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Research Article

Triallelic Pattern at INRA23 and BM1824 Loci Observed in a Sicilian Cattle

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Abstract

Short tandem repeats are routinely used in various forensic applications such as food chain traceability and pedigree verification. Typically, every STR marker yields one or two peaks from one individual depending on allelic pattern inherited from parents. In this article we describe a case of an unusual three-peak profile at INRA023 and BM1824 loci occurred in a Sicilian cattle. Two blood samples were collected from one cattle and amplified in a 11-plex PCR to obtain a unique DNA profile using a certified kit. A triallelic pattern was observed for the two loci with peak size of 197, 204 and 212 bp for INRA023 and 180, 184 and 190 bp for the locus BM1824. The reasons of the triallelic profile occurrence showed in this work was not defined; a rare double mutational event could have occurred in two different stages of development or most likely a genetic carrier would be involved at first instance. To our knowledge this is the first case reporting a triallelic pattern in a Sicilian cattle using dinucleotide microsatellite markers. In conclusion, current data provide new knowledge about triallelic pattern of BM1824 and INRA023 loci concerning cattle forensic analysis.

Keywords: DNA typing; Microsatellite; Triallelic Profile; Cattle

Introduction

Short tandem repeat (STRs) types are commonly employed in cattle for paternity testing or pedigree verification in food fraud [1] and forensic cases mainly. A unique DNA profiling is derived from the reading of the size peaks of the fragments amplified using singleplex or commercial kit. Consistent with the Mendelian inheritance, each marker usually shows one or two peaks, respectively for the homozygote and the heterozygote pattern as they are derived from both parents. Rare mutational events may occur at one or more loci causing atypical allelic profile away from inheritance rules. Chimerism [2,3], somatic or germline mosaicism [4,5], triallelic pattern [6,7], cancer cells [8] and chromosomal aberrations [9] such as translocations[10,11], duplication [6] or non-disjunction events (trisomy) [12,13] are known to cause unexpected genetic variations affecting both STR typing and results. Although all these unusual findings have been de-

scribed in human cases, no uncommon DNA profiling cases have been reported in Italian cattle until now. Here we are describing a veterinary forensic case showing a three-peak allelic pattern at INRA023 and BM1824 autosomal loci in a cattle of a Sicilian farm. Possible explanations of these findings will be discussed.

Materials and Methods

Two blood samples were collected from the same cattle in two different moments by veterinary services for a case of identity verification. Genomic DNA was extracted from both samples and purified using a commercial kit (E.Z.N.A. Genomic DNA Isolation Kits, Omega Bio-Tek, Milano, Italy) according to the manufacturer's protocols and stored at -20°C. DNA fragments were amplified in 11-plex PCRs using a certified commercial kit (StockMarks for Cattle Bovine Genotyping Kit®, Applied Biosystems, Foster City, CA, USA). Multiplex-PCR products were injected into an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, San Diego, CA, USA) using the GeneScan-350 ROX as size standard and POP-7 polymer (Applied Biosystems, Foster City, CA, USA). Genotypic profiles were read and analyzed using the GeneMapper ID v4.2 software (Applied Biosystems).

Results

A total of 11 loci was investigated in this study and a three-allelic pattern was observed for the INRA023 and BM1824 loci, as figure 1 illustrates. Electropherograms analysis reported three peaks of 197, 204 and 212 bp for the INRA023 locus; fragments of 180, 184 and 190 bp in size were observed for the BM1824 locus. Results were confirmed for both samples without any incongruence and therefore considered as authentic. Further re-extraction and amplification for both blood samples were performed to be sure not to have contaminations or mixture [14].

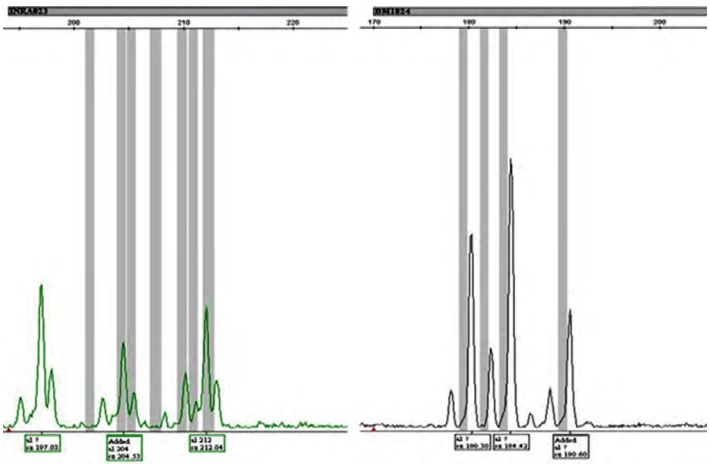

Figure 1. Three-allelic pattern at INRA023 and BM1824 loci.

Discussion

In this work we report an unusual double three-allelic dinucleotide pattern occurred in a Sicilian cattle at INRA023 and BM1824 autosomal loci (respectively located into the chromosome 3 and 1) during an investigation of identity verification. Triallelic profiles can find genetic reasons in several events such as translocations, chromosome duplication, meiotic non-disjunction events like trisomy as well as mosaicism and chimerism. A detailed overview of the main genetic anomalies described in cattle are given in Table 1 [15]. Some elucidations concerning the genetic mechanism which underlines infrequent triallelic pattern will be provided in our study. According to Clayton *et al.* [16], triallelic pattern can be classified in two different types based on the peak intensities. Type I pattern happens during development and it is the more frequent. It shows generally three alleles of uneven intensity as results of a somatic mutation at a heterozygous locus. Moreover the area of the highest peak should be the result of the sum of the peak areas of the two other minor alleles. On the other hand, type II triallelic pattern shows usually three peaks of similar height indicating chromosomal rearrangement at a heterozygous locus such as localized chromosomal duplication or non-disjunction events. In our study the uneven peak intensities seem to show a type I-like pattern even if the sum of the peak areas of the minor alleles are not comparable to the unmutated allele with a difference for both loci estimated in ~1000 (2895 vs 1880 and 5941 vs 5021, respectively for INRA023 and BM1824) (Table 2). Type II patterns in humans were extensively described in literature [5,17]. Among these, Lukka *et al.* [6] described a case of a large chromosomal duplication event in TPOX locus confirming genotyping, by the presence of two dinucleotide STRs just adjacent to the locus. In his study he displayed an electropherogram for the two microsatellites analogous to ours. In light of this, our results appear to be difficult to be inferred and included in type I/II group.

Table 1. Resume of the main chromosomal anomalies in cattle [15].

Aneuploidy of autosomes	Aneuploidy of sex chromosomes	Insertion	Translocation, centric fusion (Robertsonian)
Trisomy 12, Trisomy 16, Trisomy 17, Trisomy 18, Trisomy 20, Trisomy 22, Trisomy 23, Trisomy 24	*XXX, XXY*	*16*	*1/4, 1/21, 1/23, 1/26, 1/27, 1/28, 1/29, 2/4, 3/4, 3/27, 4/4, 4/8, 4/10, 4/21, 5/18, 5/23, 5/26, 6/28, 7/12, 7/21, 8/9, 9/23, 11/21, 11/22, 13/21, 14/20, 14/21, 14/28, 15/25, 16/18, 21/27, 27/29*

Translocation, reciprocal	Translocation, tandem	Sex Reversal	Inversion, pericentric
1/8/9, 8/13, 8/15, 10/11, 20/24, X/23, Y/17	*1/16, 1/18, X/23*	*XY female*	*14, X*

Table 2. Genotypes information derived from the GeneMapper v4.2 software.

Marker	Size 1 bp	Size 2 bp	Size 3 bp	Height 1	Height 2	Height 3	Peak Area 1	Peak Area 2	Peak Area 3
INRA023	197	204	212	313	173	301	1880	1028	1867
BM1824	180	184	190	621	864	379	3620	5021	2321

Fragments Size, Peak Height in RFU and Peaks Area are referred to each peak for the corresponding locus.

Current literatures concerning unusual STR typing are referred to human since they employ simple or complex tetra-repeat (CODIS system); no data are available for three-allelic pattern in dinucleotide microsatellite markers in cattle till now. It is plausible that proneness to produce high-frequency stutter bands in di-repeat rather than tri- tetra- or penta- can make hard the assignment to pattern type; allele-specific stutter could be also included within the true allele peak enhancing its height. Furthermore the high intensity of some peaks could be due to its preferential amplification, masking a type II pattern. These conditions could be taken into account particularly for INRA023 which shows a more homogenous peak heights (2:1:2 with Peak 2 / Peak 1 ratio ~55%) if compared to BM1824 (2:3:1 with Peak 3 / Peak 2 ratio ~43%). Defined assignment criteria related to the ratio of the peak areas and their intensity values could help in type I/II inclusion. As stated above, many genetic reasons are involved in unusual three-band profile. Chimerism is defined as the existence of more than one genetically-distinct population of cells originated from more than one zygote. Exchanging of blood between twin fetuses by placental anastomoses are not uncommon in cattle and are known as chimeras (e.g. freemartins cattle). In the same way, dispermic chimerism which is the consequence of a double fertilization of two separate ova by two sperm was also reported as source of three-allele patterns in forensic genotyping [17]. However we were prone to rule them out because of the presence of only 2 triallelic loci over 11 standard peaks. Beside any evidence of mixture or contaminated samples in other markers, the more accredited genetic reasons of unusual STRs typing in this work are represented by i) autosomal or germinal mosaicism ii) meiotic non-disjunction alone (e.g. trisomy) or followed by previous aberration (e.g. Robertsonian centric fusion) and iii) chromosomal duplications. Mosaicism denotes the co-presence of two or more genetically-distinct populations of cells derived from a single zygote. Defective mitosis can create two daughter cells, one of which with a higher number of chromosomes resulting in two different cell lines. Moreover mechanisms including meiotic non-disjunction and anaphase lag are reported to cause mosaicism. Unfortunately we can't confirm if the mutational events occurred during embryogenesis step without typing germline tissue even if, according to Brinkmann *et al.* [18], mutations in germline cells are inclined to change the number of repeats by one mainly. In line with our findings, all changes ranging from 4 to 10 repeats so that we exclude germ cell mutations. Changings in diploid number are also explained by irregular meiosis determining non-disjunction in subsequent anaphase; rare cases of trisomy were also reported in cattle. On the other hand, local chromosome duplication is caused by doubling of a portion of a chromosome. Duplication of a region containing the STR including flanking portions may be the result e.g. of an unequal or wrong meiotic crossing over. In order to generate the third allele the duplicated STR region would have undergone in polymerase slippage or towards a new recombination event. Although rare, duplication events coupled with mutation could be happened more easily at BM1824 based on its fragments size. Robertsonian translocation involving chromosomes 1/29 in heterozygous condition is the most widespread and known chromosomal aberration in cattle worldwide [19,20]. Gametogenesis in these individuals (2n=59) resulted in a more frequent degree of non-disjunction in meiosis than in normal individuals increasing the rate of aneuploidy occurrence. Moreover, the metacentric derived by Robertsonian chromosome could be segregated in meiosis leading to a trisomy. A chromosomal abnormality like this, if balanced, doesn't show visible changes in the phenotype and can be easily transmitted to a numerous progeny through selective mating strategies. With this premise, a few of male breeding cattle are today used in Sicily as unique reproducers by farmers because their own productive purposes (e.g. phenotype selection) through non-random mating between direct line consanguineous (e.g. parents-child) [21]. In line with this assertion, we supposed a two-step model involving most likely at first instance a genetic carrier followed by a somatic mutation in the bovine or a rare double mutational event could have occurred during the development. The probability of a multi-step mutational event is very low but happenings like these are recognized to be responsible for the onset of rare chromosomal aberrations. Unfortunately we have not got enough information to trace back

the parents and further investigations about the bovine will not be possible whereby only assumption will be provided. Since this is the first study describing a triallelic STRs profile in a cattle, the frequency is difficult to estimate; over a mean of 1500 individuals tested in various forensic applications, no one mutational event such this was ever detected at any locus. In agreement with these assumptions, it represents a rare event that in absence of further data would be estimable in a raw mean rate of 1:750. Lacking of three-allelic peak information concerning di-repeat in cattle from other countries strongly affects this incidence estimate. To our knowledge this is the first study reporting a triallelic profile at two dinucleotide STRs loci in a cattle; further investigations will be performed on the other animals of the same farm to shed some light on the allelic flow between the animals. These data provide new insight about triallelic pattern of BM1824 and INRA023 loci in parentage and identity verification during forensic analysis in cattle.

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