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SHORT COMMUNICATION

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A null allele for the Afm175xg3 marker at locus D17S795 caused by a primer binding failure

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Abstract We found a null allele for the marker Afm175xg3, at locus D17S795, due to primer binding failure, which makes this polymorphic marker unsuitable for genetic and forensic studies. This problem can be overcome by designing two new primers.

Key words Microsatellites · Polymorphism · Null allele

Introduction

DNA polymorphisms due to di-, tri- or tetranucleotide repeats (microsatellites) are powerful tools for genetic analysis and forensic applications due to high heterozygosity, easy and speedy identification and reliability [1–4].

In the polymorphic marker Afm175xg3 we identified a "null allele", in addition to those previously described by Gyapay et al. [5], that makes this system unreliable for genetic and forensic analysis. We suggest how to overcome this problem.

Materials and methods

As part of an independent population study, we examined 13 families with markers Afm175xg3, at locus D17S795 (EMBL Ac Z16771) and Afm107yb8, at locus D17S789 (EMBL Ac Z16583) [5].

DNA was extracted from venous blood using published techniques [6]. DNA amplification was performed for 25 cycles consisting of 30 s at 94° C, 60 s at 55° C and 150 s at 72° C on a Gene Amp PCR System 2400 (Perkin Elmer). PCR amplifications were carried out in a total volume of 25 µl containing 30–60 ng of genomic DNA, 50 pM of each primer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 50 mM KCl, 10 mM Tris-HCl pH 7.0, 0.1% Triton X-100 and 2.5 U Taq polymerase (Promega). For the analysis, the following primers were used:

Afm 175xg3, CA strand: TTTTCATGCGCTATCTCCAG

Afm 175xg3, GT strand: TGAGTTGTTAGCAATACTCGTAGG

Afm 107yb8, CA strand: ACTCCAAATCAAGTTTGTACTGAGA

Afm 107yb8, GT strand: CTGCATACGAAGGGTAGGAC

as previously described [5].

Moreover, we synthesized to new primers (Pharmacia Biotech) external to Afm 175xg3 named "Long" primers as follows:

"Long" CA strand AGCTCTAACCATGTGCTAAG

"Long" GT strand CTTCCATTAATATACCATAT

which produced fragments 43 bases longer than the corresponding normal ones.

The PCR products were separated on 4% MetaPhor agarose (FMC) in TBE 1 × and stained with ethidium bromide. Electrophoresis was performed for 7–8 h at 10V/cm.

Results and conclusions

The polymorphic dinucleotide repeat Afm175xg3 revealed an anomalous segregation in nine families, inconsistent with mendelian inheritance. Both the high frequency of the trait and its transmission also by the mother excluded illegitimate progenies. The pattern of transmission is compatible with the presence of a "null" allele (allele 0). The individuals who were heterozygous for allele 0 seemed to be homozygous for the normal allele, and the homozygous individuals gave no amplification products (Fig. 1 A). In a sample of 42 unrelated members from 13 examined families (one member for each family and 29 spouses) we found two homozygotes for the allele 0 and nine heterozygotes, giving an allele frequency of 0.15 (13/84) (Table 1). This frequency may be biased because we were not able to discriminate between a true homozygous and a heterozygous individual for the allele 0 if segregation analysis is not informative (for instance, if parents and children all appear homozygous for the same allele). The frequencies of normal alleles, calculated in the same unrelated individuals with known genotypes, but obviously with the same bias reported above, are listed in Table 1.

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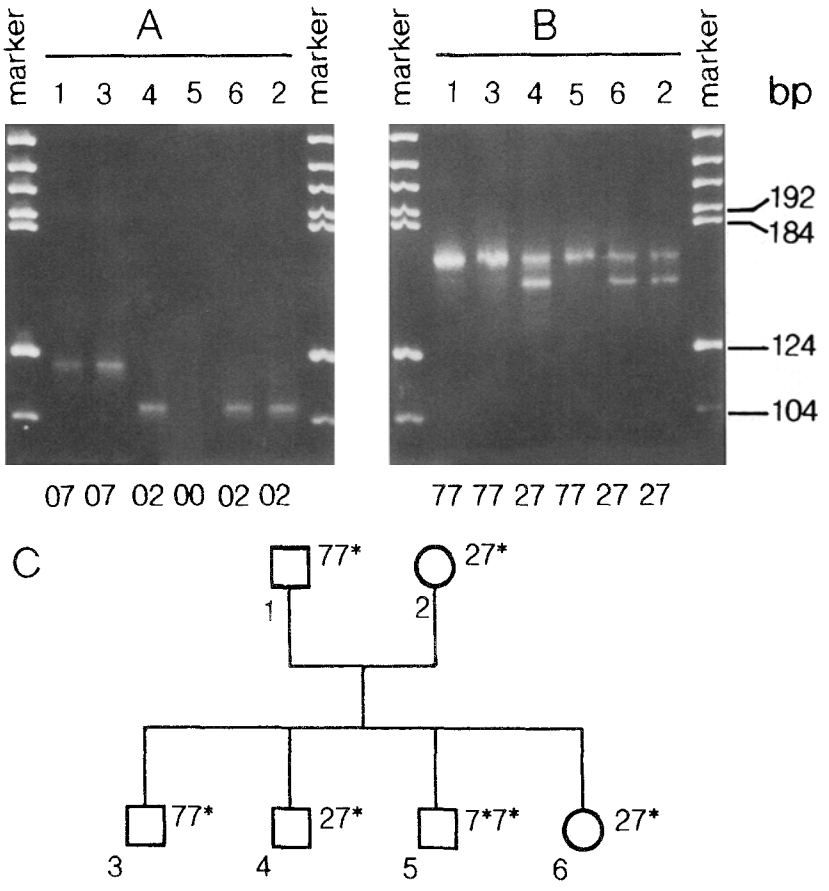

Fig. 1 PCR amplification of the microsatellite repeat polymorphism Afm175xg3. The genotypes are shown in figure 1A and 1B. A) amplification using primers previously described by Gyapay et al. [5]. B) amplification using newly synthesized primers ("long") on the same individuals as (A), numbers 1–6. C) pedigree of analysed family. The alleles* are not visible in 1A due to binding failure of the CA-primer

Table 1 Allele frequencies at locus D17S795

Agm175xg3 "Normal" primers				Afm175xg3 "Long" primers			
Allele	bp	n	freq.	Allele	bp	n	freq.
0	0	13	0.15	–	–	–	–
1	105	4	0.05	1	148	5	0.06
2	107	13	0.15	2	150	14	0.17
3	109	8	0.10	3	152	6	0.07
4	111	8	0.10	4	154	12	0.14
5	113	5	0.06	5	156	3	0.04
6	115	6	0.07	6	158	7	0.08
7	117	18	0.21	7	160	22	0.26
8	119	3	0.04	8	162	9	0.11
9	121	6	0.07	9	164	6	0.07
Total		84				84	

All families carrying this "null" allele were also tested for a closely linked marker (Afm107yb8, at locus D17S789, 0 cM from Afm175xg3) and showed a typical mendelian segregation and a correct cosegregation of the two markers (data not shown).

There are three possible explanations for this finding: 1) a microdeletion encompassing the analysed sequence, 2) an expansion too long to detect or 3) a polymorphic site in a primer which prevents normal binding. To discriminate between these three possibilities we designed two new primers, external to the ones used previously. The results are shown in Fig. 1 B. Fragments obtained by means of these latter primers are 43 bases longer (Fig. 1 A) as expected by primer design, and show a mendelian segregation (Fig. 1 C), supporting the hypothesis that the null allele is due to a primer binding failure. We therefore ruled out the presence of a total or partial deletion and the presence of an extreme expansion. By varying the primer matches we also determined that the observed modification involved the CA strand. Using these two new primers on the same sample, we estimated the allele frequency at locus D17S795 (Table 1), that represents, in this case, the true frequency of the repeat polymorphism alleles. The frequency assigned to the allele 0 by "normal" primer analysis (Table I) is divided uniformly between the other alleles, excluding an association with a particular repeat expansion (e.g. linkage disequilibrium). In some cases (alleles 3 and 5) the observed number (n) is greater using "normal" as opposed to "long" primers. This is due to the fact that all but one of the individuals considered to be homozygous for a normal allele (based on a similarly homozygous progeny) were found to be heterozygous with the new primers (and consequently they were heterozygous also for the null allele by the former analyses). Therefore, the CA strand polymorphism frequency is also greater (ca. 0.25). In conclusion, the analysis of the repeat polymorphism at locus D17S795 remains a useful and reliable system, but requires a careful selection of appropriate primers. To avoid errors in individual genetic characterization as described here we suggest carefully testing

each new microsatellite on as many families as possible to verify correct mendelian segregation.

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BOOK REVIEW

Oehmichen M, Kirchner H (eds) (1996) The wound healing process – forensic pathological aspects. Schmidt-Römhild-Verlag, Lübeck, 344 pages, DM 48.–. ISBN 3-7950-0310-5

The wound healing process including estimating wound age and distinguishing intravital from postmortem processes is a topic of forensic practice and a particular focus of forensic research. Many other medical disciplines do research on this complex topic with other goals. This book is a presentation of previous and new interdisciplinary results without claiming completeness. It presents the experience of the pathogenetic background of wound repair and describes possibilities of estimating wound age.

The individual chapters are mainly based on lectures given at the 5th Lübeck Congress of North German Forensic Scientists in 1994. The individual papers include dermatological, pathological and surgical aspects, functions and effects of various humoral factors such as cytokines and interleukines, cathepsin D, proteinase inhibitors, ions, prostaglandins, and cell adhesion molecules, cellular reactions such as RNA/DNA synthesis of epidermal basal cells, reaction of platelets, mast cells, macrophages, erythrocytes, hemoglobin degradation and synthesis and extracellular distribution of collagen. Finally the book deals with the forensic application of wound age estimation including vital and supravital reactions, estimating the age of hematomas in living subjects based on spectrometric measurements, time-dependent morphological alterations of injection marks, and early vital reactions in bleedings of the laryngeal muscles after laryngeal injuries. This recommendable book therefore gives an up-to-date overview of time-dependent mechanisms of wound healing for forensic theory and routine practice.

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