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SHORT COMMUNICATION

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Polish population study on Y chromosome haplotypes defined by 18 STR loci

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Abstract Polymorphism of 18 STR loci specific to the human Y chromosome (DYS19, DYS388, DYS389I, DYS 389II, DYS390, DYS391, DYS392, DYS393, DYS426, DYS437, DYS438, DYS439, DYS460, GATA H4.1, DYS 385 a/b, and YCAII a/b) was evaluated by means of a multiplex (octadecaplex) PCR reaction and capillary electrophoresis in a Polish population sample of 208 unrelated males. A total of 192 different haplotypes and 183 unique haplotypes were identified. The observed haplotype diversity was 0.998, while discrimination capacity was 92.3%. DYS389 was shown to be the most valuable in discrimination of similar haplotypes, whereas DYS388, DYS393, DYS426, and DYS438 did not affect the discrimination power of the multiplex.

Keywords Y chromosome · STRs · Haplotypes · Multiplex PCR · Polish population

Introduction

Due to presence of the largest nonrecombining region in the whole human genome, the Y chromosome is characterised by a unique inheritance pattern and specificity to males. Forensic geneticists' interest has focussed on Y-chromosomal short tandem repeat (Y-STR) markers, a constantly growing number of which is made available for population-genetic, evolutionary, genealogical, and forensic investigations [1]. Two sets of Y-STR systems called minimal and extended haplotypes have been widely accepted by the forensic community, and genotyping results for different human populations throughout the world have been collected in publicly available databases [2]. Discimination capacity of well-characterised minimal haplotypes was shown to be relatively low in the Polish population, reaching 61.2% [3], so that genotyping data for haplotypes extended by other Y-chromosome microsatellites are highly desirable. Thus, the aim of this study was to evaluate usability of Y-STR loci hitherto unstudied in forensic practice in Poland by means of a multiplex PCR system.

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Material and methods

DNA samples

Whole EDTA-treated blood samples were collected from 208 randomly selected, unrelated Polish males participating in paternity testing at the Department of Forensic Medicine, Medical University of Gdansk. DNA was extracted using a nonenzymatic method [4].

Amplification conditions

Sequences of primers used in the multiplex system were obtained from the literature [5]. Forward primers were labelled at 5′ ends with 6FAM, TET and HEX (virtual filter set C). PCR amplification conditions generally remained unchanged [5], with the exception of a reduced reaction volume (5 μl instead of 20 μl). For most cases, 0.2 ng of DNA (2 μl) were used for amplification. Thermal cycling was performed on a Mastercycler Gradient thermocycler (Eppendorf) with the ramp speed set at 1°C/s.

Detection and genotyping

The separation and detection of PCR products were performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Fragments of distinctive sizes underwent se-

quencing as described elsewhere [6]. Allele nomenclature for DYS437, DYS438, DYS439, DYS460, GATA H4.1, and YCAII was as proposed by Gusmão et al. [7] and Schmidt et al. [8]. Allele designation was based on comparison with the constructed allelic ladder. The proficiency was successfully achieved by the Y-STR haplotyping quality assurance exercise 2004 (http://www.yhrd.org).

Statistical analysis

Observed gene and haplotype diversities were computed using Arlequin 2.000 software [9]. Discrimination capacity (DC) and fraction of unique haplotypes (FUH) were estimated as percentage proportions of different and unique haplotypes in the population sample, respectively. DC contribution of each system was calculated as a difference between DC of the whole multiplex and DC of the multiplex after exclusion of the system, divided by DC of the whole multiplex and expressed in percents. FUH contribution of each system was estimated analogically. In all calculations, DYS389 was treated as a haplotype of two independent loci: DYS389I and DYS389II-I.

Results and discussion

Multiplex optimisation

Initial testing of the multiplex showed necessity for modification of originally described primer concentrations. In case of DYS460, the primer concentration needed even a sevenfold decrease. Allele range overlapping was observed between DYS393 and YCAII, and between DYS460 and GATA H4.1. For this reason, a very rare YCAII*11 allele was excluded from the ladder. For the same reason, forward primers for DYS391 and DYS460 had to be relabelled with different dyes so that DYS391 replaced DYS460 in the yellow panel, while DYS460 was transferred to the blue one.

Haplotype analysis

By combined analysis of all 18 loci studied in a sample of 208 unrelated males, 192 different haplotypes and 183 unique haplotypes could be defined (supplementary Table S1). The most frequent haplotype occurred as many as eight times (3.8%). The overall haplotype diversity was 0.9982. The discrimination capacity of the multiplex was 92.3%. Although males born in northern Poland dominated in the studied population sample, this Y-STR haplotype database can be treated as representative for the Polish population as a whole due to postwar migrations and present-day genetic homogeneity within Poland [3].

The most polymorphic system was DYS385, while the least polymorphic ones were DYS392 and DYS393 (Table 1), which belong to the most popular set of loci analysed in forensic laboratories and define so-called minimal haplotypes. Since it was demonstrated that comparison of haplotype diversities does not illustrate well the value of addition of a marker to the Y-STR locus set [10], the influence of the marker's elimination from the octadecaplex on the DC and FUH was analysed. Values of DC contribution and FUH contribution of the Y-STR marker correlate with the number of haplotypes identical at all loci except for the marker tested, thus showing the marker's potential for resolution of similar haplotypes. Comparison of these values revealed that the most discriminative marker was DYS389 (Table 1). DYS388, DYS393, DYS 426, and DYS438 did not have any effect on DC and FUH, although all four were removed from analysis. It was suggested that the decision on the inclusion of new markers to the established STR core set should by preceded by exten-

Table 1 Characterisation of Y-STR markers (in order of gene diversity) in the studied population sample

System	Allele range	No. of alleles/ haplotypes	Genetic diversity	DC contribution	FUH contribution
DYS385	9–18	30	0.8746	6.77	12.02
DYS389	I: 12–15 II-I: 15–19 II: 27–32	14	0.7990	9.37	12.57
DYS19	13–17	5	0.7575	5.73	8.20
DYS439	10–14	5	0.6881	5.73	8.74
DYS390	21–26	6	0.6540	3.12	4.92
YCAII	11, 18–25	17	0.6381	1.56	2.73
DYS438	9–13	5	0.5835	0	0
DYS460	9–13	5	0.5634	3.12	6.01
H4.1	18–23	6	0.5576	2.60	4.92
DYS391	9–12	4	0.5038	1.56	1.64
DYS426	11–13	3	0.4721	0	0
DYS437	14–16	3	0.4573	0.52	1.09
DYS388	12–17	6	0.3997	0	0
DYS393	12–15	4	0.3419	0	0
DYS392	11–14	4	0.3214	0.52	1.09

sive empirical studies in different populations [11]. Since a limited forensic value of DYS438 was demonstrated in such distant European populations as those of Poland and Portugal [12], its redundancy may be confirmed also in other populations and should be taken into consideration if nonexclusion has to be verified by typing additional Y-STR markers.

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