Original Article

Cytogenetic and Genome Research

Cytogenet Genome Res 2013;139:97–101
DOI: 10.1159/000346193

Accepted: September 26, 2012
by M. Schmid
Published online: January 16, 2013

Cytogenetic Elaboration of a Novel Reciprocal Translocation in Sheep

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Key Words
Chromosome banding · Cytogenetic analysis · FISH · Reciprocal translocation · Sheep

Abstract
Reciprocal translocations represent one of the most common structural chromosomal rearrangements observed in both humans and domestic animals. In these translocations, the balanced forms are most frequent but may remain undetected because the carriers show a normal phenotype. For this reason, routine cytogenetic analysis of domestic animals should necessarily rely on banded karyotypes. In fact, during a screening analysis, carried out on phenotypically normal young sheep (*Ovis aries*, OAR, 2n = 54) from Laticauda-Comisana hybrids, a new structural rearrangement was detected. Two abnormal acrocentric chromosomes (the smallest and the largest one) were found in all metaphases of this carrier animal, suggesting the presence of a reciprocal translocation (rcp). CBA and RBA banding were performed in order to characterize the translocation, and FISH with chromosome-specific BAC probes and telomere probes was applied to confirm the cytogenetic data. The translocation was classified as rcp(4q;12q)(q13;q25).

Chromosomal translocations are the most frequent chromosome aberrations found in both humans [De Braekeleer and Dao, 1994] and domestic animals [Bruère, 1974; Gustavsson, 1980; Long, 1985; Ducos et al., 2008]. In particular, centric fusions are a major feature of karyotype evolution in bovids, where the fundamental number has almost always remained constant at FN = 60 [reviewed in Iannuzzi et al., 2009]. The domestic sheep karyotype obviously results from 3 Robertsonian translocation events, with sheep (*Ovis aries*, OAR) chromosome 1 being equivalent to goat (*Capra hircus*, CHI) and cattle (*Bos taurus*, BTA) chromosomes 1 and 3, OAR2 being equivalent to CHI/BTA 2 and 8, and OAR3 being equivalent to CHI/BTA 5 and 11 [ISCNBD2000, 2001]. Actually, centric fusions are the most important and investigated autosomal abnormalities in sheep with 6 cases reported. On the other hand, there are only 3 cases of reciprocal translocations in sheep: rcp(1p;19q) [Glahn-Luft et al., 1978], rcp(13q;19q) [Anamthawat-Jonsson et al., 1992] and rcp(2q;3q) [Popescu and Tixier, 1984; Popescu, 1990]. In our opinion, this low rate does not reflect the reality, mostly due to the difficulty to detect this kind of abnormality when chromosomes are conventionally stained without any type of banding. In fact, convention-

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al Giemsa staining on acrocentric chromosomes can detect only abnormally large or small chromosomes and pericentric inversions, without giving any indication of chromosomal regions involved. In addition, the phenotypic effects of reciprocal translocations on the carriers depend on whether they are present in a balanced or unbalanced form. While unbalanced forms usually cause physical problems to the carrier, making it easy for the breeders to eliminate these animals, the balanced forms do not have any phenotypic effect on the carriers and are often the cause of low fertility or sterility [Gustavsson, 1980; Ducos et al., 2008].

In our lab, a cytogenetic screening of some endangered breeds of cattle, sheep, goat, pig and horse raised in Campania Region (southern Italy) has been performed with the aim to cytogenetically characterize these animals. It is supported by a regional project which is trying to increase the value of these animals and their products. In the present study, a new reciprocal translocation detected in a phenotypically normal young sheep from Laticauda-Comisana hybrids is reported.

Materials and Methods

Animals Studied

The cytogenetic screening analysis was carried out on 15 phenotypically normal young sheep (4 males and 11 females) from Laticauda-Comisana hybrids, reared in the province of Naples. Several cytogenetic analyses were performed to characterize their karyotypes.

Cell Culture Techniques

Peripheral blood samples were cultured in RPMI medium enriched with 10% fetal calf serum, 1% antibiotic-antimycotic mixture and 15 μg/ml concanavalin A. Two types of cell cultures were performed: one without adding any base analog and the other with 5-bromodeoxyuridine (BrdU) plus Hoechst 33258. One hour before harvest, all cultures were treated with 0.1 μg/ml colcemid. Chromosome preparations were obtained by after hypotonic treatment and 3 successive fixations in methanol/acetic acid (3:1).

BrdU Cultures. In order to gain cells in the metaphase stage, synchronized peripheral blood lymphocytes were cultured for 72 h and treated for late-incorporation of BrdU and Hoechst to obtain R-banded chromosome preparations. Three drops of fixed cells were spread on wet slides and air-dried. Slides were aged for 1 week at room temperature and subjected to several banding and FISH techniques as reported earlier [Iannuzzi and Di Berardino, 2008].

Banding Techniques

All banding techniques are reported in detail in Iannuzzi and Di Berardino [2008].

CBA Banding. Slides from conventional cultures were CBA-banded. Briefly, chromosomes were treated with HCl solution for 30 min at room temperature and denatured in 5% barium hydroxide solution at 50°C for 10–15 min. Then they were rinsed in 2× SSC and air-dried. Finally, slides were stained with acridine orange (0.01%). At least 100 metaphases were studied.

RBA Banding. Slides from BrdU cultures were treated for RBA banding. Firstly, slides were stained with Hoechst 33258 for 20 min, rinsed and air-dried. Then they were exposed to UV light for 30 min. Finally, slides were stained with acridine orange (0.01%). At least 100 cells were analyzed. R-banded karyotypes were prepared using the GENIKON software and the latest international standard nomenclature for domestic sheep [ISCNDB2000, 2001].

Fluorescence in situ Hybridization

Two different kinds of FISH analyses were conducted using both a telomere PNA probe and bovine BAC clones.

Telomere PNA Probe. The telomere PNA probe, mapping on all telomeres, was hybridized on metaphase cells using the telomere PNA FISH kit/FITC (Dako Cytomation).

BAC Clones. Two different BAC clones were selected from the bovine genomic library (INRA) and used for our study: BAC 876G9 mapping proximally to BTA4 and BAC 387F08 mapping distally on BTA16. These data were obtained by blasting the 4 GSS sequences against Btau6 genome assembly (UMD_3.1) [Eggen et al., 2001].

FISH Mapping. The analyses were carried out according to Iannuzzi and Di Berardino [2008]. The probes were biotinylated by nick translation (Roche Diagnostic kit), precipitated in absolute ethanol with Cot DNA and sonicated salmon sperm DNA and then resuspended in the hybridization solution. At the same time, selected slides were treated with pepsin solution at 37°C for 30 min, rinsed in ddH_2O and dehydrated in an ice-cold ascending ethanol series and air-dried. The hybridization mixtures were co-denatured with the slides on a hot plate at 74°C for 4 min. Hybridization was performed under a sealed coverslip in a moist chamber at 37°C for 24 h. Hybridization sites of biotinylated probes were visualized by indirect staining using fluorescein-avidin DCS A-2011 (Roche) and anti-avidin antibody D BA-0300 (Roche). Chromosomes were counterstained with Vectashield DAPI H1500 in Vectashield H 1000 (Vector Lab) antifade solution. At least 30 metaphases per probe were analyzed using a Nikon E1000 fluorescence microscope equipped with a digital camera and computer.

Results and Discussion

The 15 animals investigated presented normal karyotypes (2n = 54) except for a 1-year-old female which showed 2 abnormal acrocentric chromosomes, one shorter and the other larger than the shortest and largest normal acrocentric chromosomes, in each metaphase analyzed (fig. 1A). At this point, in order to distinguish between derivate chromosomes (der) and fragments, it was necessary to show the presence of centromeres and telomeres on both aberrant chromosomes by CBA banding and telomere PNA FISH analysis, respectively. Indeed, after CBA banding, all chromosomes showed a distinct

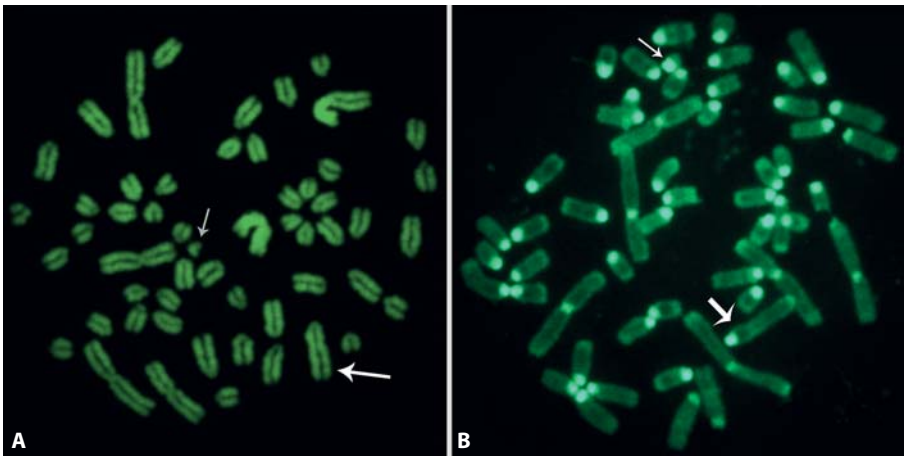

Fig. 1. Metaphase plates of the female carrier of rcp(4;12). **A** Conventionally stained metaphase showing both the larger (large arrow) and the smaller (small arrow) translocation chromosome. **B** CBA-banded metaphase showing the presence of centromeres on both ders (arrows).

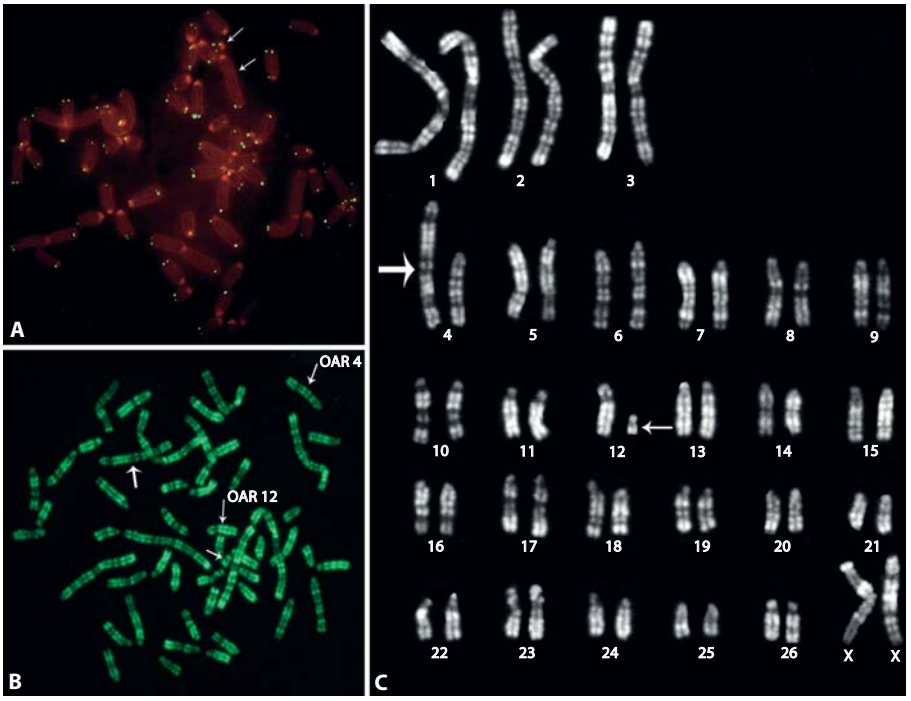

Fig. 2. A Metaphase of the translocation-carrying sheep after FISH with the telomere PNA probe. Telomere signals are present in all chromosomes including the ders (arrows). **B**, **C** RBA-stained metaphase plate showing both ders (larger and smaller arrows, respectively), OAR4 and OAR12 (**B**) and the corresponding karyotype (**C**).

and entire centromere in all metaphases investigated (heterochromatin block) (fig. 1B). On the other hand, FISH with the telomeric probe revealed the presence of telomeres in all chromosomes, abnormal chromosomes included (fig. 2A).

The RBA banding analysis (fig. 2B, C) revealed the presence of a reciprocal translocation between chromosomes identified as OAR4 and OAR12. The confirmation of both chromosomes and chromosome regions involved was validated by FISH mapping analysis using 2 specific BAC clones: BAC 876G9 mapped on the subcentromeric regions of both OAR4q and the smaller abnormal chromosome (smaller der) (fig. 3A); BAC 387F08 mapped on the distal telomeric regions of both OAR12q and the smaller abnormal chromosome (fig. 3B). Eventually, according to the results of cytogenetic analysis, the chromosomal translocation was then classified as rcp(4q;12q)(q13;q25).

Finally, figure 4 shows an idiogrammatic representation of the chromosomes and possible rearrangements involved in this new reciprocal translocation and the FISH-mapping localizations of the BACs.

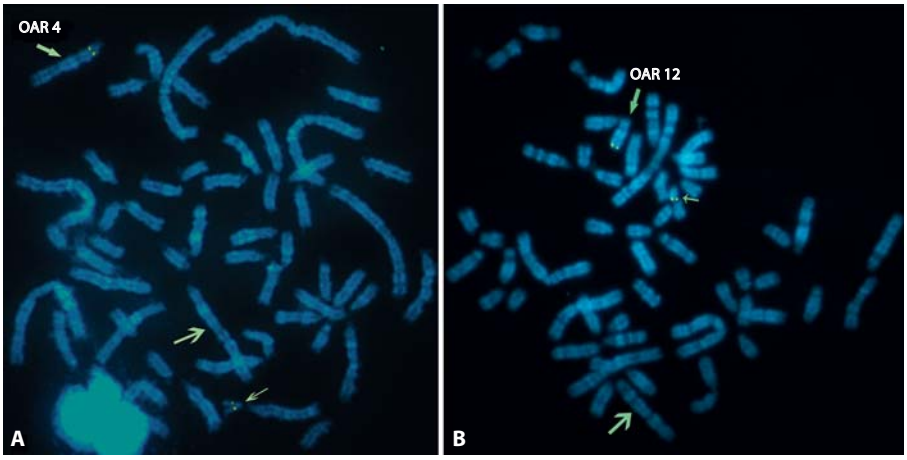

Fig. 3. FISH with BAC 876G9 (**A**) and BAC 387F08 (**B**) on RBA-banded metaphases. Both probes hybridize to the smaller translocation chromosome (small arrows) as well as to OAR4 (BAC 876G9) and OAR12 (BAC 387F08), respectively. Large arrows indicate the larger translocation chromosome.

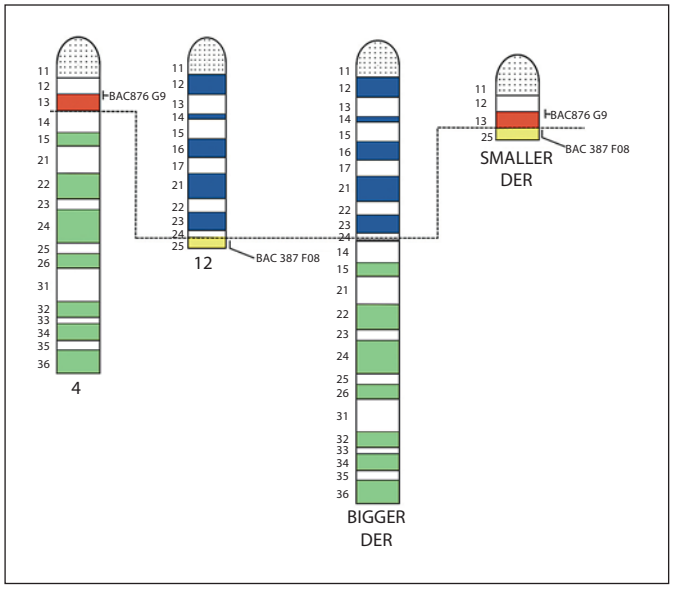

Fig. 4. Idiogrammatic representation of the chromosomes involved in the reciprocal translocation rcp(4;12)(q13;q25). The location of BAC probes as determined by FISH and the breakpoints (dotted line) are indicated.

In this study, we found a new type of reciprocal translocation in sheep, underlining the importance to perform banding analysis during cytogenetic screening of domestic animal populations. Nowadays, the cytogenetic techniques allow scanning the genome for aberrations involving both gains and losses of portions of the genome as well as rearrangements within and among chromosomes. Cytogenetic analysis still represents one of the most important tools for the genetic improvement of livestock, research purposes and economical profit for breeders. Even if the staining patterns produced on the chromosomes by banding procedures are sometimes ambiguous, the analysis of banded karyotypes still remains the best way to evaluate chromosome aberrations which can be later confirmed by FISH analysis with specific markers or chromosome painting probes.

Actually, centric fusions in sheep are more frequent than reciprocal translocations while in human newborns the frequency of these anomalies is quite similar. In cattle, only 16% of all reciprocal translocations can be detected by analyzing the conventional karyotype and, consequently, the real frequency could be 5 times higher than de novo Robertsonian translocations [De Lorenzi et al., 2012].

It is important to remember that chromosomal abnormalities are a cause of economic loss in animal production, due to the production of unbalanced gametes which are known to give rise to chromosomally unbalanced embryos, destined to die in early embryonic life or to be aborted.

Eventually, we are going to analyze both germ cells and embryos of the carrier in order to study the percentage of unbalanced gametes and their segregation.

Acknowledgements

The authors wish to thank Mr. Domenico Incarnato, CNR-ISPAAM for excellent technical assistance and Dr. Giuseppe Auriemma and Mr. Giuseppe Grazioli for blood collection. This study was supported by 'PSR, Misura 214-e2, Conservazione e Valorizzazione di Animali allevati in Campania e in via di estinzione', and project 'Razze Autoctone a rischio di estinzione della Regione Campania RARECa'.

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