TECHNICAL NOTE

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The DRPLA CAG Repeats in an Italian Population Sample: Evaluation of the Polymorphism for Forensic Applications

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ABSTRACT: The DRPLA CAG repeats polymorphism has been studied in an Italian population sample. PCR amplification, manual PAGE and silver staining were employed. A total of 16 different alleles, spanning the range from 5 to 21 CAG triplettes, was observed. The heterozygosity was 0.81 and no significant deviation from Hardy-Weinberg equilibrium was found. 81 meioses from parentage testing were also analyzed and a Mendelian pattern of inheritance was observed in all cases. In addition, we could successfully type DRPLA locus in some forensic specimens, 1 ng of DNA allowing clear definition of alleles. The authors conclude that the DRPLA CAG repeats analysis may be useful for forensic applications.

KEYWORDS: forensic science, dentatorubral-pallidoluysian atrophy, polymorphism, polymerase chain reaction, CAG repeat, population genetics, Italy, DNA typing

Short tandem repeats (STR) loci are highly informative polymorphic markers that are gaining popularity for genetic identification. The Dentatorubral-pallidoluysian atrophy (DRPLA) is an autosomal dominant neurodegenerative disorder more common in Japan and rare in other parts of the world (1–3), that results associated with an unstable CAG-repeat expansion in a gene located on chromosome 12p (1,4). Recent studies via PCR (1,3,5) have reported that the normal population at the DRPLA locus is very polymorphic (heterozygosity $> 80\%$) with bimodal distribution ranging between 7–25 CAG repeats that are inherited under Mendelian rules, while affected individuals have a range between 49–75 repeats; intermediate alleles were not observed. Analysis (3) of the distribution of CAG repeats at the DRPLA locus in 10 geographically and ethnically diverse populations: 3 of African origin, 4 Caucasian, 2 Asian Mongoloid populations and a Polynesian sample from Samoan island, detected a total of 20 alleles in the range 6–25 CAG repeats. The alleles >18 repeats were more frequent in population of Asian and Pacific origin in comparison with Africans and Caucasians: the parallel decreasing incidence of DRPLA from Japanese to Caucasian to African populations agrees with the proposition (6) that larger alleles in the Japanese population constitute a source for expansion to the disease-causing range. We have analyzed a sample of Italian population to evaluate the polymorphism of DRPLA locus; besides, to verify the forensic potential of this STR, 81 meioses from parentage testing, PCR sensitivity and forensic samples were submitted to the study.

Materials and Methods

Blood samples from 200 unrelated healthy individuals from the Bologna area were extracted using "salting out" method (7).

In addition 30 forensic samples aging from 1 week to 6 years were analyzed: DNA was recovered with Chelex 100 (8) from 8 blood stains, 6 semen stains and 6 buccal scrapings; from 2 cigarette butts the Chelex extracts were concentrated using Centricon™ 100 tubes (9); an organic extraction procedure was applied to 4 hair roots (10) and to 4 paraffin embedded tissues (11).

A commercial kit (ACES™ 2.0^+ Human Quantitation System, Gibco BRL, Bethesda, MD) was employed according to manufacturer's instructions to estimate DNA content. PCR was performed in a Thermal Cycler 480 (Perkin Elmer Cetus) with 10–20 ng of genomic DNA derived from blood donors in a 25 μL reaction volume containing amplification buffer, 1.5 mM $MgCl_2$, 100 μM dNTPs, 1 unit of Taq DNA Polymerase (Amplitaq, Perkin Elmer Roche, USA) and 0.2 μM of each primer: 5′CACCAGTCTCAACACATCACCATC, the same forward primer of the original pair of Li et al. (5) for clone CTG-B7 (PC/GENE sequence 699-722); 5′CAGGGAGGGAGACATGGCGTAAG, corresponding to PC/GENE sequence 886-864, external to the original reverse primer (PC/GENE sequence 839-816).

PCR conditions for 30 cycles were: 94°C-1 min, 55°C-1 min, 72°C-1 min with final extension of 72°C for 10 min. PCR products were resolved on a 8% polyacrylamide horizontal native gel as described by Allen et al. (12) and silver stained according to Budowle et al. (13). For determination of number of CAG repeats,

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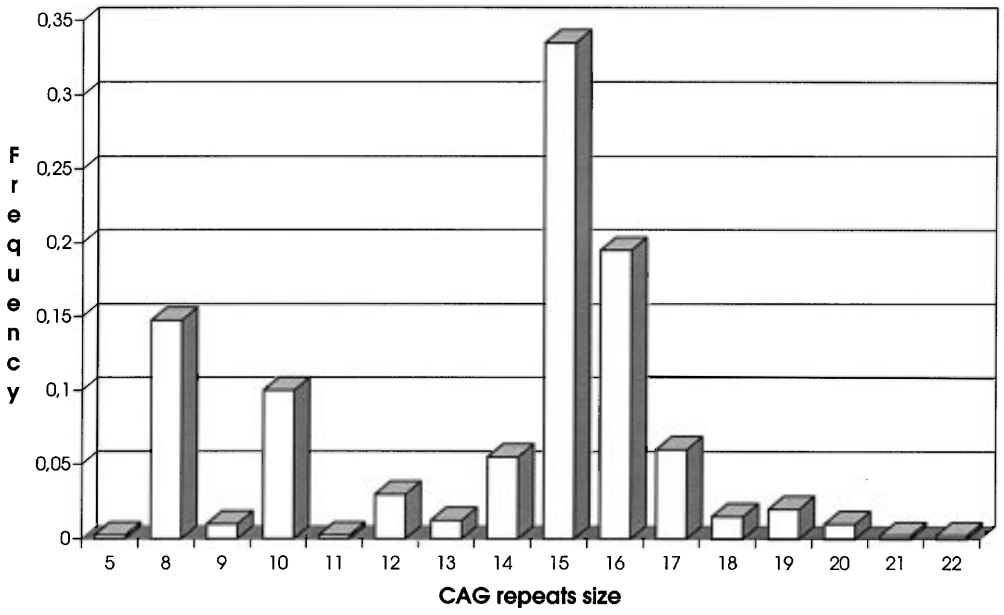

5	0.0025
8	0.1475
9	0.0100
10	0.1000
11	0.0025
12	0.0300
13	0.0125
14	0.0550
15	0.3350
16	0.1950
17	0.0600
18	0.0150
19	0.0200
20	0.0100
21	0.0250
22	0.0250
h	0.84
p	0.43 ±0.01
PIC	0.78

FIG. 1—*Frequency distribution of CAG repeat at DRPLA locus in the Italian population sample.*

some samples were amplified with a radiolabeled primer and compared with a sequenced allelic ladder.

Statistical analysis for Hardy-Weinberg equilibrium was performed using an exact test (14) based on a Markov chain method to estimate the exact p-value. The standard error of this estimate is also reported. The Polymorphism Information Content (PIC) (15) and the heterozygosity value (h) (16) were calculated as previously described. An R × C contingency table was used to test homogeneity of the allele frequency distribution between different populations: the allele frequencies for Caucasian and Japanese samples were calculated from histograms published by Deka et al. (3) and Nagafuchi et al. (4).

Results and Discussion

A total of 16 different alleles, spanning the range from 5 to 21 CAG triplets, was observed in the sample of unrelated individuals. The allele frequency distribution of CAG-repeats is presented in Fig. 1. No significant deviation from Hardy-Weinberg equilibrium was found. The exact test (9) revealed a p value of 0.43 ± 0.01. Our study has also shown that the DRPLA locus is highly polymorphic in the Italian population (PIC = 0.78) and the allele frequencies demonstrate a bimodal distribution (Fig. 1) with modes corresponding to 8–10 and 14–17 CAG repeats, similar to that reported in CEPH families (5), Japanese controls (4) and Caucasian controls (2). The unbiased estimate of heterozygosity (10) was 0.81, corresponding to values reported in other populations. A small allele 5 was found while no alleles larger than 21 repeats were observed in the 400 chromosomes tested and this result is consistent with the reported low frequency of DRPLA in Caucasian populations. The pairwise comparisons showed homogeneity between our data and Caucasian allele frequencies (χ^2 = 20.86, p = 0.184, d.f. = 16) and a significant heterogeneity with the Japanese population (χ^2 = 231.96, p = 0.0000, d.f. = 20). In our family studies a Mendelian pattern of inheritance was observed in 81 meioses, in accordance with the findings obtained using traditional paternity tests and other STR analysis. This confirms the previously reported meiotic stability exhibited by the normal range allele. With DNA quantitation assays PCR sensitivity was of 1 ng and, from all the forensic specimens investigated, sufficient DNA was obtained allowing a correct typing (data not shown). In conclusion PCR analysis of the CAG repeats at DRPLA locus may be useful for forensic applications.

The rare chance of detection of a mutant allele may be in itself of great value for individual diagnosis in criminal investigations. If it occurred in a paternity test, the interested person should be informed in any case, either of a pathological range (in an unrecognized desease) or of an intermediate range for which there is risk of transmitting a longer repeat (17).

References

1. Koide R, Ikeuchi T, Onodera O, Tanaka H, Igarashi S, Endo K, et al. Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). Nat Genet 1994;6:9–13.
2. Warner TT, Williams L, Harding AE. DRPLA in Europe. Nat Genet 1994;6:225.
3. Deka R, Miki T, Yin S-J, McGarvey ST, Shriver MD, Bunker CH, et al. Normal CAG repeat variation at the DRPLA locus in world populations. Am J Hum Genet 1995;57:508–11.
4. Nagafuchi S, Yanagisawa H, Sato K, Shirayama T, Ohsaki E, Bundo M, et al. Dentatorubral and pallidoluysian atrophy expansion of an unstable CAG trinucleotide on chromosome 12p. Nat Genet 1994;6:14–8.
5. Li S-H, McInnis MG, Margolis RL, Antonarakis SE, Ross CA. Novel triplet repeat containing genes in human brain: cloning, expression, and length polymorphisms. Genomics 1993;16:572–9.
6. Burke JR, Ikeuchi T, Koide R, Tsuji S, Yamada M, Pericak-Vance MA, et al. Dentatorubral-pallidoluysian atrophy and Haw River syndrome. Lancet 1994;344:1711–2.
7. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
8. AmpliTipe™ Users Guide, Version 2, Cetus Corporation 1990.
9. Hochmeister MN, Budowle B, Jung J, Borer UV, Comey CT, Dirnhofer R. PCR-based typing of DNA extracted from cigarette butts. Int J Leg Med 1991;104:229–33.

10. Higuchi R, von Beroldingen CH, Sensabaugh GF, Erlich HA. DNA typing from single hairs. Nature 1988;332:543–6.
11. Wrigh DK, Manos MM. Sample preparation from paraffin-embedded tissues. PCR Protocols, Academic Press Inc., San Diego, California, 1991;153–8.
12. Allen RC, Graves G, Budowle B. Polymerase chain reaction amplification products separated on rehydratable polyacrylamide gels and stained with silver. Biotechniques 1989;7:736–44.
13. Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen R. Analysis of the VNTR locus D1S80 by PCR followed by high resolution PAGE. Am J Hum Genet 1991;48:137–44.
14. Guo SW, Thompson EA. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 1992;48: 361–72.
15. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 1980;32:314–31.
16. Nei M, Roychoudhury AK. Sampling variances of heterozygosity and genetic distance. Genetics 1974;76:379–90.
17. Ikeuchi T, Koide R, Onodera O, Tanaka H, Oyake M, Takano H, Tsuji S. Dentatorubral-Pallidolusyan Atrophy (DRPLA). Clin Neurosci 1995;3:23–7.

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