STUDIES ON THE HUMACTBP2 (SE33)

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INTRODUCTION

The short tandem repeat (STR) HumACTBP2 (human beta-actin related pseudogene) displays a highly variable polymorphism of length. Therefore it has a high information content in trace analysis and paternity examinations. The purpose of the study was to answer the question of how exact a determination of the alleles of the HumACTBP2 system may be obtained with the ABI 373A sequencer by means of a genetic population study involving 327 non-related persons of the greater Munich area. Apart from the internal standard GS350, an allelic ladder was added to each sample in order to increase the accuracy of allele determination. In addition, families with proven paternity were examined and scanned for mother/child or father/child mismatches.

MATERIALS AND METHODS

DNA was prepared from whole blood using the QIAmp Blood Kit, Qiagen or standard methods (Manniatis et al. 1989).
Amplification conditions: After quantification PCR reactions were carried out using 1-5 ng of genomic DNA in a 25 µl reaction volume. Reaction mixtures consisted of 10 mM Tris(HCl) pH 8,3; 50 mM KCl; 1,5 mM $MgCl_2$; 0,1% Tween 20; 200 µM dNTPs; 1,25 U Taq polymerase (Perkin Elmer, USA); 0,15 µM of each Primer (HumACTBP2/1 5′ AAT CTG GGC GAC AAG AGT GA 3′ labelled with "6-FAM" or with "HEX" (ABD) for the internal allelic ladder, HumACTBP2/2 5′ AAT CTG GGC GAC AAG AGT GA 3′, Polymeropoulos et al. 1992). Amplification reactions were carried out on a Perkin Elmer Cetus 9600 thermal cycler and consisted of 30 cycles of 95 °C for 20s, 59 °C for 40s, 72 °C for 20s followed by a final 7 min incubation at 72°C.
Detection: Aliquots of each amplification reaction were combined with the internal lane standard GS350 (ROX) and a HEX-labelled allelic HumACTBP2-ladder. Samples were loaded onto 6% denaturing gels (19:1, 24 cm well to read) and run in 1xTBE on an ABD 373A Sequencer for 7 h at constant power (30 Watts). Fragment sizes were determined using Genescan 672 Software employing the Local Southern method.
Taq-Cycle-Sequencing: After Etidiumbromid-staining DNA bands were cut from a 6% PAA gel, eluated using the "crush and soak" method (Maniatis et al. 1989) and

reamplified in a 100 µl reaction volume. Amplification products were desalted and concentrated using Microcon 50 (Amicon, Beverly, USA). Sequencing reactions were carried out using the Taq Dye-Deoxy-Terminator Cycle Sequencing Kit (ABD) with 100 - 300 ng template DNA and 6 pmol sequencing primer (5'AAT CTG GGC GAC AAG AGT GA 3'), 25 cycles total of 96 °C 15s, 58 °C 15s, 60 °C 4 min.

RESULTS

A genetic population study was carried out involving the STR-system HumACTBP2 of 327 non-related persons of the greater Munich area. In order to increase accuracy, an allelic ladder was added to all samples as additional internal standard. The frequency distribution obtained is shown in Fig. 1. Nomenclature from the allelic ladder followes Kratzer et al. 1994.

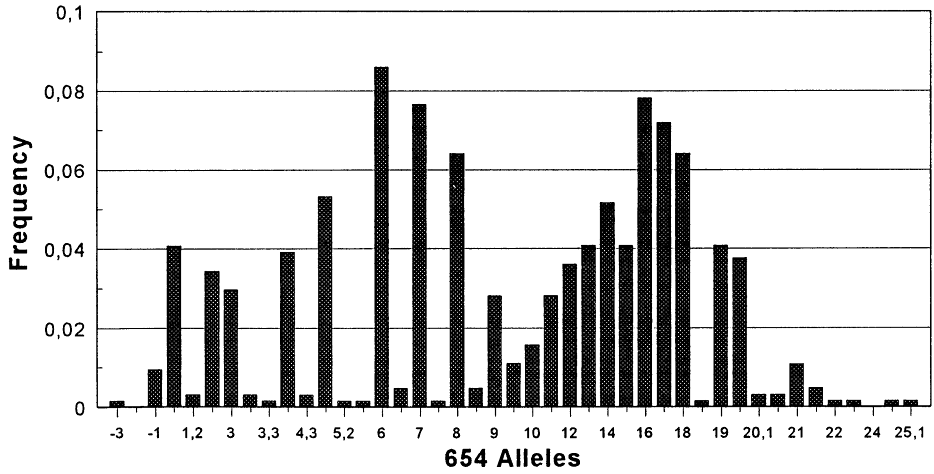

Fig. 1: Distribution of the HumACTBP2 Alleles

Using Genescan analysis in the 223 to 325 bp range 41 alleles with frequencies between 0,15% and 8,26% could be differentiated. Twenty of these alleles displayed an allele frequency of less than 1%. Data found in other examinations concerning population genetics let the discovery of additional alleles seem possible (Wiegand 1993 et al., Kratzer et al. 1994 Cabrero et al. 1995).
The power of discrimination of HumACTBP2 was 0,995 and heterocygosity was 0,938.

The alleles of the additional allelic ladder showed gel-to-gel differences between 0,8 and 2,3 bp, depending on allele size, in the 235 to 318 bp segment. Gel-to-gel differences bigger than 1% were found only in alleles larger than 250 bp. Lane-to-lane differences in gels with 24 or 36 tracks were less than 0,01 bp.

Examination of families with proven paternity showed one mutation per 176 meioses. A total of 2 mutations was found. After sequencing with the Taq Cycle sequencing reaction one (GAAA) repeat unit was lost in both cases respectively.

Sequencing of the allele 16 (291 bp, Genescan) shows a $(GAAA)_{11}$(GAAAAA) $(GAAA)_{15}$ repeat with a total fragment length of 285 bp. The Allele coresponds to allel 27 from Möller et al. 1994.

SUMMARY

A genetic population study was carried out involving the STR-system HumACTBP2 of 327 non-related persons of the greater Munich area. We detected 41 different HumACTBP2 alleles with frequencies between 0,15% and 8,26%. Some of these alleles differing by as little as one base. Data found in other examinations concerning population genetics let the discovery of additional alleles seem possible.

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