a deviation from linkage equilibrium for 1 of the 6 locus pairs, HUMTHO1 and HUMFES/FPS, which had a lower number of matching individuals than expected under linkage equilibrium ($P = 0.02$, Table 3). However, this is not significant considering the fact that 6 tests were conducted (improved Bonferroni [14], rejection level for the smallest P-value at an overall level of $\alpha = 5\%$ is $0.05/6 = 0.008$).

Comparison of HUMTHO1 and HUMVWA31/1 allele distributions with other populations

The allelic composition at the HUMTHO1 and HUMVWA31/1 loci of several populations is displayed in Fig. 2. More insight into the genetic differences between the populations can be obtained from the corresponding biplots (Fig. 3). The interpretation of these plots is explained in the "Materials and methods" section. Rare alleles are not included in the analysis. In the biplot of

Table 1 Genotype and allele distributions for HUMTHO1, HUMFES/FPS, HUMVWA31/1, and HUMF13A1 in a sample of 195 Dutch Caucasians. Standard errors are shown in parentheses. Below each part of the table are shown: – the observed fraction of heterozygotes and the expected fraction assuming HWE; – the power of discrimination; – the intragel standard deviation of fragment size estimates, obtained from 35 runs of an allelic ladder. The table shows the maximum observed standard deviation

HUMTHO1

Allele	5	6	7	8	9	9.3/10
5	–					
6	–	11				
7	2	15	10			
8	–	10	7	2		
9	1	15	10	11	4	
9.3/10	–	27	26	9	11	24
freq	0.01 (0.004)	0.23 (0.02)	0.21 (0.02)	0.11 (0.02)	0.14 (0.02)	0.31 (0.02)

Heterozygosity: obs 0.74, exp 0.78 (0.030)
Power of discrimination: 0.915 (0.006)
Max. SD of fragment size est. 0.15 bp (allele 7)

HUMFES/FPS

Allele	8	9	10	11	12	13
8	–					
9	–	–				
10	–	–	11			
11	1	1	53	47		
12	1	1	17	40	13	
13	1	–	4	3	1	1
freq	0.01 (0.004)	0.01 (0.004)	0.25 (0.02)	0.49 (0.03)	0.22 (0.02)	0.03 (0.01)

Heterozygosity: obs 0.63, exp 0.65 (0.034)
Power of discrimination: 0.810 (0.013)
Max. SD of fragment size est. 0.24 bp (allele 13)

HUMVWA31/1

Allele	14	15	16	17	18	19	20	21
14	–							
15	1	4						
16	4	5	10					
17	8	7	22	21				
18	7	7	23	22	7			
19	3	2	4	15	11	4		
20	1	1	1	1	1	1	–	
21	1	–	–	1	–	–	–	–
freq	0.06 (0.01)	0.08 (0.01)	0.20 (0.02)	0.30 (0.02)	0.22 (0.02)	0.11 (0.02)	0.02 (0.01)	0.01 (0.004)

Heterozygosity: obs 0.76, exp 0.80 (0.029)
Power of discrimination: 0.928 (0.006)
Max. SD of fragment size est. 0.24 bp (allele 21)

HUMF13A1

Allele	3	4	5	6	7	8	14	15	16
3	–								
5	7	2	7						
6	5	3	20	20					
7	12	9	31	39	27				
8	–	–	–	–	2	–			
14	–	–	–	1	3	–	–		
15	–	–	1	2	1	–	–	–	
16	–	1	–	–	1	–	–	–	–
freq	0.06 (0.01)	0.04 (0.01)	0.19 (0.02)	0.28 (0.02)	0.39 (0.02)	0.01 (0.004)	0.01 (0.01)	0.01 (0.01)	0.01 (0.004)

Heterozygosity: obs 0.72, exp 0.73 (0.032)
Power of discrimination: 0.884 (0.009)
Max. SD of fragment size est. 0.38 bp (allele 16)
Combined power of discrimination: 0.99986 (0.00002)

Table 2 *P*-values[a] of the exact test for Hardy-Weinberg equilibrium for a Dutch Caucasian population sample (195 individuals)

Locus	P-value
HUMTHO1	0.26
HUMFES/FPS	0.09
HUMVWA31/1	0.34
HUMF13A1	0.86

[a] *P*-values estimated using a Markov-Chain method [11] (dememorization period = 1000 steps); frequencies of HUMTHO1 alleles 9.3 and 10 were pooled

Table 3 *P*-values[a] for linkage equilibrium test [32] between 2 loci for a Dutch Caucasian population sample (195 individuals)

	HUMTHO1	HUMFES/FPS	HUMVWA31/1
HUMFES/FPS	0.02[b]		
HUMVWA31/1	0.66	0.75	
HUMF13A1	0.80	0.67	0.24

[a] *P*-values estimated by a bootstrap method using 1000 resamples. HUMTHO1 alleles 9.3 and 10 are pooled
[b] The rejection level at an overall significance level of 0.05 is 0.05/6 = 0.008 (Improved Bonferroni [14])

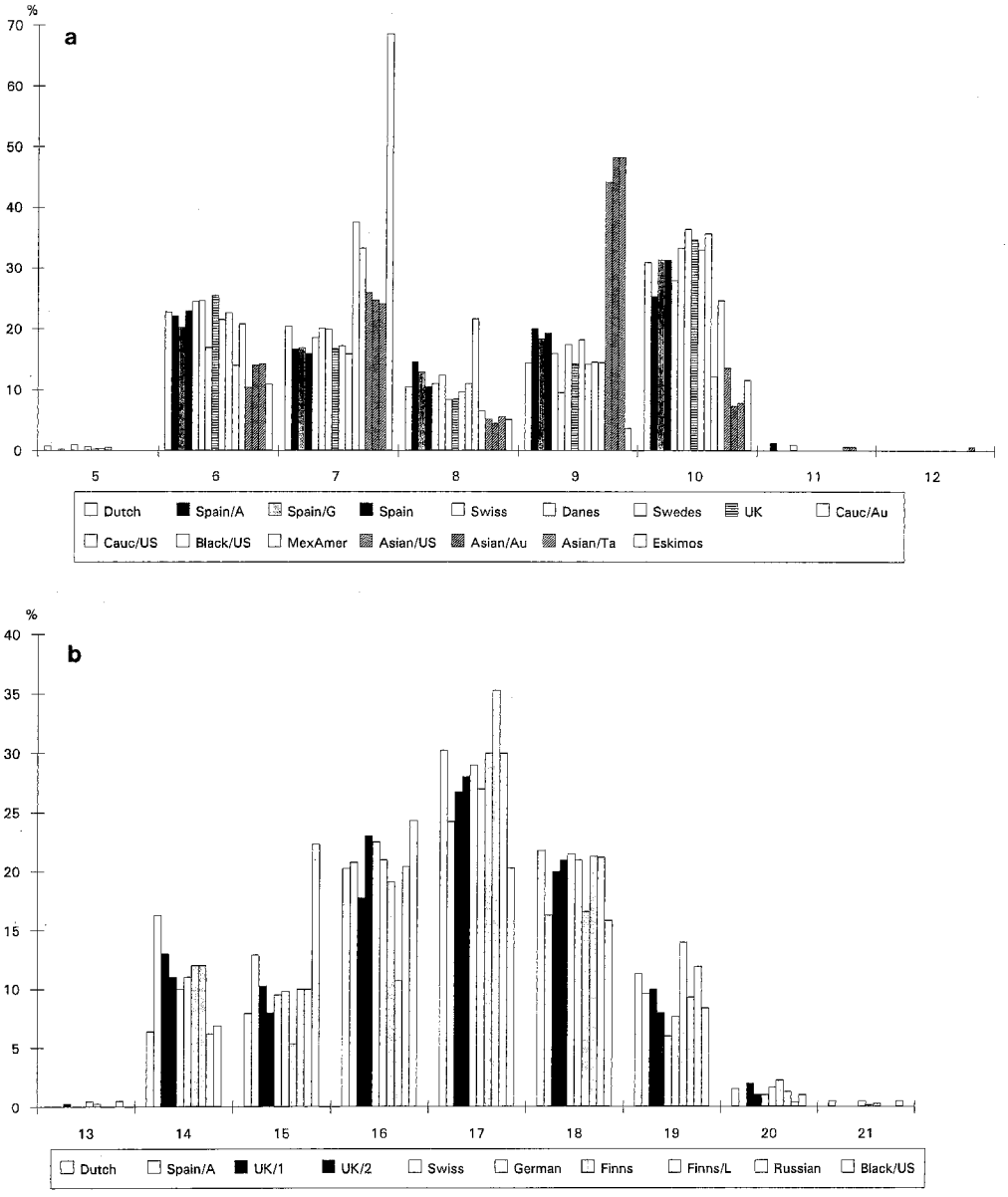

Fig. 2 Histograms of **a** HUMTHO1 and **b** HUMVWA31/1 frequencies in different populations. References as in Table 4. (**a** *Spain/A* Spain/Andalucia, *Spain/G* Spain/Galicia, *Cauc/Au* Caucasian/Australia, *Cauc/US* Caucasian/US, *MexAmer* Mexican Americans, *Asian/Au* Asian/Australian, *Asian/Ta* Asian/Taiwan; **b** *Spain/A* Spain/Andalucia, *UK/1* UK [6], *UK/2* UK [19], *FinnsL* Finland/Larsmo)

HUMTHO1 (Fig. 3a), the Caucasian populations appear to be a relatively homogeneous group and are clearly separated from the non-Caucasian populations. The Caucasians score low on allele 7, whereas the non-Caucasians score high, especially the Eskimos. Furthermore, the Caucasians score high on allele 10, whereas the non-Caucasians score low. The three Asian populations appear close together and are thus similar in allelic composition. They score high on allele 9 compared with the other populations. The biplot of HUMVWA31/1 (Fig. 3b) shows

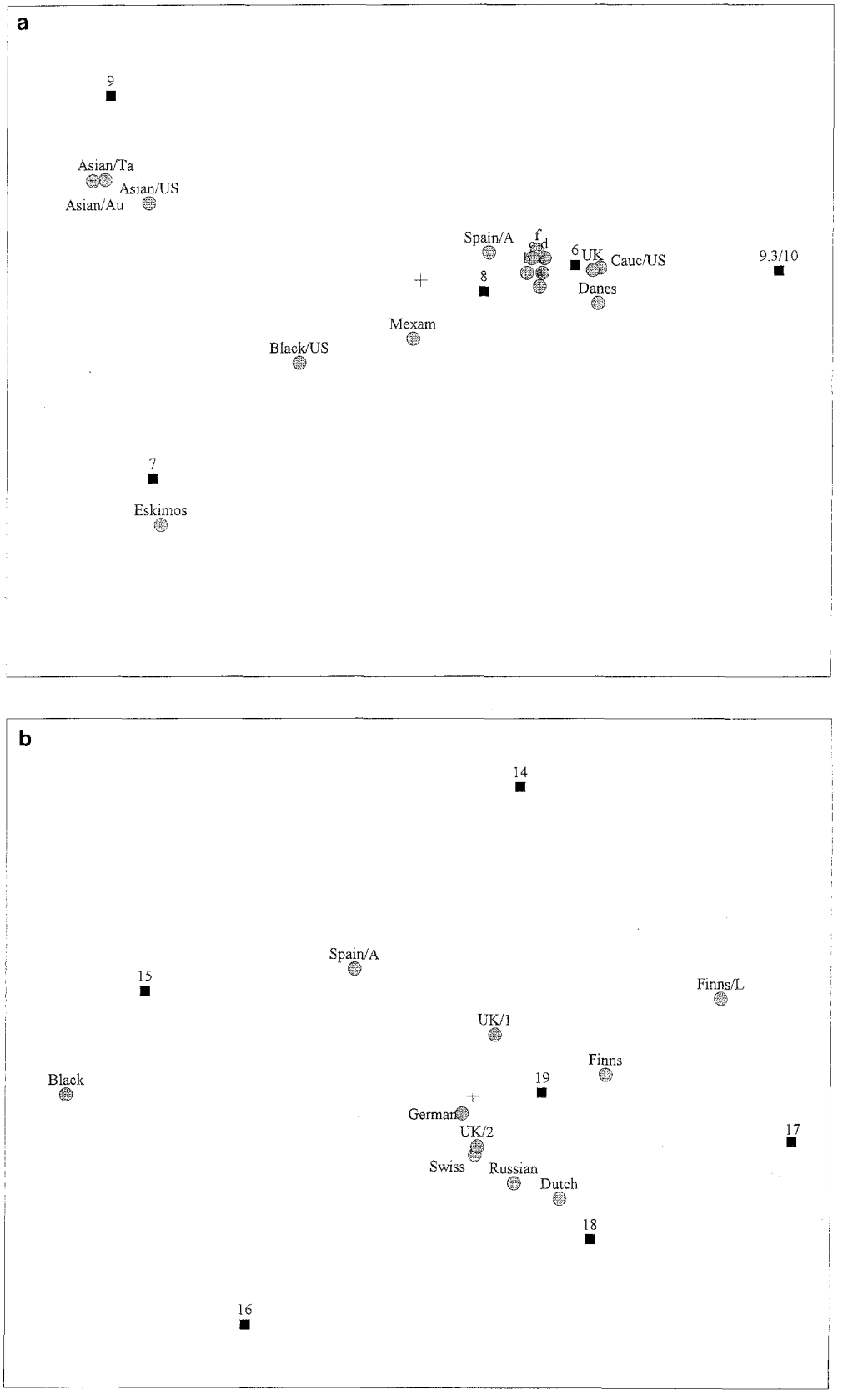

Fig. 3a, b Biplots of HUMTHO1 and HUMVWA31/1. The interpretation is explained in the "Materials and methods" section. References as in Table 4. **a** HUMTHO1, accounting for 95% of the total variance (horizontal and vertical direction 52% and 42% respectively). Alleles 9.3 and 10 are pooled, and alleles 5, 11, and 12 are excluded from the analysis (**a** Dutch, **b** Switzerland, **c** Spain/Galicia, **d** Caucasian/Australia, **e** Sweden, **f** Spain) **b** HUMVWA31/1, accounting for 75% of the total variance (horizontal and vertical direction 57% and 18% respectively). Alleles 13, 20 and 21 are excluded from the analysis

Table 4 P-values of adjusted LR test for pairwise comparisons of Dutch Caucasian allele frequencies with other populations for HUMTHO1 and HUMVWA31/1 (195 individuals)

Population	Origin	Region	No. individuals	Ref.	*P*-value
HUMTHO1					
Caucasian	Spain	Andalucia	120	[24]	0.01*
Caucasian	Spain	Galicia	210	[22]	0.34
Caucasian	Spain		205	[3]	0.09
Caucasian	Switzerland		100	[16]	0.54
Caucasian	Denmark		189	[28]	0.12
Caucasian	UK		200	[22]	0.46
Caucasian	Sweden		231	[17]	0.16
Caucasian	Australia	Victoria	151	[29]	0.74
Caucasian	US	Houston	186	[31]	0.72
Black	US	Houston	185	[31]	< 0.001*
Mexican	US	Houston	192	[31]	0.002*
Asian	US	Houston	77	[31]	< 0.001*
Asian	Australia	Victoria	89	[29]	< 0.001*
Asian	Taiwan		116	[18]	< 0.001*
Eskimo	Greenland		147	[28]	< 0.001*
HUMVWA31/1					
Caucasian	Spain	Andalucia	120	[23]	< 0.001*
Caucasian	Switzerland		100	[16]	0.42
Caucasian	Germany		321	[26]	0.06
Caucasian	UK		200	[6]	0.04*
Caucasian	UK		60	[19]	0.71
Caucasian	Finland		175	[33]	0.09
Caucasian	Finland	Larsmo	75	[33]	0.07
Caucasian	Russia	Vologda	130	[33]	0.72
Black	US	Houston	185	[31]	< 0.001*

that the black population differs from the other populations, scoring exceptionally high on allele 15 but low on allele 17.

The results of the adjusted likelihood ratio test for pairwise comparisons between the Dutch Caucasian population and other populations concerning the HUMTHO1 and HUMVWA31/1 allele frequencies are presented in Table 4. The allelic composition of the Dutch population differs at both loci from that of all reported non-Caucasian populations and from the Spanish Caucasian population from Andalucia. Of the other Caucasian populations, only one of the British populations showed a deviation at the HUMVWA31/1 locus.

Discussion

This study confirms that the automated detection technique is very precise [17, 21, 25]. The largest standard deviation, 0.38 bp, was found at the HUMF13A1 locus for allele 16. This precision allows the separation of adjacent alleles for all 4 tetrameric loci. Furthermore, the precision at the HUMTHO1 locus (largest sd = 0.15 bp) allows a distinction between the common 9.3 allele and the rare 10 allele.

The combination of the 4 STR loci is highly discriminating: in the Dutch Caucasian population, the combined power of discrimination exceeded 0.9998. The powers of discrimination are similar to those reported in other studies [3, 13, 22–24, 26, 29, 33]. In the database containing 195 individuals, giving 18,195 pairs of individuals, only one pair matched at all 4 loci.

Our data show no deviations from HWE and linkage equilibrium, except for a minor indication that there are fewer matching (HUMTHO1, HUMFES/FPS) profiles than expected under linkage equilibrium. However, in a forensic context such negative correlation between loci will favour an innocent suspect, because it reduces the chance that an innocent suspect matches at both loci with the offender. Moreover, in the case of a match it is likely that the profile frequency will be overestimated. Our results are consistent with other studies, which in most cases showed only slight deviations or none at all from HWE [3, 7, 13, 16–18, 22–24, 26, 28, 29, 31] and linkage equilibrium. Deviations from HWE have been reported for locus HUMVWA31/1for a Russian population [33] and a British population [6].

Since a single statistical test only detects certain types of deviations from HWE or linkage equilibrium, some authors combine several tests in order to increase the power to detect deviations (e.g. [7, 13, 16, 18, 24, 31]). However, it is questionable whether performing several tests of the same hypothesis on the same data set is an appropriate method. The disadvantage of performing several tests is that the probability that one or more tests falsely rejects the null hypothesis increases with the number of tests. To keep the probability of false rejection at an acceptable level, some kind of combination procedure of the depen-

dent test results has to be followed. Often, the Bonferroni procedure is used. However, this procedure is rather conservative [12], and may well result in a lower instead of a higher power to detect deviations from the null hypothesis.

In our opinion, one should either perform a single powerful test or use a less conservative combination procedure. We chose to test each hypothesis with a single test, and to use the improved Bonferroni procedure [14] when this test was performed several times, for instance when testing for HWE at several loci.

A feature of the biplot is that it allows a quick grasp of the differences and similarities between the allele distributions of many populations, which is difficult to obtain from a combined histogram such as Fig. 2. However, some of the information in the histogram is lost in the biplot because it reduces a high dimensional space to only 2 dimensions. Nevertheless, the biplots in this paper reproduce 95% and 75% of the total variation, which means most information is retained.

Our analysis of the loci HUMTHO1 and HUMVWA31/1 shows that the Dutch Caucasian population is similar in allelic composition to other Caucasian populations, except for the Spanish population sample from Andalucia. However, there are differences from the non-Caucasian populations. Hence, when other databases are used to estimate the frequency of a profile, we expect the largest differences to occur when non-Caucasian databases are used. Nevertheless, the frequency estimates are expected to be low in all databases.

In conclusion, we can confirm that the present typing method is very precise, and others have shown that it is quick, cost-effective and reliable even with small or degraded samples [21, 25, 35]. Its high precision allows the distinction of discrete allele classes, which simplifies the statistical analyses [17]. Furthermore, DNA profiles of the STR loci investigated are highly discriminating. The frequency of the profiles in the Dutch population may be estimated from our database by multiplying frequencies within and across loci. Therefore, this set of STR loci is a valuable tool for forensic DNA casework in The Netherlands.

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