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SHORT COMMUNICATION

M. Klintschar · F. Neuhuber

A study on the short tandem repeat system ACTBP2 (SE33) in an Austrian population sample

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Abstract Population genetic studies were carried out on 932 caucasians from Austria using the short tandem repeat system ACTBP2 (SE33). A sequenced allelic ladder was used for typing (Möller et al. 1995). After native polyacrylamide gel electrophoresis all 26 alleles of the ladder were found as well as 194 alleles which migrated differently from those in the ladder. Forensically relevant parameters were calculated (discrimination power: 0.989, mean exclusion chance: 0.854, observed heterozygosity 0.946). An allele consisting of 9 repeats which is not part of the allelic ladder was also found. In 692 meioses 5 mutations were found (mutation rate 0.72%).

Key words ACTBP2 · SE33 · Population study · Mutation rate · STR

Introduction

The short tandem repeat (STR) ACTBP 2 (SE33) (Polymeropoulos et al. 1992) was selected for a population survey from Austria as the polymorphism is outstanding among the STRs commonly used in forensic casework (Wiegand et al. 1993). Nevertheless this fact implies several disadvantages, e.g. difficult typing and limited interlaboratory controls due to the use of differing allelic ladders or electrophoretic systems (Lareu et al. 1993; Gill et al. 1994) and the necessity of large population samples for reliable allele frequencies. Furthermore there is evidence of a more frequent occurrence of mutations in this system (Brinkmann et al. 1995). This study was performed to further investigate the frequency of mutations at this locus and to obtain allele frequency estimates for an Austrian population sample using a standardized allelic ladder and a standardized electrophoretic protocol (Möller et al. 1995) to enable comparisons between different populations.

M. Klintschar (✉)
Institut für Gerichtliche Medizin, Karl Franzens Universität Graz, Universitätsplatz 4, A-8010 Graz, Austria
FAX: +43 (316) 380 9655

F. Neuhuber
Institut für Gerichtliche Medizin,
Paris Lodron Universität Salzburg, Ignaz-Harrer Straße 79,
A-5020 Salzburg, Austria

Materials and methods

DNA was extracted from 932 blood samples of unrelated Austrian caucasians and from 346 children in family studies as previously described (Klintschar et al. 1997). PCR amplification was performed using 0.4 μM each primer, 200 μM each nucleotide, 2.5 μl 10X buffer (1X is 10 mM Tris-HCl pH 8.8 at 25°C), 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100), 0.5 U DynaZyme II DNA-polymerase (Finnzymes Oy, Espoo, Finland) diluted to a final volume of 25 μl with double distilled H_2O. PCR cycling conditions were 94° for 2 min followed by 95°C for 15 s, 61°C for 30 s, 72°C for 30 s for 29 cycles and 72°C for 5 min in a programmable heatblock (DNA Thermal Cycler 9600, Perkin Elmer/Cetus). Typing was performed using native horizontal polyacrylamide gel electrophoresis and a sequenced allelic ladder (Möller and Brinkmann 1994). Sequencing of the rare allele was performed according to Möller et al. 1995.

The statistical analyses were performed using the HWE-Analysis program (C.Puers, Münster, Germany). The frequency profile comparison between different populations was carried out using a test for genetic heterogeneity (RxC contingency test; G. Carmody, Ottawa, Canada).

Results

All 26 alleles of the ladder were observed in the subjects tested, however, 194 alleles migrating differently from those in the ladder were found. Most of these interalleles migrated between alleles 22 and 23 and 28 through 31. As proposed by Möller et al. (1995) they were assigned to the anodal allele and marked with the letter "i". Classification of these interalleles as discrete alleles resulted in 309 genotypes compared to 216 genotypes when pooling the interalleles. The pooled allelic frequencies of the ACTBP2 system in Austria are given in Table 1. Using these data, the observed heterozygosity rate (H obs.) was 0.946 and

Table 1 Allele frequencies for HumACTBP2 in Austrians (this study; 932 ind.), Germans (Möller et al. 1995; 278 ind.), Turks (Alper et al. 1995; 204 ind.) and Hungarians (Csete et al. 1996; 105 ind.)

Allele	Austrians	Hungarians	Turks	Germans
9	0.001	0	0	0
< 12	0	0	0.012	0
12	0.005	0.019	0.002	0.006
13	0.011	0.052	0.012	0.004
14	0.028	0.043	0.034	0.037
15	0.049	0.043	0.027	0.032
16	0.056	0.090	0.034	0.055
17	0.072	0.038	0.080	0.058
18	0.066	0.033	0.095	0.068
19	0.073	0.038	0.088	0.083
20	0.048	0.024	0.056	0.064
21	0.039	0.010	0.037	0.051
22	0.047	0.014	0.022	0.032
23	0.024	0.024	0.020	0.015
24	0.028	0.010	0.032	0.041
25	0.024	0.038	0.037	0.028
26	0.031	0.086	0.027	0.036
27	0.051	0.057	0.033	0.064
28	0.077	0.086	0.042	0.058
29	0.094	0.062	0.095	0.105
30	0.085	0.105	0.078	0.105
31	0.035	0	0.032	0.021
32	0.027	0.048	0.034	0.023
33	0.014	0.033	0.037	0.013
34	0.007	0.033	0.007	0
35	0.006	0	0.010	0.002
36	0.001	0.014	0.015	0
37	0.001	0	0.006	0
> 37	0.001	0	0.000	0

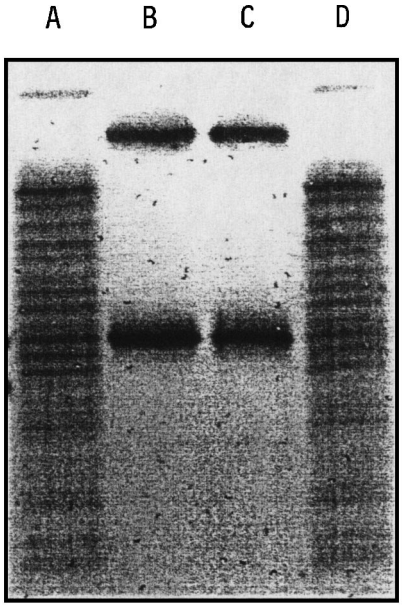

Fig. 1 Pherogram of the rare allele. Lane A and D: Allelic ladder. Lane B and C: Alleles 9 and 21

Table 2 The constellations of the five new mutations found in this study. The alleles probably involved are underlined. Mo: Mother; Ch: Child; Pf: Putative father

	Mo	Ch	Pf
Case 1	15,32	14i,32	13i,14
Case 2	17,24	17,18	20,29
Case 3	17,28	17,18	15,17
Case 4	28,30i	21,30i	20,28
Case 5	17i,29	19,29	16,17

the expected heterozygosity rate (H exp.) was 0.943 ± 0.018. The mean exclusion chance (MEC) was 0.855 and the discriminating power (D) was 0.989. No differences were found to a German population sample (Möller et al. 1995), but the differences to a Hungarian (Csete et al. 1996), and to a Turkish (Alper et al. 1995) population sample were highly significant (< P 0.01). No significant deviations from Hardy-Weinberg expectations were found in the exact test, $\chi 2$ test and G-test. An allele migrating between 7 and 12 was found in two individuals (Fig. 1). Sequencing data of these alleles revealed a fragment length of 221 bp consisting of a 142 bp long 5′-flanking region, 9 AAAG repeats, and a 43 bp long 3′-flanking region. At position 182 a transversion from G to A was found. Furthermore alleles migrating slower than 37 were observed in 2 persons. For segregation analysis, ACTBP2 was also tested in families where no exclusions were found using classical bloodgroups and a variable number of DNA loci and the combined W-value (probability of paternity) was at least 99.99% (692 meioses). However, in 5 cases a mismatch between putative father and child was found in the ACTBP2 locus (Table 2). These findings were regarded as mutations (mutation rate: 0.72%). Amplification and typing of these samples was repeated two times to rule out artifacts. In 85 trios where the putative father was already excluded from paternity, an additional exclusion in ACTBP2 was found in 93.1%.

Discussion

The forensically relevant parameters are considerably higher for ACTBP2 than for other STRs (Klintschar et al. 1995; Neuhuber et al. 1996b) or AmpFLPs (Klintschar and Kubat 1995; Neuhuber et al. 1996a) tested in the Austrian population. Because of this outstanding poly-morphicity and the frequent occurrence of interalleles, which reflect the large sequence variation at this locus (Möller and Brinkmann 1994; Rolf et al. 1997), this STR is difficult to type on native horizontal gels. Nevertheless, the sequenced allelic ladder used in this study simplified this process considerably compared to other ladders previously used (Wiegand et al. 1993). The frequency profile comparisons between the different populations were possible as all studies applied the same electrophoretic conditions and the same allelic ladder (Möller et al. 1995; Wiegand et al. 1993). The mutation rate was 0.72% in our

study and thus slightly higher than that found by Brinkmann et al. (1995) (3 in 453 meioses: 0.66%). Pooling the data of both studies would result in a mutation rate of 0.70%. However, further studies are required to narrow down the still large 95% confidence interval (0.22–1.18%). While four of the five mutations occurred on the paternal chromosome, the mutation in case 3 could not be allocated to either the paternal or maternal chromosome (Table 2). The excess of paternal mutations is in accordance with observations in RFLP systems (Henke and Henke 1995) and earlier observations on the ACTBP2 locus (Brinkmann et al. 1995) and can be explained by the fact that there are at least 10 times more cell divisions between the zygote and sperm than between the zygote and ova (Crow 1993). As observed for other loci (Weber and Wong 1993), most mutations generated larger alleles and the allele of the child was smaller than that of the putative father only in case 2. On the other hand, unlike prior observations (Brinkmann et al. 1995; Weber and Wong 1993), base changes for 1 and for 2 repeats were equally frequent in our sample. It is noteworthy that all five mutations affected alleles shorter than 21 repeats and the three mutations described by Brinkmann et al. (1995) also affected short alleles (17, 19, 20). Theoretical considerations for simply repeated DNA (Jeffreys et al. 1988; Richards and Sutherland 1992) however postulate that the probability of mutation of a STR be a direct function of copy number. From the data presented in this study it might be concluded that these considerations are not applicable to all STR loci. Nevertheless, due to its high discriminating power and chance of exclusion, this system has proved to be a useful tool for both paternity testing and forensic casework.

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