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SHORT COMMUNICATION

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Identification of a base pair substitution at the tetranucleotide tandem repeat locus DHFRP2 $(AAAC)_n$ in a worldwide survey

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Abstract An allele heterogeneity in a short tandem repeat at the human dihydrofolate reductase pseudogene (DHFRP2) was detected using non-denaturing gel electrophoresis and ethidium bromide staining. Sequence analysis of the allele, designated 9A, revealed the C to A substitution in the 8th AAAC repeat. A survey of 16 worldwide human populations showed that this mutation was spread through five continents at a relatively high frequency (up to $p = 0.19$ in Europeans). Some statistical parameters of forensic interest were also calculated (h, PD, EC and PIC) for this polymorphism. This type of heterogeneity stresses the complexity of STR variation.

Key words STR · Mutation · DHFRP2 · PCR · Population genetics

Introduction

The human dihydrofolate reductase psi-2 pseudogene (DHFRP2) localized on chromosome 6, contains a polymorphic 4 bp STR locus [1]. The present study describes the identification of a repeat sequence heterogeneity at this tetranucleotide STR locus. In a large population survey 702 chromosomes from 16 different human populations covering five continents have been analysed (Table 1).

Materials and methods

PCR amplifications were performed using primers and PCR conditions as previously described [1] in a Thermal Cycler 480 (Perkin Elmer Cetus). The amplification products were run in 8% non-denaturing polyacrylamide gel electrophoresis for 15 h at 140V, and stained in 5% ethidium bromide solution for 1 h. DNA from three heterozygous individuals containing alleles 7 and 9, 7 and 8, 6 and 10, was used to construct an allelic ladder (Fig. 1, lane L). Sequence analysis indicated that alleles constituting the ladder differed from each other by multiples of the AAAC repeat. Samples homozygote for allele 9A were also sequenced. Purification and sequencing of PCR products was performed as described [2] using the same primers as for PCR amplifications. Sequencing of Alleles 9, 10 and 9A was repeated using an ABI 373A sequencer. Individual alleles (Table 1) were designated according to the repeat number in line with the recomendations of the DNA commission of the International Society for Forensic Haemogenetics [3]. Hardy-Weinberg equilibrium was assessed through conventional Pearson's χ^2 methods. Some statistical parameters of genetic and medico-legal interest (Table 1) were calculated. The power of discrimination (PD) was calculated following Fisher's method [4], the heterozygosity value (h) [5], the chance of exclusion (CE) [6] and the polymorphism information content (PIC) [7] were calculated as previously described.

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Results and discussion

During the population analysis of DHFRP2, an allele was discovered which migrated faster than allele 10 but slower than 9 (Fig. 1). It was called 9A because although the nomenclature for complex repeats has been widely discussed in forensic circles [3], allele designations for fragments with the same size but different sequence are problematical and non-standardized. This allele was suspected to be a non-integral repeat or another anomaly as largely described in other STR loci [8–10]. While sequences for alleles 9 and 10 were shown to be $(AAAC)_9$, and $(AAAC)_{10}$ respectively, allele 9A was shown to have one substitution C to A giving $(AAAC)_7$ AAAA $(AAAC)_1$. This allele has been found quite frequently (Table 1) and in populations from five continents. The sequences obtained with the automated sequencer did not show any base pair differences within the flanking region of the microsatellite repeat between the different alleles. Allele frequencies, PD, h, CE and PIC values for 16 worldwide populations are given in Table 1.

Table 1 Allele frequencies, polymorphism information content (PIC), discrimination power (PD), heterozigosity (h) and chance of exclusion (CE) for locus DHFRP2 in 16 different worldwide populations; (*CPY* Biaka pygmies, *ZPY* Mbuti pygmies, *LIS* Lisongo, *MEL* Melanesian, *AUS* Australian, *NGU* New Guinean, *MAY* Mayan, *SUR* Surui; *KAR* Karitiana; *CHI* Chinese; *JAP* Japanese, *CAM* Cambodian, *NIT* North Italian, *NEU* North European, *CAT* Catalan, *BAS* Basque)

DHFRP2	n	6	7	8	9A	9	10	11	PIC	PD	h	CE
CPY	20	0.150	0.450	0.050	0.050	0.100	0.200	0	0.685	0.886	0.720	0.504
ZPY	20	0	0.800	0.050	0	0	0.050	0.100	0.326	0.551	0.345	0.190
LIS	20	0	0.650	0	0.100	0.100	0.150	0	0.498	0.747	0.535	0.318
MEL	22	0.136	0.181	0.682	0	0	0	0	0.434	0.684	0.484	0.256
AUS	20	0.150	0.200	0.550	0.050	0.050	0	0	0.587	0.819	0.630	0.397
NGU	20	0.150	0.350	0.450	0	0.050	0	0	0.584	0.811	0.650	0.379
MAY	20	0.150	0.150	0.100	0.050	0.250	0.300	0	0.759	0.925	0.790	0.590
SUR	18	0.444	0.444	0	0	0	0.112	0	0.505	0.746	0.593	0.299
KAR	26	0.077	0.192	0	0	0.423	0.308	0	0.626	0.842	0.683	0.420
CHI	20	0.100	0.350	0.250	0	0.150	0.150	0	0.722	0.905	0.760	0.540
JAP	20	0.050	0.450	0.300	0.050	0.050	0.100	0	0.643	0.857	0.690	0.454
CAM	20	0.100	0.450	0.250	0.100	0	0.100	0	0.663	0.871	0.705	0.475
NIT	28	0.071	0.572	0.179	0	0.071	0.107	0	0.583	0.819	0.619	0.399
NEU	32	0.094	0.500	0.125	0.188	0.062	0.031	0	0.651	0.866	0.678	0.469
CAT	176	0.074	0.455	0.171	0.159	0.085	0.057	0	0.691	0.891	0.722	0.513
BAS	220	0.045	0.555	0.177	0.141	0.032	0.045	0.005	0.610	0.838	0.636	0.425

n = number of chromosomes analysed

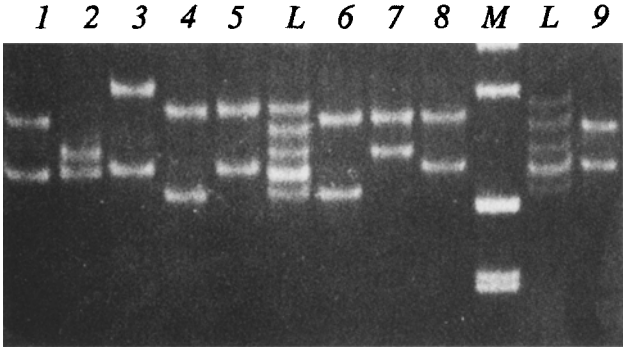

Fig. 1 Separation of PCR amplified fragments of the DHFRP2 polymorphism in non-denaturing acrylamide gel electrophoresis. Lanes 1–9 contain alleles: 7/9A, 7/8, 7/11, 6/10, 7/10, 6/9A, 8/9A, 7/9A, and 7/9 respectively. Lanes marked L contain the allelic ladder made from alleles 6–10. Lane marked M containsthe molecular weight marker PBR 322 (Msp 1)

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