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ORIGINAL ARTICLE

A. Möller · M. Schürenkamp · B. Brinkmann

Evaluation of an ACTBP2 ladder composed of 26 sequenced alleles

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Abstract A total of 90 alleles found in white Caucasians from North-West Germany were sequenced and 26 alleles chosen to construct a uniform and reliable allelic ladder for the STR system ACTBP2 (SE33). In addition 3 new aspects concerning the sequence structure were observed. Population studies were carried out on white Caucasians (n = 278) from North-West Germany using the new improved ladder. A total of 24 alleles and 14 "interalleles" were found and reproducible results obtained. No significant deviation from Hardy-Weinberg-equilibrium could be observed.

Key words STR system ACTBP2 · Sequencing · Sequence variation · Allelic ladder · Validation study

Introduction

Among the STR systems known so far, HumACTBP2 tends to be one of the most polymorphic ones. While the allele number usually ranges from 7 to 15, ACTBP contains a multiplicity of alleles with at least 30 length variants and an even higher number of additional sequence variants (Urquhart et al. 1993; Möller and Brinkmann 1994). The disrimination index (DI) of > 99% (Wiegand et al. 1993) is equivalent to the combination of 3 other STR systems each with a DI of approximately 80%.

Due to the high degree of polymorphism the characterization of alleles causes considerable problems. It is especially difficult to compare international population data obtained with this system. Additionally, structural variants can have different electrophoretic mobilities depending on the gel system and the gel composition applied (Lareau et al.1993).

Dedicated to Prof. Dr. U. Heifer on the occasion of his 65th anniversary

A. Möller · M. Schürenkamp · B. Brinkmann (✉)
Institue of Legal Medicine, Westfälische Wilhelms-Universität, Von-Esmarch-Strasse 86, D-48149 Münster, Germany

The aim of this study was to establish and validate a uniform allelic ladder for the ACTBP2 system using a defined horizontal discontinuous gel system and test the reproducibility of the results. Also in this paper we will describe additional sequence variations.

Materials and methods

Human genomic DNA of white Caucasians from North-West Germany was extracted from blood according to Brinkmann et al. (1991) and quantified using the slot blot technique and the probe D17Z1 (Gibco BRL, Waye et al. 1989).

PCR amplification was performed as described by Wiegand et al. (1993).

Gel composition: 5% T, 3% C; piperazine diacrylamide as crosslinker (0.16%); 80 mM formate; 28 mM CHES (cyclohexylaminoethane sulfonic acid); 2% agarose plugs; gel dimensions: 26 cm × 24 cm × 0.75 mm; separation distance: 18 cm; well dimensions: 0.5 cm × 0.25 cm (for 4 µl volume), 0.5 cm × 0.5 cm (for 8 µl volume), 0.5 cm × 1 cm (for 16 µl volume).

Electrophoresis conditions were an initial 1000 V, 40 mA and 3 W with ramping every 90 min up to 15 W which was continued until the bromophenol blue marker had reached the anode. Bands were visualized by silver staining (Budowle et al. 1991).

Isolation of the DNA fragments from the gel, Taq-Cycle-Sequencing, analysis of the sequence data and construction of the allelic ladder were carried out as described previously (Möller and Brinkmann 1994).

The allele designation was according to the number of AAAG repeats including the hexamer unit ARAAAG (Nomenclature Committee of the International Union of Biochemistry 1985; DNA recommendations 1994). But for easier handling in routine work the alleles were numbered arbitrarily and continuously with the prefix "N" (native gels) to avoid confusion (see Fig. 1).

The discrimination index and the heterozygosity rates were calculated according to Jones et al. (1972). The Hardy-Weinberg equilibrium was checked using the exact test (Guo and Thompson 1992) with the software HWE- Analysis, Version 3.0 (Ch. Puers, Institute of Legal Medicine Münster, Germany, 1995).

Results and discussion

Construction of the ACTBP2 allelic ladder

A total of 90 Caucasian alleles were sequenced of which 26 defined alleles were selected and chosen to construct

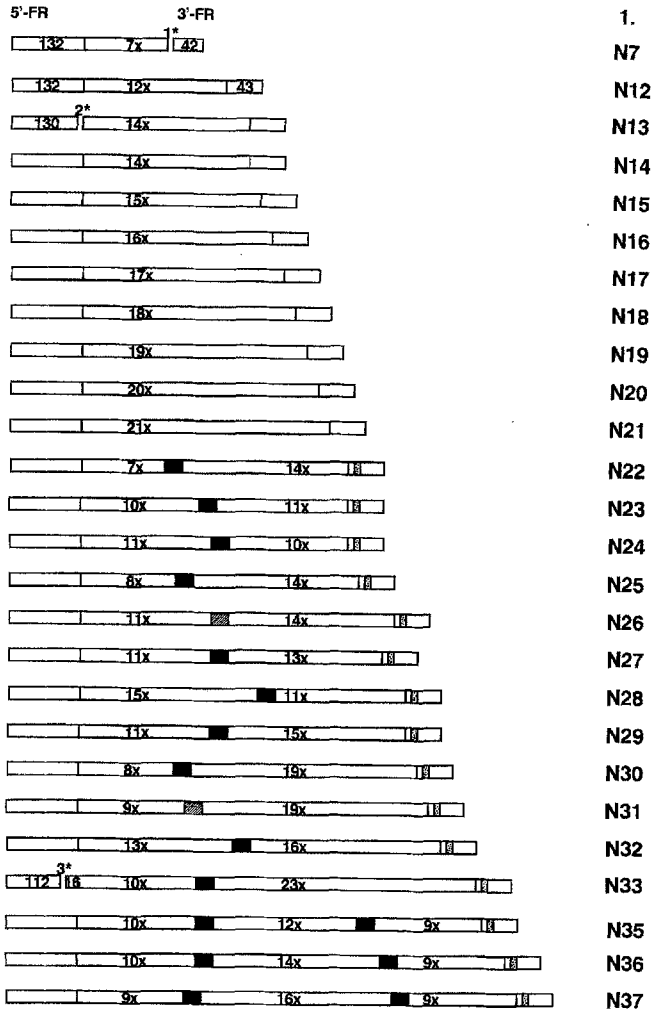

Fig. 1 Sequence structure of the 26 alleles of the ACTBP2-ladder (□ AAAG, ■ AAAAAG, ▨ AGAAAG, ▯ A to G transition). 1. arbitrary allele designation according to Table 1. 1* 1 bp-deletion next to the repeat region, 2* AG-deletion at position 131-132, 3* AAAG-deletion at position 113-116

Table 1 Frequency values, heterozygosity rate (H), mean exclusion chance (MEC) and discrimination index (DI) for the ACTBP2 locus in a Caucasian population study ($n = 278$). (1): arbitrary allele designation. (2): total number of repeats including the hexamer unit (according to Fig. 2), deletions (–) are given in brackets together with the number of base pairs (bp). (3): fragment length in bp as determined by Taq-Cycle-Sequencing. In the case where interalleles (allele > N20) occurred the frequency of the interallele was combined with the frequency of the next anodal allele. Individual phenotypes can be made available to interested persons.

Allele (1)	Repeat number (2)	Fragment length (bp, 3)	Frequency (%)
N7	7 (–1)	202	0
N12	12	223	0.56
N13	14 (–2)	229	0.38
N14	14	231	3.67
N15	15	235	3.20
N16	16	239	5.45
N17	17	243	5.83
N18	18	247	6.77
N19	19	251	8.27
N20	20	255	6.39
N21	21	259	5.07
N22	22	265	3.20
N23	22*	265	1.51
N24	22**	265	4.14
N25	23	269	2.82
N26	26	281	3.57
N27	25	277	6.39
N28	27	285	5.83
N29	27*	285	10.54
N30	28	289	10.53
N31	29	293	2.07
N32	30	297	2.26
N33	34 (–4)	309	1.32
N34	–	–	0
N35	33	311	0.19
N36	35	319	0
N37	36	323	0
H	93.23%		
MEC	0.873		
DI	99.62%		

an almost regularly spaced and uniform allelic ladder (Fig. 1).

Figure 1 in combination with Table 1 show the structure of the alleles, their designation (N-nomenclature) and the comparison between N-nomenclature, repeat nomenclature and fragment lengths (in base pairs as obtained by sequencing).

Sequence data of the alleles revealed considerable positional polymorphism of the AAAAAG unit within the repeat region resulting in anomalous electrophoretic mobilities in a horizontal discontinuous gel system (Möller and Brinkmann 1994). Because of this phenomenon the ACTBP2 ladder contained some alleles having the same fragment sizes, but different sequence structure i.e. alleles 22, 22*, 22** (Figs.1 and 2). The electrophoretic mobilities of certain alleles change under non-denaturing conditions (Fig. 2, see alleles 25 and 26).

In addition to the published data (Möller and Brinkmann 1994) 3 new aspects can be demonstrated:

1. In some alleles the hexamer AAAAAG showed an A to G transition at position 2 leading to the sequence AGAAAG (Fig. 1, see alleles N26 and N31). Thus 2 types of hexamer unit exist.

2. An additional type of structural variation was found. Alleles ranging from 311–323 bp showed 2 variable hexanucleotide units within the repeat region (Fig. 1) resulting in an increased level of polymorphism.

3. One extremely short allele with a fragment size of 202 bp and the repeat structure $(AAAG)_7$ showed a 1 bp deletion (G) next to the repeat region in the 3′ flanking region (Fig. 1, allele N7).

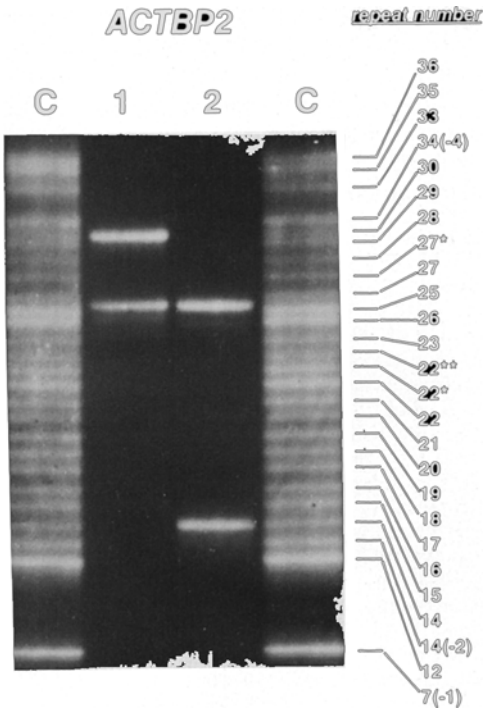

Fig. 2 Representation of the allelic ladder composed of 26 sequenced alleles according to Table 1 (magnification 2.4fold). Samples were separated on a non-denaturing, horizontal discontinuous gel system (Allen et al. 1989) and visualized by silver staining (Budowle et al. 1991). DNA typing of the alleles is according to Table 1. Interalleles are designated with the suffix "i". C = allelic ladder, 1 = N27i, N31i (repeat number 25, 29), 2 = N14, N27i (repeat number 14, 25)

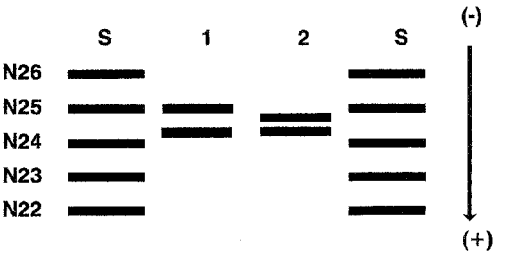

Fig. 3 Schematic demonstration of the assignment of interalleles. S = Standard (part of the ACTBP2 ladder), 1 = N25, N24i, 2 = N24i, N24i

Table 2 Comparison of the allele distribution of the ACTBP2 ladder in a horizontal, discontinuous (native) gel system and in a vertical, denaturing gel system using the automatic fragment length analysis software 672 (ABI, Foster City, CA).

Allele designation	
Native (ABI)	Denaturing
N7	7 (–1)
N12	12
–	13 (–2)
N13	14 (–2)
N14	14
N15	15
N16	16
N17	17
N18	18
N19	19
N20	20
N21	21
N22	–
N23	–
N24	22
N25	23
N26	25
N27	26
N28	–
N29	27
N30	28
N31	29
N32	30
N33	34 (–4)
N34	–
N35	–
N36	35

Applicability

Population genetics

Unrelated Caucasian individuals (n = 278) from North-West Germany were typed applying the horizontal discontinuous gel system and the new ACTBP2 ladder. The allele frequencies were determined (Table 1) and Hardy-Weinberg calculations showed no significant deviation from equilibrium applying the exact test ($P > 0.05$; Guo and Thompson 1992). The heterozygosity rate was 93.23%, the discrimination index (DI) 99.62% and the mean exclusion chance 87.3%.

– Reproducibility of the ladder was checked by several loadings of 8 defined DNA samples within a gel (intra gel variation). In addition 8 defined DNA samples were loaded on different gels to examine possible inter gel variations which could lead to false typing results.

Handling

Due to the construction of the ladder (Figs. 1, 2) in combination with a distance of 3.3 cm between extreme ladder alleles a resolution of all ladder alleles was achieved (Fig. 2). From case work experience it has been found to be very advantageous that the ladder is not purely and evenly spaced and the rungs not equally expressed. These gap and intensity irregularities are on the contrary very important because they constitute a guidance within the complex framework of numbers and rungs. Due to the extensive optimization and the application of singleplex rather than multiplex PCR, extra bands caused by single-stranded DNA and/or heteroduplex formation were not observed.

"Interalleles"

Approximately 14 alleles which did not match the ladder alleles (so-called "interalleles") occurred between alleles

N20 and N35 and were statistically always assigned to the anodal allele, as schematically exemplified in Fig. 3.

Denaturing gels

When separating the ACTBP2 ladder the vertical denaturing gel system, the automatic laser fluorescence detection system and the internal standard GS2500 (Applied Biosystems, Foster City, CA) were used. Allele designation was performed relative to the allelic ladder and not based on the fragment sizes measured relative to the internal lambda standard. As recent studies show, the precision of allele sizing is increased by the use of an internal allelic ladder (Smith 1995). Only 23 instead of 26 alleles could be detected (Table 2). This is so, because the electrophoretic mobilities of the alleles depend only on the fragment length and not on the sequence structure. As a consequence alleles N22 (22), N23 (22*) and N24 (22**) cannot be distinguished while the mobilities of the alleles N26 (26) and N27 (25) now correspond logically to the fragment lengths. From this one has to conclude that DNA typing results obtained under non-denaturing and denaturing gel conditions are not comparable. The conversion of typing results between both strategies is therefore impossible.

Nomenclature

Although the ISFH recommendations (1994) prescribe a notation according to the repeat number, this is not sufficient in the case of ACTBP2 because there exist multiple alleles which have the same repeat number but different structures being therefore genetically different and necessitating an additional structural description (Fig. 1, Table 1). Such a nomenclature would cause confusion in each laboratory and does not seem applicable. The best way out of this dilemma is the usage of a continuous nomenclature as proposed for the ladder described. This has been quickly adapted in our laboratory. But it must be emphasized that this approach must be based on precise knowledge of the structural background. Otherwise it would lack comparability.

Despite the results obtained in this study one has to consider that ACTBP2 needs a high interlaboratory standardization concerning the experimental conditions, especially if native gel conditions are used. Especially the gel composition and electrophoresis conditions must be standardized. Other prerequisites must be a sufficient spacing of the ladder alleles (3.3 cm) and the visualization of all 26 alleles in the ladder. ACTBP belongs to a series of STR systems which need a good laboratory skill.

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