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Further sequence data of allelic variants at the STR locus ACTBP2 (SE33): Detection of a very short off ladder allele

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Abstract. Genetic characterization of more than 15,000 individuals (mainly Caucasians) was performed using different primers. The study presents sequence structures of regular alleles ranging from 8 to 38 in comparison with variant alleles. Half of the variant alleles have insertions or deletions within the central polymorphic region. Other variations are located in the 120 bp 5′-flanking part and the 20 bp 3′-flanking part.

Keywords: STR; SE33; Variant allele

1. Introduction

SE33 is one of the most powerful STR markers in forensic use. A high number of alleles have been described, some of which may vary by as little as 1 bp. In addition to the length polymorphism, a number of different sequence variants have been observed [1–6]. The goal of this study is to add the sequence structure of some rare variants to the known data, and examine a very short off ladder allele which is described here for the first time.

2. Materials and methods

Genetic characterization of more than 15,000 individuals was carried out using buccal cell swabs or blood (DNA extraction: Chelex method or the Qiagen BioRobot 9604). At

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least 90% of the tested persons were Caucasians living in Germany. PCR products of SE33 were generated either using the Nonaplex I and II kits (Mentype® Twin, Biotype AG, Dresden, Germany) or in a single PCR with the primer pair described by Polymeropoulus et al. [7]. Automated fragment analysis was carried out on the ABI PRISM® 310 or 3100 Genetic Analyzers (Applied Biosystems, Foster City, CA, USA). The direct Taq-cycle-sequencing method was performed [8].

3. Results and discussion

Fig. 1 shows the structure of the polymorphic region. A very short off ladder allele (A) was found in a Somali individual. Amplification with Nonaplex II failed, indicating that there is a variation in the primer binding region. Sequence analysis revealed 14 tetranucleotide repeats in the central region and a deletion of 15 tetranucleotide repeats (60 bp) in the 5′ flanking region. Resulting fragment length was one repeat shorter than the length of a theoretical allele with 0 repeats.

Allele 6.3 (B) was sequenced in four different Caucasians showing an identical repeat structure. This irregular allele seems to be comparatively frequent and was described by other authors as having 7 tetranucleotide repeats with a 1 bp deletion in the 3′ flanking region [1,3,9].

Allele 9* (E), which originated from a Portuguese individual, was based upon 16 AAAG repeats in the central region, but deletion of 7 summarized tetranucleotides resulted in length allele 9.

Alleles 11.2 (F) and 15.2 (L) have 12 or 16 AAAG repeats, respectively, and an AG deletion in the 5′ flanking region. These structures have already been described in a Taiwanese population by Hsieh [6]. Allele 13.3 (H) was the result of a single G insertion in the central region but with an AG deletion in the 5′ flanking region (similar to F and L).

		5' flanking region																				Central region							3' flanking region						
Probe	Allele	5'-CTCCGTC	A	AAAG	AAAG	AGAC	AAAG	AGAG	TTAG	AAAG	AGAG	AAAG	GAAG	AA	AAAG	AA	G	AAAG	AG	AAAG	AG	AAAG	A	AAAG	A	G	AAAG	AA	AAAG	G	AAGG	AAAG	AGCG	AGC	AAGT-3'	Ethnic origin
A	<0	1	1	2	0	0	0	0	0	0	0	0	1	1	2	1		3	1	3	1	14								1		3		1	1	Somali
B	6.3	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	7								0		3		1	1	4 Caucasian
C	8	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	8								1		3		1	1	Caucasian
D	9	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	9								1		3		1	1	Caucasian
E	9*	1	1	2	2	1	1	1	1	3	3	1	3	0	0	0		0	1	2	1	16								1		3		1	1	Caucasian
F	11.2	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	0	12								1		3		1	1	1 Cauc., 1Afr.
G	12.1	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	1	1	11						1		3		1	1	Caucasian
H	13.3	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	0	11				1	3			1		3		1	1	unknown
I	14.1	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	2	1	12						1		3		1	1	Caucasian
J	15*	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	15								1		2	1	1	1	unknown
K	15.1*	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1	1	2	1	3	1	16								1		3		1	1	unknown
L	15.2	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	0	16								1		3		1	1	Caucasian
M	16.1	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	3	1	13						1		3		1	1	Caucasian
N	16/17	1	G	1	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	17								1	1	2		1	1	Caucasian
O	17.1	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	7	1	10						1		3		1	1	Caucasian
P	18	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	18								1		3		1	1	Caucasian
Q	18.1	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	12	1	6						1		3		1	1	Caucasian
R	18.1*	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1	1	2	1	3	1	19								1		3		1	1	Uzbek
S	19.2	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	10	2	9						1	1	2		1	1	Caucasian
T	21.1	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	18				1	3			1		3		1	1	Asian
U	23.2*	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	6	2	18						1		3		1	0	Caucasian
V	32.1	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	10	2	2	1		9	1	10	1	1	2		1	1	Caucasian
W	35.2	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	12	2	23						1	1	2		1	1	Caucasian
X	37	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	10	2	14	1	1	12			1	1	2		1	1	Caucasian
Y	38	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	12	2	15	1	1	10			1	1	2		1	1	Caucasian
Z	38	1	1	2	2	1	1	1	1	3	3	1	3	1	2	1		3	1	3	1	9	1	18				1	10	1	1	2		1	1	Caucasian

Fig. 1. Sequence structure of SE33 alleles. The central repeat region declared by Rolf et al. [3] is boxed. Probes C, D, P, S, W to Z show common repeat structures ranging from allele 8 to 38. Irregular deletions or insertions are shadowed in grey.

We found three classes of X.1 alleles: Probes G, I, M, O, and Q resulted from a single A insertion between the AAAG repeats in the central region. Probes K (15.1*) and R (18.1*) exhibited different structures: we detected 16 and 19 AAAG repeats, respectively, and a deletion of AAA in the 5′ flanking region. In contrast, the longer alleles 21.1 (T) and 32.1 (V) resulted from a single base pair (G or A) insertion in the central region.

Allele 15* (J) exhibited fragment length between alleles 15 and 15.1 (ABI Prism™ 310). An AA to CG conversion in the 3′ flanking region was found.

A deletion of four base pairs upstream of the central repeat region caused a genotype discrepancy (probe N: 16/29.2 with Nonaplex I, 17/29.2 with Nonaplex II).

Allele 23.2* (U) has a 4 bp deletion in the 3′ flanking region failing amplification with Nonaplex II.

4. Conclusions

This study shows the sequence structure of a very short off ladder allele which has never been previously described, as well as some rare variant alleles. Only half of the X.1 and X.3 alleles have insertions or deletions in the central repeat region. Therefore, it is difficult to compare our sequence structures with the existing data. Using different primer pairs, variations in the primer binding regions can prevent primer binding resulting in false homozygotes or giving discordant typing results. Although the X.1 and X.3 alleles are rare, accuracy is important for distinguishing them from the common alleles.

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