FORENSIC EFFICIENCY AND GERMAN POPULATION DATA FOR THE TETRAMERIC STR POLYMORPHISM DHFRP2 (HUMFOLP23)

Mark Benecke, Cornelia Schmitt, Mechthild Prinz

Institute for Forensic Medicine, University of Cologne, DNA Research Dept., D-50923 Koeln

Introduction

Hypervariability in short tandem repeated sequences of genomic DNA (STRs) is one of the most reliable ways of personal identification from biological samples. We examined the allelic distribution of the human dihydrofolate reductase psi-2 pseudogene (Polymeropoulos et al. 1991) in a Western German population (Rhine area). These were compared to population data first described by Polymeropoulos et al. and by Kimpton et al.. To reach highest possible detection sensitivity, separation and detection of PCR products were performed using an Automatic Laser Fluorescence Detection Unit (A.L.F. sequencer, Pharmacia) (Schmitt & Prinz, in press).

Material and Methods

Whole blood DNA of unrelated West Germans was amplified using fluorescent primers (FPLCpure, Pharmacia) as given in Polymeropoulos et al. 1991 (20-/21-mer, resp.) on the Perkin Elmer GeneAmp PCR System 2400 (29 cycles, 58°C annealing) and on the Biometra Thermoblock (28 cycles, 60°C annealing). Mastermix: Primer 0,5 µM each in 25 µl, 2 U Taq Polymerase (Promega), 150 µM dNTPs, 10 mM Tris-HCl pH 9,0, 0,1% Triton X-100 (Promega buffer).
1-5 µL of PCR product each were loaded on a Pharmacia A.L.F. Sequencer (green laser); data processing was performed by internal software exclusively. Quantitation of DNA was done with the ACES Human DNA Quantification System (Gibco Life Technologies).

Results

Analysing the allelic distribution of 102 unrelated Germans we detected six alleles; minor differences to the allelic distribution given in the original paper were found (Fig. 1). Heterozygosity was 72 %. Using the exact test (Guo & Thompson 1992; formula implemented in the „DNA View" software package (Charles Brenner, Berkeley)) and assuming 15 degrees of freedom, alleles were in Hardy-Weinberg equilibrium. No slippage bands were observed.

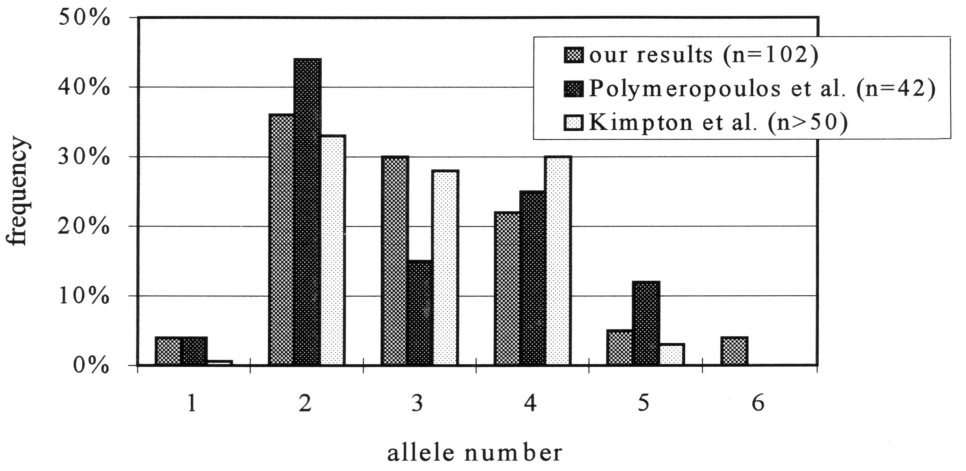

Figure 1: Allele frequencies of DHFRP2 (HUMFOLP23) in Caucasians. Alleles 1-5 are alleles A5-A1 at Polymeropoulos et al.. Accordingly, #1 is the shortest allele.

Amplification of samples containing less then 50 pg of DNA led to clear signals on the A.L.F. sequencer (Fig. 2) in homozygous genotypes. No DNA-dependent band shifts occurred but baseline sometimes got triflingly fuzzy at lowest DNA level.

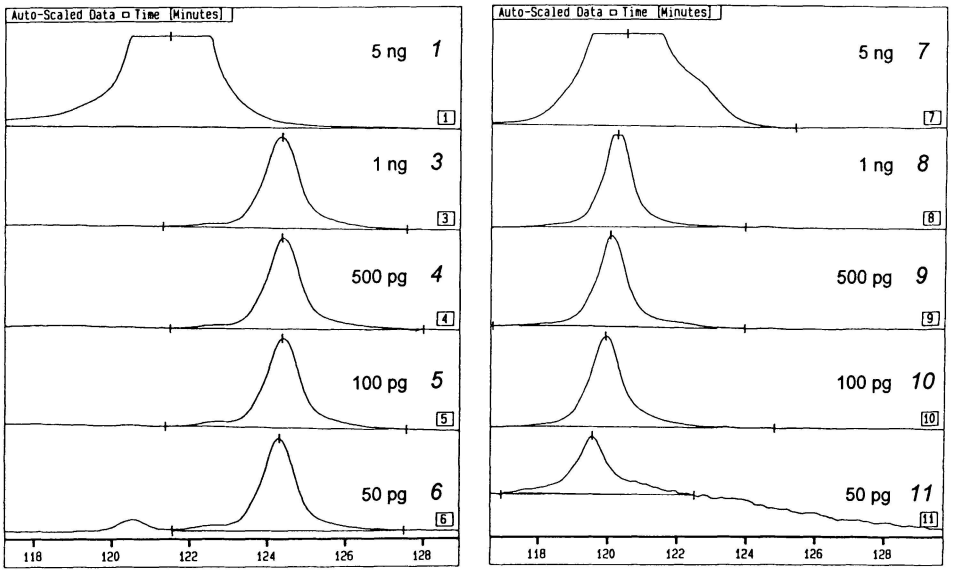

Figure 2: Sensitivity of DHFRP2/HUMFOLP23. 1-6: Decreasing amounts of homozygous DNA of person A. Band/allele shift of the 5 ng signal is due to the use of the first slot of the A.L.F.-PAG. 7-11: Decreasing amounts of DNA of person B.

Discussion

Due to
- the simple repeat structure (Urquhart at al. 1994),
- the short fragment length,
- the low detection limit and
- the non-occurrence of slippage bands

this locus should be a valuable forensic marker.
Another primer sequence for the same locus which we did not try out was suggested by Urquhart et al. 1994.

References

GUO SW, THOMPSON EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48, 361-372

KIMPTON CP, GILL P, WALTON A, URQUHART A, MILLICAN ES, ADAMS M (1993) Automated DNA profiling multiplex amplification of short tandem repeat loci. PCR Methods and Application, 13-22

POLYMEROPOULOS MH, XIAO H, RATH DS, MERRIL CR (1991) Tetranucleotide repeat polymorphism at the human dihydrofolate reductase psi-2 pseudogene (DHFRP2). Nucleic Acids Research 19, 4792

SCHMITT C, PRINZ M (1995) Anwendbarkeit eines DNA-Sequenzierautomaten für die PCR-Typisierung von forensischen Spuren. (Applicability of a DNA sequencer for forensic PCR typing.) Rechtsmedizin, in press

URQUHART A, KIMPTON CP, DOWNES TJ, GILL P (1994) Variation in short tandem repeat sequences-a survey of twelve microsatellite loci for use as forensic identification markers. International Journal of Legal Medicine 107, 13-20