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A New and Unusual Reciprocal Translocation in Cattle: rcp(11;25)(q11;q14–21)

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Key Words
Agerolese breed · Cattle · Chromosome abnormality · Chromosome banding · FISH

Abstract
A new and unusual reciprocal translocation was detected in a heifer of the Agerolese cattle breed during a routine cytogenetic screening carried out on 13 animals (2 males and 11 females) kept at the ConSDABI Conservation Center in Benevento (Southern Italy). The 13 animals investigated had a normal karyotype except for a 1-year-old female, which carried one autosome smaller than the smallest normal bovine autosomes. This small autosome showed very little C-banding in comparison to the other autosomes, while another medium-sized autosome showed 2 distinct and prominent C-bands. RBA-banding and karyotype analysis revealed that these 2 chromosomes were the result of a reciprocal translocation between chromosomes 11 and 25. FISH analysis with BAC142G06 mapping to the proximal (subcentromeric) region of both BTA25 and der11, BAC513H08 *(ELN)* mapping to BTA25q22dist and der25, and BAC533C11 mapping to the proximal region of BTA11 and der11 confirmed the localization of the breakpoints on band q11 (centromere) of chromosome 11 and q14–21 of chromosome 25. Ag-NOR and sequential RBA/Ag-NOR techniques detected the presence of NORs on both BTA11 and BTA25 and both der11 and der25. To our knowledge, this is the first report of a reciprocal translocation event in cattle with the breakpoint located in the centromeric region.

While variation in the number of autosomes often causes abnormal body conformation making it easy for the breeders to eliminate carrier animals, structural and balanced autosomal aberrations escape normal breeding selection because the carriers have normal body conformation. These chromosome abnormalities are often the cause of low fertility or sterility [Gustavsson, 1980; Molteni et al., 2007; Ducos et al., 2008] and without cytogenetic control they can easily spread in the progeny, especially when artificial insemination is applied.

In cattle, the most common chromosome abnormalities are the Robertsonian or centric-fusion translocations [for review see Ducos et al., 2008; Iannuzzi et al., 2009]. In fact, to date few cases of reciprocal translocations have been reported in cattle [de Schepper et al., 1982; Mayr et

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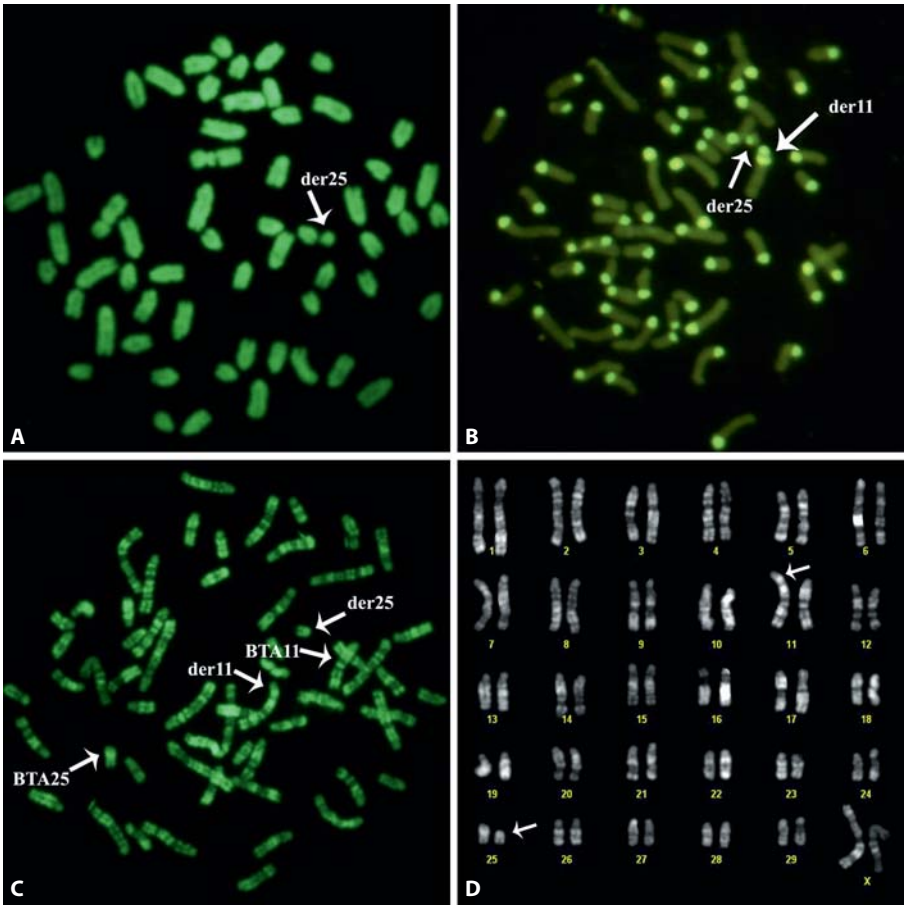

Fig. 1. A Female cattle metaphase spread showing one very small autosome (der25). **B** CBA-banding pattern on a metaphase plate of the carrier showing both der25 and der11 with a very small C-band and double and prominent C-bands, respectively. **C** RBA-banding of chromosomes from the carrier and the corresponding karyotype (**D**) showing that BTA11 and BTA25 are involved in a reciprocal translocation.

al., 1983; Kovács et al., 1992; Ansari et al., 1993; Villagómez et al., 1993; Ducos et al., 2000, 2008; Iannuzzi et al., 2001a, b; De Lorenzi et al., 2007; Molteni et al., 2007; Switonski et al., 2008, 2011], probably because of the difficulty in detecting this kind of abnormality when chromosomes are conventionally stained without banding. Indeed, conventional staining can detect only chromosomes unusually large or small compared to the remaining autosomes, and then further cytogenetic analysis can be performed to ascertain the nature of the abnormality and identify the chromosomes involved.

In the present study, we report a new and unusual reciprocal translocation detected in a heifer of the Agerolese cattle breed during a routine cytogenetic screening carried out at the ConSDABI Conservation Center in Benevento (Southern Italy). The chromosomes involved in this new translocation were identified as chromosomes 11 and 25 by banding analysis and fluorescence in situ hybridization (FISH) with chromosome-specific BAC clones.

Materials and Methods

Animals

Thirteen animals (2 males and 11 females) belonging to the Agerolese cattle breed and kept at the ConSDABI Conservation Center in Benevento (Southern Italy) were analyzed in this study.

Cell Cultures

Peripheral blood samples were cultured in RPMI medium enriched with fetal calf serum (10%), antibiotic-antimycotic mixture (1%), L-glutamine (1%) and Concanavalin A (15 μg/ml). Two types of cell cultures were performed: either without adding any base analog (normal cultures) or with BrdU to produce an R-banding pattern as follows. After 48 h of cell culture, thymidine (300 μg/ml) was added to block cells in S-phase and then removed 17 h later by washing cells twice, recovering the cells in fresh medium containing BrdU (15 μg/ml) and Hoechst 33258 (30 μg/ml) and further culturing for 6 h including a colcemid treatment (0.1 μg/ml) for the last hour. Chromosome preparations were obtained by hypotonic treatment and 3 successive fixations in methanol/acetic acid (3:1).

Banding Techniques

Slides obtained from normal and BrdU-treated cells were used to perform CBA- and RBA-banding, respectively. Chromosomes

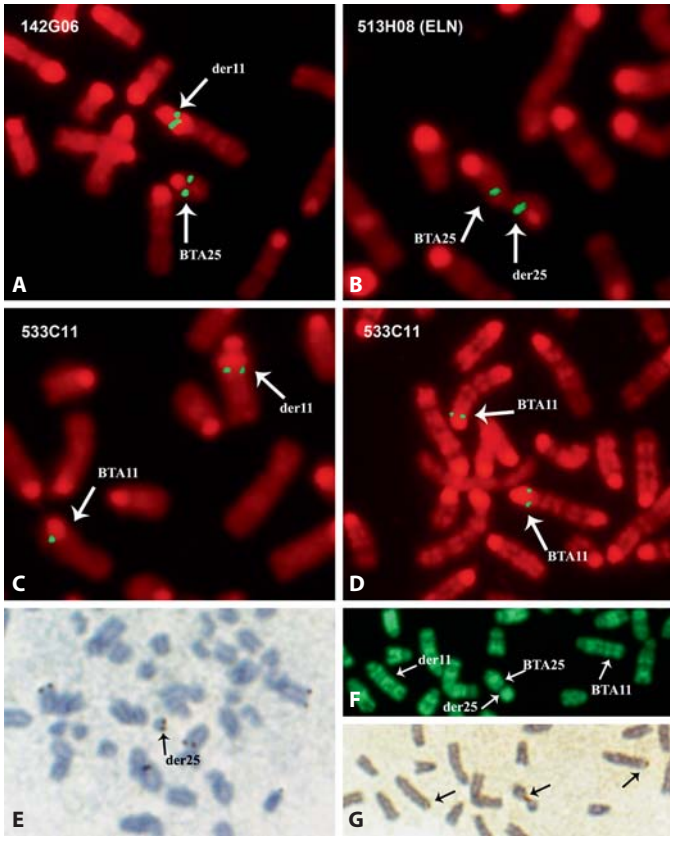

Fig. 2. Details of FISH-mapping on metaphase spreads of the carrier with BAC142G06 mapping to both BTA25q14 and der11 (**A**), BAC513H08 *(ELN)* mapping to both BTA25q22dist and der25 (**B**) (in this detail BTA25 and der25 are in nucleolar association) and BAC533C11 mapping to both BTA11q12 and der11 (**C**), as well as to both BTA11 on a metaphase from a normal animal (**D**). Ag-NOR (**E**) and sequential RBA/Ag-NOR (**F**, **G**) techniques confirmed the presence of NORs on der25 (**E**) and both BTA11 and BTA25, as well as on both der11 and der25 (**F**, **G**). Note that BTA25 and der25 are in nucleolus organizer association.

were also treated with sequential RBA- and Ag-NORs techniques. Details concerning these techniques can be found in Iannuzzi and Di Berardino [2008].

FISH-Mapping

FISH-mapping was carried out according to the previously reported protocol [Iannuzzi and Di Berardino, 2008]. The following bovine BAC clones obtained from the INRA bovine BAC library were used as probes: BAC533C11 located on BTA11q12 (our result), BAC142G06 on BTA25q14 (our result) and BAC513H08 containing the ISCNDB reference marker of BTA25 *(ELN)* located on BTA25q22dist [Hayes et al., 2000; ISCNDB2000, 2001]. Biotin was incorporated by nick translation using Biotin-Nick Translation Mix (Roche Diagnostic), ethanol precipitated in the presence of bovine COT1-DNA and sonicated salmon sperm DNA and then resuspended in the hybridization solution. FITC-signal detection and RBPI-banding (R-banding using propidium iodide staining) were carried out as earlier reported [Iannuzzi and Di Berardino, 2008]. Thirty metaphase plates for each probe were analyzed.

Chromosome Nomenclature

Chromosome banding nomenclature followed the most recent standard chromosome nomenclature [ISCNDB2000, 2001]. RBA-banded karyotypes were constructed using GENIKON software.

Results

The 13 animals investigated had a normal karyotype except for a 1-year-old female, which carried one autosome smaller than the smallest normal bovine autosomes (fig. 1A). After CBA-banding, this small chromosome showed very little C-band staining while another large autosome showed 2 distinct and prominent constitutive heterochromatin (HC) blocks (fig. 1B). After RBA-banding (fig. 1C) and analysis of the corresponding karyotype (fig. 1D), it was evident that these 2 chromosomes were the result of a reciprocal translocation between chromosomes 11 and 25. This was further confirmed by FISH analysis with the 3 selected BAC clones: BAC142G06, mapped to the proximal (subcentromeric) region of both BTA25 and der11 (fig. 2A), BAC513H08 *(ELN)* mapped to BTA25q22dist and der25 (fig. 2B), and BAC533C11 mapped to the proximal region of BTA11 and der11 (fig. 2C). Furthermore, Ag-NOR staining (fig. 2E) and sequential RBA/Ag-NOR banding (fig. 2F, G) confirmed the presence of NORs on both BTA11 (and der11) and BTA25 (and der25), 2 of the NOR-bearing bovine chromosomes [ISCNDB2000, 2001; Iannuzzi et al., 2009]. Figure 2D also shows FISH-mapping results of BAC533C11 to BTA11q12 from a normal cattle metaphase plate. Figure 3 shows a schematic representation of the possible origin of this unusual reciprocal translocation with the FISH-mapping localizations.

Discussion

The Agerolese cattle breed is mainly reared in the province of Naples. Since only a few hundred animals are still present, the population is considered as 'endangered' by the F.A.O. Over the years, this breed has suffered a substantial reduction in size because of the continuous use of crossbreeding with the Italian Friesian to increase milk production. However, since 1985 it has been included into the registry of indigenous cattle populations with

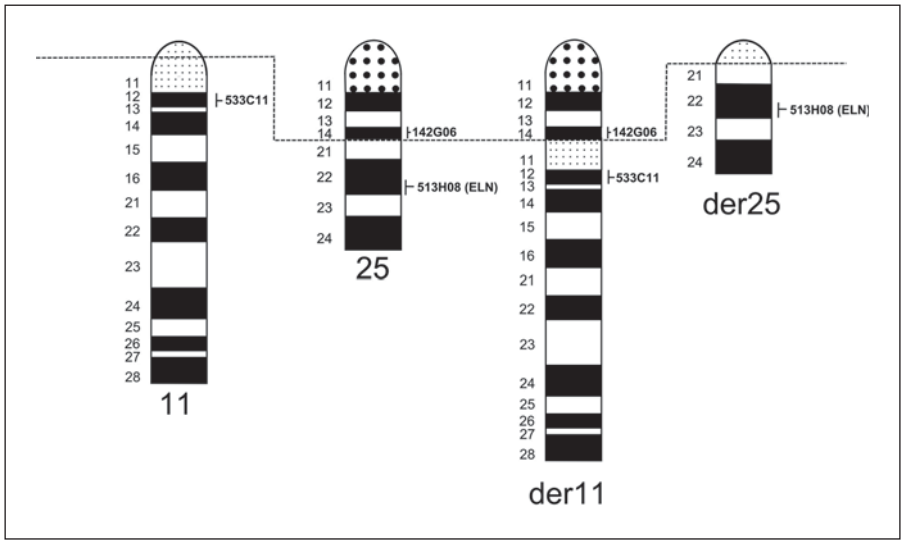

Fig. 3. Idiogrammatic representation (R-banding) of the possible origin of rcp(11;25)(q11;q14–21). A proximal part of BTA25 containing BAC142G06 with a breakpoint situated in bands q14–q21 translocated to the centromeric region of BTA11 (der11) which had a breakpoint very proximal to centromeric region (BTA11q11prox). This small centromeric region of BTA11 is translocated to the remaining part of BTA25 resulting in der25 which shows a very small C-band. The dotted line indicates the breakpoints involved in this reciprocal translocation. FISH-mapping localizations with the 3 BACs used are reported on both BTA11 and BTA25, as well as on both der11 and der25.

limited distribution in Italy and regional programs are currently developed in attempt to save this breed, the milk of which is used to produce a typical D.O.P. (Protected Origin Denomination) local cheese named 'Caciocavallo del Monaco'.

The rcp(11;25) identified in the female Agerolese carrier is not only new in cattle but it also appears unusual, because one break has occurred in the centromeric region of BTA11, as demonstrated by the small C-band and 2 distinct and prominent HC blocks found in der25 and der11, respectively (fig. 1B). However, since BAC533C11 maps very close to the centromeric region of BTA11 and very close to the second and proximal C-band of der11 (fig. 2C, D), we confirm that the first C-band of der11 originates from BTA25 while the second (and proximal) one from BTA11 (figs. 1–3). To our knowledge, this is the first report on a bovine reciprocal translocation with a break occurring in the centromeric region of 1 of the 2 involved chromosomes. This event is also rare in humans where only a few cases of reciprocal translocation involving chromosome breaks in centromeric regions have been reported [Wang et al., 2009].

Bovine chromosome 11 has been involved in 2 other reciprocal translocations with BTA9 [De Lorenzi et al., 2007] and BTA21 [Molteni et al., 2007]. It is interesting to note that both BTA11 and BTA25 are NOR-bearing chromosomes (fig. 2E–G) [ISCNDB2000, 2001; Iannuzzi et al., 2009] and their presence on both normal BTA11 and BTA25, as well as on both der11 and der25 (fig. 2E–G) confirms that the proximal regions of BTA25 are involved in the reciprocal translocation with BTA11. FISH-mapping of BAC clones specific for BTA25 and BTA11 not only confirmed the chromosome involved in this reciprocal translocation, but allowed us to delineate the breakpoints of this new reciprocal translocation defined as rcp(11;25)(q11;q14–21) (fig. 3). Furthermore, this study allowed us to more precisely localize the BAC533C11 to BTA11q12 and the BAC142G06 to BTA25q14 (figs. 2A, D and 3).

Unfortunately, both parents and related subjects of the reciprocal translocation carrier were not present at the ConsDABI Center. Thus, it was impossible to establish if the abnormality has a de novo origin or not. Generally, reciprocal translocations are the cause of reduced fertility or sterility [for review see Molteni et al., 2007; Ducos et al., 2008]. This is mainly due to abnormal meiotic segregations of quadrivalent configurations originating in unbalanced gametes (and embryos) as revealed in meiotic preparations [reviewed in Villagómez and Pinton, 2008]. Based on these considerations, this heifer should be sterile or with reduced fertility. For this reason it will be accurately screened to check its reproductive performance.

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