ORIGINAL ARTICLE

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PCR analysis of the short tandem repeat (STR) system HUMVWA31

Allele and genotype frequencies in an Italian population sample

Abstract A population study in a sample of 211 unrelated individuals from 2 cities in North and Central Italy was carried out to investigate the short tandem repeat (STR) system HUMVWA31. Separation of PCR- amplified DNA fragments was performed by high-resolution horizontal denaturing polyacrylamide gel electrophoresis (PAGE) followed by silver staining. The 7 common alleles were found, together with a new smaller allele. Distribution of the observed genotypes did not deviate from Hardy-Weinberg (H-W) equilibrium. The power of discrimination for this locus was 0.93 and the chance of exclusion was 0.61. Good agreement was found between the allele frequencies in 2 Italian population samples and previous studies on Caucasians. The results of this study suggest that this STR system may be a useful tool in forensic investigations.

Key words STR-HUMVWA31 · PCR-DNA · Polymorphism · Population genetics

Zusammenfassung Eine Populationsstudie an einer Stichprobe von 211 nicht verwandten Individuen aus 2 Städten in Nord- und Zentralitalien wurde mit dem Short-Tandem-Repeat (STR)-System HUMVWA31 durchgeführt. Die Auftrennung der PCR-amplifizierten DNA-Fragmente

erfolgte mittels hochauflösender, horizontaler, denaturierender Polyacrylamidgelelectrophorese (PAGE) mit anschließender Silberfärbung. Die 7 bekannten Allele wurden gefunden, zusammen mit einem neuen, kleineren Allel. Die Verteilung der beobachteten Genotypen wich nicht vom Hardy-Weinberg-(H-W)-Gleichgewicht ab. Die Diskriminationsstärke betrug für diesen Locus 0.93 und die Ausschlußchance 0.61. Ein Vergleich der Allelfrequenzen zwischen den 2 italienischen Populationsstichproben und mit zuvor durchgeführten Studien an Kaukasiern zeigte gute Übereinstimmungen. Die Ergebnisse dieser Studie legen nahe, daß dieses STR-System für forensische Untersuchungen gut geeignet ist.

Schlüsselwörter STR-HUMVWA31 · PCR-DNA · Polymorphismus · Populationsgenetik

Introduction

Many laboratories worldwide are studying a series of highly polymorphic loci whose polymorphism derives from dimeric-trimeric-tetrameric tandem repeated core sequences, named short tandem repeat (STR) (Weber and May 1989; Edwards et al.1991). The present study investigated the polymorphism of the STR system HUMVWA31, first described by Kimpton et al. (1992). Before being routinely introduced into forensic investigations, further studies are needed on this system: 1) to investigate allele frequency distribution; 2) to establish the real number of alleles; and 3) to compare samples for intra- and inter-ethnic differences.

Materials and methods

A collaborative study was carried out on blood samples obtained from healthy unrelated donors living in Ancona ($n=114$) and Parma ($n=97$).

DNA was extracted as described by Budowle and Baetchel (1990). DNA concentration was estimated by spectrophotometry (Bausch and Lomb) or on a quantitation test gel with K562 DNA (Promega).

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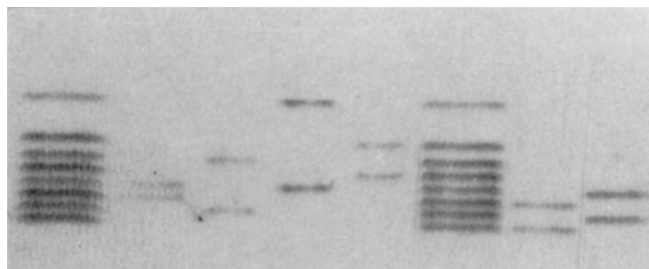Istituto di Medicina Legale, Via Falloppio 50, Padova, Italy

Fig. 1 Silver-stained HUMVWA31 system polyacrylamide gel of the amplified fragments. From left to right: allelic ladder is in lane 1, 6. HUMVWA31 phenotypes: lane 2: 17–18; lane 3: 15–19; lane 4: 11–17; lane 5: 14–16; lane 7: 18–20; lane 8: 17–19

Table 1 Allele frequencies in two Italian population samples

HUMVWA31 allele	Ancona 114 individuals	Parma 97 individuals	Combined data
11	0.000	0.005	0.002
14	0.096	0.077	0.087
15	0.074	0.123	0.097
16	0.197	0.175	0.187
17	0.280	0.314	0.296
18	0.223	0.221	0.223
19	0.096	0.072	0.085
20	0.030	0.010	0.021

Heterogeneity test: $\chi^2 = 7.6700$, $P = 0.3420 \pm 0.0150$;
G statistic $= 8.2023$, $P = 0.3390 \pm 0.0150$

H-W equilibrium expectation
$\chi^2 = 22.116$ $0.75 < P < 0.90$ $df = 28$

PCR amplification was performed according to Wiegand et al. (1993a) in a PTC 100–60 thermal cycler or Minicycler (MJ Research Inc.).

Electrophoresis was carried out as suggested by Wiegand et al. (1993b) on denaturing PAGE using a discontinuous buffer (Allen et al. 1989). Bands were visualised by silver staining (Budowle et al. 1991). Unknown samples were compared with an allelic ladder consisting of a mix of commonly found alleles (Fig. 1). Taq-Cycle-Sequencing of an uncommon fragment was performed as described by Möller and Brinkmann (1994) on an ABI 373A Sequencer using the Taq-Dye-Deoxy-Terminator Cycle Sequencing Kit (Applied Biosystems).

The 2 Italian population samples were tested for heterogeneity with an $R \times C$ contingency table using a computer program kindly provided by G. Carmody (Carleton University, Ottawa, Canada). The same program was used to compare allelic observations between Italian and other populations.

The chi-square test between observed and expected genotype frequencies was calculated assuming the Hardy-Weinberg law. Results were also verified by comparing observed and expected heterozygosity frequencies. The expected value is equivalent to allelic diversity (Nei 1978) and $\sqrt{\left[h \left(1 - h \right) / N \right]}$ is the formula needed to compute the standard error for h, where h the expected heterozygote frequency and N the number of subjects examined.

The power of discrimination (PD) was calculated using Fisher's (1951) equation. The exclusion chance was calculated from allele frequencies (Garber and Morris 1983).

Results

The 2 Italian population samples (Table 1) were tested for the STR system HUMVWA31; a total of 8 discrete alleles, identified in the range 126–162 bp, was found. A new smaller allele was also found (Fig. 1) composed of 11 repeats showing the following sequence: TCTA (TCTG)₃ (TCTA)₇. The 2 Italian population samples were preliminarily tested for heterogeneity with an $R \times C$ contingency table and showed a similar distribution of allele frequencies ($\chi^2 = 7.6700$, $P = 0.3420 \pm 0.0150$; G statistic $= 8.2023$, $P = 0.3390 \pm 0.0150$).

The chi-square test showed good agreement between observed and expected values ($0.75 < P < 0.90$) (Table 1). The heterozygosity rate and allele diversity value were 0.82 and 0.80 ± 0.027, respectively. The chance of exclusion was 0.61, the PD was 0.93.

When the allele frequencies of our work were compared with those reported for samples of 148 Swiss (Gehrig and Coquoz 1993) and 100 Caucasians (Kimpton et

al.1992), no heterogeneity was found ($\chi^2 = 9.4256$, $P = 0.8350 \pm 0.0117$; G statistic $= 9.9069$, $P = 0.8160 \pm 0.0123$). Comparison with data calculated from the histograms of Hou et al. (1994) in Chinese subjects gave no agreement (data not shown).

Discussion

A total of 211 subjects living in 2 Italian regions were typed for the STR system HUMVWA31. The 7 common alleles (Kimpton et al. 1992; Gehrig and Coquoz 1993), classified according to the number of repeat sequences, were found, together with a new shorter allele. This allele, sequenced and identified as HUMVWA31*11, showed a (TCTG)₃ block in its sequence. Studies in Caucasians proved that 4-TCTG repeats are more frequent (Möller et al. 1994). A further new allele, assigned as HUMVWA31*13, was described by Hou et al. (1994), suggesting that other alleles are present in other populations.

The results of statistical analysis were satisfactory and highly informative, suggesting that this STR system may be a powerful tool for forensic purposes. Preliminary tests on bloodstains and other biological material (data not shown) demonstrate the potential efficiency and suitability of this marker for routine application in paternity and stain investigations.

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