Int J Legal Med (2002) 116:365–367
DOI 10.1007/s00414-002-0306-y

TECHNICAL NOTE

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Sequence variations in the primer binding regions of the highly polymorphic STR system SE33

Received: 30 October 2001 / Accepted: 20 March 2002 / Published online: 25 May 2002

Abstract Five cases were found with sequence variation in the primer binding region of the highly polymorphic STR system SE33 used in the German genetic database. This variation can produce homozygote mistyping because of failed primer binding. We calculated a variation rate of 0.0022 (0.0006–0.0056) that could lead to complications in database matching. To avoid errors in individual genetic characterisation for SE33 as described here, it is suggested that two different primer pairs should be used.

Key words SE33 · STR · Sequence data · Mutation · Null allele

Introduction

Most reports have described mutations involving the addition or deletion of repeats from the polymorphic tandem array, presumably occurring during meiosis (Brinkmann et al. 1998). However, increasingly, there are observations about variations in the primer binding region for several STR systems (Gusmão et al. 1996; Mochi et al. 1996; Boutrand et al. 2001; Han et al. 2001) and some cases of failed primer binding were detected using different STR typing kits (Kline et al. 1998; Allen et al. 2000; Budowle 2000; Cotton et al. 2000).

The STR system SE33 (Polymeropoulos et al. 1992) which is involved in the central genetic database of the Federal Criminal Police Office of Germany (BKA), is one of the most informative systems with a heterozygosity index of 0.93–0.95 (Wiegand et al. 1993; Huckenbeck et al. 1997). It is therefore essential that when only one allele is detected this represents a true homozygote and not a non-detected heterozygote, as "null" alleles will not necessarily be recognized when there is a product from the other homologue.

The scope of this paper was to investigate the underlying mechanism of primer sequence mutations identified for the SE33 locus and to suggest how to overcome the problem.

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Material and methods

Samples (blood or buccal cell swabs) for paternity testing were obtained from Germans from the Dresden area. Genetic characterization of criminal offenders for the database was carried out using buccal swabs and approximately 95% of the tested persons were Caucasians living in Germany. Amplification of SE33 was performed either in a single PCR (Wiegand et al. 1993, annealing temperature 61°C), in a triplex PCR, with TH01 and FGA (annealing temperature 63°C, PCR primers according to Polymeropoulos et al. 1992) or using the commercially available multiplex PCR kits Mentype Nonaplex I and II (Biotype AG, Dresden, Germany; PCR conditions according the manufacturer's instructions). Samples with suspected variations in the SE33 primer binding region were further amplified in a single PCR by lowering the annealing temperature to 50°C and using the Mentype Nonaplex II kit. The primer binding sequences in the Mentype Nonaplex II kit (sequence information not available) are shifted compared to Nonaplex I kit (SE33 primers according to Polymeropoulos et al. 1992) by at least 6 base pairs with the specific aim of detecting null alleles (manufacturer's information). Analysis was carried out on an ABI PRISM 310 Genetic Analyzer (Perkin Elmer, Foster City, Calif.).

For sequencing analysis of the five cases with variation, a new primer pair (Fig. 1) was designed according to the GenBank sequence information (Moos M. and Gallwitz D. accession V00481). The direct Taq-cycle sequencing method was performed as previously described (Hering and Szibor 2000).

The sequence variation rate is given with an exact 95% confidence interval assuming a binomial distribution.

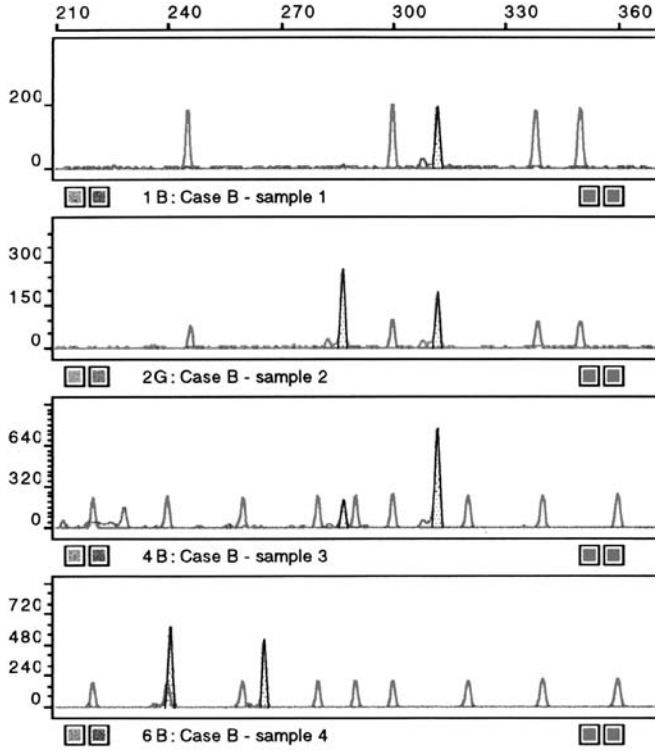

Fig. 1 Electropherogramm of case B (alleles 27.2/34) where samples 1 and 2 were amplified in a single PCR and the annealing temperatures were 63°C and 50°C, respectively. Samples 3 and 4 were amplified using the Mentype Nonaplex I and II kit, respectively, both with annealing at 58°C

Results and discussion

During paternity testing with the STR system SE33 one case (among 373 paternal and 518 maternal meioses) of isolated father/child mismatch was found with opposite homozygosity and alleles differing by more than two repeats. A null allele was taken into consideration (case A).

Analysis of SE33 for database characterization using the Mentype Nonaplex I (annealing temperature 58°C) detected 2 heterozygous samples (cases B and C) out of 554 persons with strikingly lower amplification yields of the smaller or neighbouring alleles. The most likely explanation for the discrepant amplification yield is that changes within the DNA sequence complementary to the primers, may inhibit or completely prevent annealing. In multiplex reactions amplification conditions are not optimal for all systems. Using annealing temperatures lower than the optimal temperature for specific primer/template hybridisation, it is possible to get amplification products from templates changed in the primer binding sequence.

Retyping of these samples with a reduced annealing temperature resulted in equal amplification of the second allele (cases B and C) and detected a further allele in the apparent homozygous father and child in the paternity case (case A).

Amplification results of the variation samples are given in Table 1: cases A and B gave equal amounts of amplification product for both alleles of the heterozygous genotype with Nonaplex II, while case C appeared to be homozygote – the variant allele was completely lost. Reduction of the annealing temperature from 58°C to 50°C with Nonaplex II revealed amplification of the variant allele in case C with reduced yield compared to the single PCR. An electropherogramm of case B is given as an example in Fig. 1.

To detect the presumed mutations, a new primer pair localised largely outside the common amplicon was designed but there was still an overlap of five base pairs in the forward primer region (Fig. 2).

Substitutions responsible for the null alleles observed at the SE33 locus occurred in the forward primer binding region, GenBank position 42, C to T transition (case A), position 43, G to T transversion (case B) and reverse primer, GenBank position 311, G to A transition (case C).

Retyping (annealing 50°C) of all homozygous individuals (n=24) from a sample of 361 offenders from criminal cases typed originally with an annealing temperature of 63°C, detected 2 further mutations (alleles 17 and 18, mutation type C in the reverse primer sequence). Therefore we found 4 variant alleles in a cumulated database of 915 persons (or 1,830 chromosomes) The calculated variation rate for this population is then at least 0.0022 (0.0006–0.0056).

A strong possibility must be entertained that there are a significant number of apparent homozygote SE33 genotypes in the German DNA database because of the different testing systems used by the crime laboratories. Comparing of genetic profiles would need special search algorithms to avoid false exclusions.

Table 1 Amplification results of the mutant alleles compared to the common alleles under different amplification conditions

Case	Variant allele substitution position	Common primer binding region (Polymeropoulos et al 1992)			Changed primer pair	
		Single PCR, 61/63°C annealing	Single PCR, 50°C annealing	Nonaplex I, 58°C annealing	Nonaplex II, 58°C annealing	Nonaplex II, 50°C annealing
A	42C	No product	Full amplification	Reduced yield	Full amplification	Not tested
B	43T	No product	Full amplification	Reduced yield	Full amplification	Not tested
C	311A	No product	Full amplification	Reduced yield	No product	Reduced yield

Fig. 2 GenBank sequence for SE33: the common PCR primers according Polymeropoulos et al. (1992) are underlined, the new primers designed for sequencing analysis are in capitals. The variations in the primer binding region responsible for the different null alleles are noted in bold letters

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ctacagtgag ccgaggtcAT GCCATTGCAC TCCAATCTgg gcgacaagag   50
tgaaactccg tcaaaagaaa gaaagaaaga gacaaagaga gttagaaaga  100
aagaaagaga gagagagaga aaggaaggaa ggaagaaaaa gaaagaaaaa  150
gaaagaaaga gaaagaaaga aagagaaaga aagaaagaaa gaaagaaaga  200
aagaaagaaa gaaagaaaga aaaagaaaga aagaaagaaa gaaagaaaga  250
aagaaagaaa gaaagaaaga aagaaagaaa ggaaggaaag aaagagcaag  300
ttactatagc ggtaggggag atgttgtaga aatatatata aacctcctta  350
caccgcggag accgcgtcaG CCCAGCGAGC ACAGAACCTT GTCcttgccg  400
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Conclusions

In this study, five sequence variations in the common primer binding region of SE33 were investigated. Amplification of these variant alleles under highly specific conditions failed to assist, while a reduction of the annealing temperature up to 50°C resulted in a partial or full amplification yield and detected the variant allele.

To avoid errors in individual genetic characterization result from sequence variation in the primer binding region, amplification with different primer pairs is a good strategy. But for some systems it is not possible to design non-overlapping primer pairs especially in multiplex reactions. Retyping of the homozygous genotypes at a low annealing temperature is a simple way to confirm homozygosity and reduce false exclusions in database matching.

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