Int J Legal Med (2004) 118: 361–363
DOI 10.1007/s00414-004-0473-0

SHORT COMMUNICATION

Marielle Heinrich · Miriam Müller · Steven Rand · Bernd Brinkmann · Carsten Hohoff

Allelic drop-out in the STR system ACTBP2 (SE33) as a result of mutations in the primer binding region

Received: 20 April 2004 / Accepted: 29 June 2004 / Published online: 20 August 2004

Abstract In the course of routine genotyping of forensic reference samples by multiplex PCR, an allelic drop-out due to mutations in the primer binding regions of the highly polymorphic STR marker ACTBP2 was observed in 17 samples. The variation rate was estimated to be 0.0014 (95% confidence interval: 0.0006–0.003). The most frequently found mutation was an G to A transition in the reverse primer binding region which was present in 14 out of 17 cases. To overcome the problem we have added a modified reverse primer to different multiplex kits that led to the correct genotype.

Keywords ACTBP2 · Mutation · Primer binding region · Multiplex · Database

Introduction

ACTBP2 (SE33) is one of the eight STR systems of the German DNA databank. It is one of the most informative markers used in forensic genetics with the power of discrimination ranging from 96.6% to 98.7% [1] and a heterozygosity index of 0.94–0.966 [2, 3]. Here we report on 17 samples which were apparently homozygous in ACTBP2 when using a multiplex kit but were identified as heterozygous by reanalysing these samples in a singleplex PCR. Direct sequencing of the 17 affected alleles revealed primer bindig mutations to be responsible for the occurrence of apparent null alleles. Furthermore, we have elaborated a method to correctly type samples carrying the most frequent mutation even in a multiplex PCR format.

M. Heinrich · M. Müller · S. Rand · B. Brinkmann (✉) · C. Hohoff
Institut für Rechtsmedizin, Universitätsklinikum Münster,
Röntgenstrasse 23,
48149 Münster, Germany
e-mail: remed@uni-muenster.de
Tel.: +49-251-8355160
Fax: +49-251-8355158

Materials and methods

DNA from buccal swabs in routine cases was extracted using the Chelex/proteinase K method [4]. The following multiplex kits were used according to the manufacturers' recommendations: PowerPlex ES-System (Promega, Mannheim, Germany), genRES MPX-2 (Serac, Bad Homburg, Germany) [5], and AmpflSTR SEfiler (Applied Biosystems, Darmstadt, Germany) [6]. ACTBP2 singleplex amplification was carried out as described previously [2]. For sequence analysis primers upstream of the forward and downstream of the reverse primer were designed using the GenBank sequence information (accession no: NG_000840): ACTBP2_F_seq: 5'-acagtgagccgaggtcatgc-3' and ACTBP2_R_seq: 5'-gacaaggttctgtgctcgctg-3' (Fig. 1). Sequence analysis was performed using the Big Dye Terminator cycle sequencing kit and the ABI PRISM 310 Genetic Analyzer (Applied Biosystems). An additional reverse primer (ACTBP2_R*: 5'-aca tct ccc cta cTg cta ta-3') was designed to amplify alleles carrying the G to A transition in the binding region of the reverse primer. This primer was added to the multiplex mastermixes in different amounts (1, 2, 5 and 8 pmol, respectively).

Results and discussion

In all 17 samples apparently homozygous at ACTBP2 we were able to identify sequence variations in the primer binding regions of the affected alleles. The most frequently found mutation was a transition from G to A in the binding region of the reverse primer (407A, nomenclature according to NG_000840). This mutation was responsible for allelic drop-out in 14 cases and was observed in alleles 14, 15, 16 and 18. Another mutation in the binding region of the reverse primer is represented by a T to C transition (403T; found in allele 17). A second G to A (142T) mutation was found in the binding region of the forward primer twice in allele 27.2 (all mutations are shown in Fig. 2). Surprisingly, none of the mutations caused a mismatch located at the ultimate 3'-end of a primer but

Fig. 1 GenBank sequence for ACTBP2 (NG_000840): the commonly used PCR primers published by Polymeropoulos et al. [14] are shaded in black, the new primer pair designed for sequence analysis are shaded in grey. The localisation of the mutations found in the primer binding regions are given in capital letters

```
61  ggctgaggca ggataatcgc ttgaacctgg gaggtggagg ctacagtgag ccgaggtcat
    ccgactccgt cctattagcg aacttggacc ctccacctcc gatgtcactc ggctccagta

121 gccattgcac tccaatctgg gcgacaagag tgaaactccg tcaaaagaaa gaaagaaaga
    cggtaacgtg aggttagacc cGctgttctc acttgaggc agttttcttt ctttctttct

181 gacaaagaga gttagaaaga aagaaagaga gagagagaga aaggaaggaa ggaagaaaaa
    ctgtttctct caatctttct ttctttctct ctctctctct ttccttcctt ccttcttttt

241 gaaagaaaaa gaaagaaaga gaaagaaaga aagagaaaga aagaaagaaa gaaagaaaga
    ctttcttttt ctttctttct ctttctttct ttctctttct ttctttcttt ctttctttct

301 aagaaagaaa gaaaaagaaa gaaagaaaga aagaaagaaa gaaagaaaga aagaaagaaa
    ttctttcttt ctttttcttt ctttctttct ttctttcttt ctttctttct ttctttcttt

361 gaaagaaaga aagaaaggaa ggaaagaaag agcaagttac taTagcGgta ggggagatgt
    ctttctttct ttctttcctt cctttctttc tcgttcaatg atatcgccat cccctctaca

421 tgtagaaata tatataaacc tccttacacc gcggagaccg cgtcagccca gcgagcacag
    acatctttat atatatttgg aggaatgtgg cgcctctggc gcagtcgggt cgctcgtgtc

481 aaccttgtcc ttgccgctgc gccttgcgtc cgcacccgcc gccagctcac catggatgat
    ttggaacagg aacggcgacg cggaacgcag gcgtgggcgg cggtcgagtg gtacctacta
```

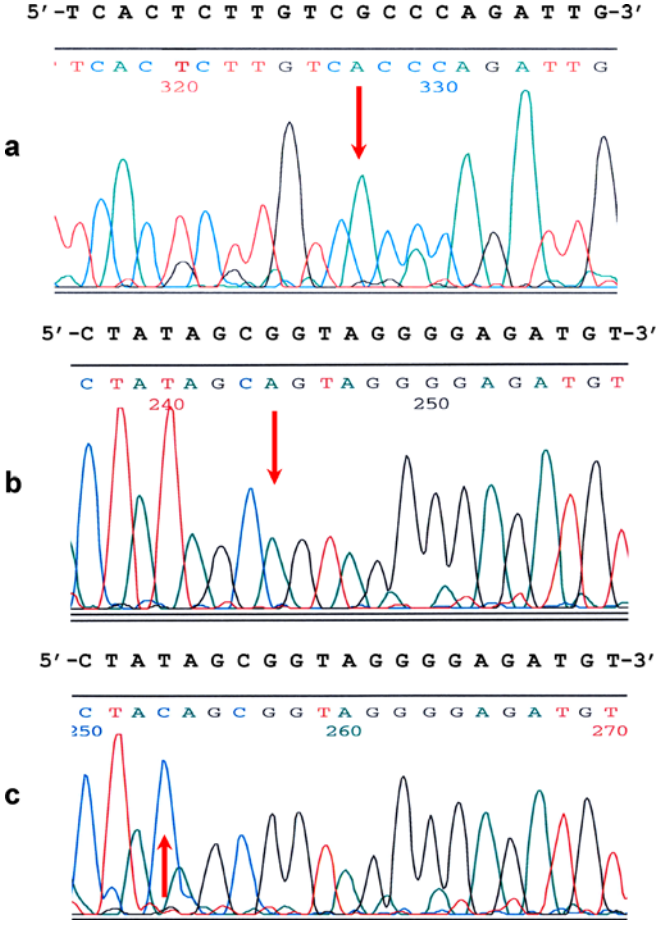

Fig. 2 Mutations found in the primer binding region of ACTBP2, **a** transition G to A in the binding region of the forward primer (142T), **b** transition G to A in the binding region of the reverse primer (407A), **c** transition T to C in the binding region of the reverse primer (403C). The wild-type sequences are given in the top line of each sequence

were located at least 4 nucleotides from the 3'-end of a primer. Nevertheless, these mutations led to a complete failure of amplification and thereby to a false homozygote result, at least in a multiplex reaction. A similar phenomenon is already known from mutations in the primer binding regions of other STRs [7, 8, 9, 10, 11, 12].

The most frequent mutation in this study, 407A, as well as 142T has been previously described by Hering et al. [13]. Additionally, they described one mutation in the forward primer binding region which was not observed in our samples. The mutation 403T has not yet been observed.

The allele distribution of the mutations (Table 1) shows that the most frequently found transition (407A) seems to be mainly associated with alleles 15 and 16 (11/14 cases).

The mutation 407A was found in 8 samples from Germany, 3 from Iraq and 1 each from Ethiopia, Turkey and Lebanon. The mutation 403C was found in 1 German sample and the mutation 142T was found in 1 sample from Togo and Germany, respectively.

The frequency of these primer binding site mutations was estimated for the systematically studied German samples as follows: in a total of 2,154 samples (4,308 chromosomes) analysed for the German DNA databank with SEfiler and PowerPlexES, 138 samples were apparently homozygous at ACTBP2 and were retyped by singleplex amplification. Out of these 138 samples 6 were in fact heterozygous. Thus, the variation rate is 0.0014 (95% confidence interval: 0.0006–0.003).

Our strategy to amplify alleles with the mutation 407A was to include a redesigned reverse primer (R*) into the

Table 1 Alleles affected by mutations in the primer binding regions of ACTBP2

Allele	407A	403C	142T
14	1[a]	-	-
15	7	-	-
16	4	-	-
17	-	1[b]	-
18	2	-	-
27.2	-	-	2[c]

Sequences were submitted to EMBL/GenBank/DDBJ as AJ746166[a], AJ746167[b]and AJ746168[c].

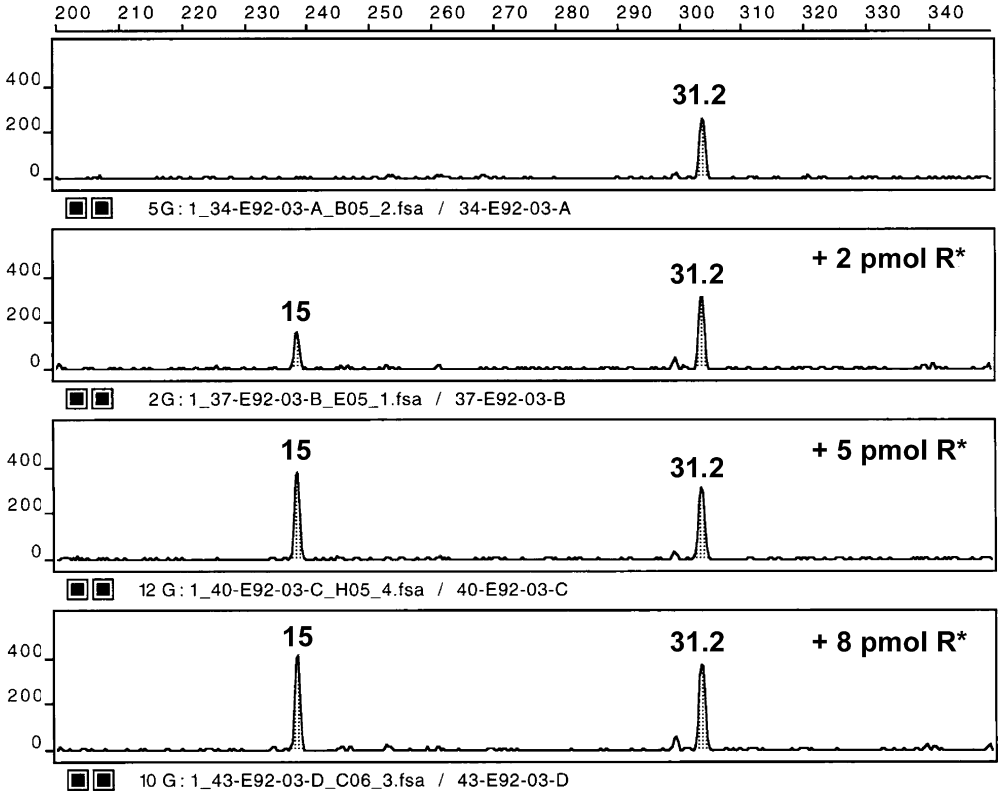

Fig. 3 Electropherogram showing the results of admixture of the modified primer R* in different amounts to the AmpflSTR SEfiler kit. Addition of 2 pmol of primer R* is sufficient for amplification of allele 15 carrying the transition G to A in the reverse primer binding region

multiplex mastermix. This primer carries a substitution C to T located 7 nucleotides from the 3' end. The admixture of primer R* to the mastermix of the SEfiler kit resulted in the amplification of both the mutated and the non-mutated alleles (Fig. 3). Similar results were obtained by using the PowerPlex ES and genRES MPX-2 kits (data not shown).

Additionally, we have tried to overcome the problem by reducing the annealing temperature of the three multiplex kits used in order to eliminate allelic drop-out of alleles with mutations in the primer binding site. A reduction of 5°C below the manufacturers' recommendations leads to amplification of both alleles (data not shown).

The admixture of a modified reverse primer to the multiplex mastermix is a simple and economical way to overcome the problem in 82% of the apparent null alleles. However, there are other mutations found in the ACTBP2 primer binding sites leading to a null allele and thus a false homozygous finding. Therefore, it is still necessary to check every apparent homozygous result obtained by multiplex analysis, e.g. by performing a singleplex analysis, preferably with a different primer pair.

Acknowledgement The authors thank Karin Rauße, Marianne Schürenkamp, Marjon Eppink and Jenny Bartsch for excellent technical assistance. This work is part of the Ph.D. thesis of M.H.

References

1. Lászik A, Sotonyi P, Rand S, Hohoff C (2001) Frequency data for the STR locus ACTBP2 (SE33) in eight populations. Int J Legal Med 115:94–96
2. Shimada I, Rand S, Brinkmann B, Hohoff C (2002) Kurdish population data for 11 STR loci (ACTBP2, CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D13S317 and D21S11). Int J Legal Med 116:301–303
3. Shimada I, Brinkmann B, Tuyen NQ, Hohoff C (2002) Allele frequency data for 16 STR loci in the Vietnamese population. Int J Legal Med 116:246–248
4. Banaschak S, Rolf B, Brinkmann B (2000) Influence of different staining techniques on the DNA analysis of histological sections. Int J Legal Med 113:114–116
5. Junge A, Lederer T, Braunschweiger G, Madea B (2003) Validation of the multiplex kit genRESMPX-2 for forensic casework analysis. Int J Legal Med 117:317–325
6. Coticone RS, Oldroyd N, Philips H, Foxall P (2004) Development of the AmpflSTR SEfiler^{TM}PCR amplification kit: a new multiplex containing the highly discriminating ACTBP2 (SE33) locus. Int J Legal Med 118:224–234
7. Leibelt C, Budowle B, Collins P et al. (2003) Identification of a D8S1179 primer binding site mutation and the validation of a primer designed to recover null alleles. Forensic Sci Int 133:220–227
8. Han GR, Song ES, Hwang JJ (2001) Non-amplification of an allele of the D8S1179 locus due to a point mutation. Int J Legal Med 115:45–47
9. Hendrickson BC, Leclair B, Forrest S et al. (2004) Accurate STR allele designations at the FGA and vWA loci despite primer site polymorphisms. J Forensic Sci 49:1–5
10. Boutrand L, Egyed B, Füredi S, Mommers N, Mertens G, Vandenberghe A (2001) Variations in primer sequences are the origin of allele drop-out at loci D13S317 and CD4. Int J Legal Med 114:295–297
11. Watanabe G, Umetsu K, Yuasa I, Suzuki T (1998) Simultaneous determination of STR polymorphism and a new nucleotide substitution in its flanking region at the CD4 locus. J Forensic Sci 43:733–737
12. Clayton TM, Hill SM, Denton LA, Watson SK, Urquhart AJ (2004) Primer binding site mutations affecting the typing of STR loci contained within the AMPFlSTR SGM Plus kit. Forensic Sci Int 139:255–259
13. Hering S, Edelmann J, Dreßler J (2002) Sequence variations in the primer binding regions of the highly polymorphic STR system SE33. Int J Legal Med 116:365–367
14. Polymeropoulos MH, Rath DS, Xiao H, Merril CR (1992) Tetranucleotide repeat polymorphism at the human beta-actin related pseudogene H-beta-Ac-psi-2 (ACTBP2). Nucleic Acids Res 20:1432