Molecular Characterization of Xp Chromosome Deletion in a Fertile Cow

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Key Words

Cattle · CGH array · Deletion Xp · FISH

Abstract

A young cow of the Marchigiana breed (central Italy) with normal body conformation and external genitalia underwent routine cytogenetic analyses prior to its use for reproduction. After normal chromosome staining, only one X chromosome was observed with a normal diploid number ($2n = 60$) in all 200 studied cells. Subsequent cytogenetic analyses by using both CBA- and RBA-banding techniques evidenced that almost all the p arms of the other X chromosome were lacking. Detailed FISH-mapping analyses with BAC covering this Xp arm region demonstrated that this large chromosome region was deleted. RBA-banding showed that the deleted X was late replicating. CGH array analysis evidenced that deletion involves the Xp arm from the telomere to around 39.5 Mb, referring to the BosTau6 cattle genome assembly. This abnormality deletes about 40 Mb of the X chromosome sequence, but, despite the large number of genes deleted, none of them are programmed to escape from inactivation. This can explain the normal phenotype of

the female which is actually pregnant. Finally, we evidenced, by analysis of an SNP mapped to the deleted region (SNP rs29024121), that the only normal (e.g. nondeleted) X chromosome present derives from the father. Hence, the deletion has a maternal origin.

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In mammals, the X chromosome is present in both males and females. In male cells, it is present together with the Y chromosome, which carries the testis determining gene SRY, while the female cells show 2 X chromosomes. The X chromosome is usually very large. In humans, it is about 155.3 Mb long and, to date, around 1,270 RefSeq genes (excluding splicing versions, hg19 genome assembly) have been annotated on this chromosome. In cattle, the X chromosome is the second longest chromosome after BTA1, and its sequence is estimated to be 148.8 Mb long. To date, 521 RefSeq genes have been annotated (BosTau6 genome assembly). In order to compensate for the difference in the quantity of the genes expressed between males (which possess only one copy of X-linked genes) and females (which have 2 copies of X-

linked genes), in early female embryonic development, one of the 2 X chromosomes is randomly and permanently inactivated in cells [Morey and Avner, 2011]. This phenomenon, called dosage compensation, involves apparently all X-linked genes, although, as some genes escape inactivation, 2 copies of a few genes are expressed.

The inactivation process is the main reason why genomic alterations involving the X chromosome are usually more tolerated in XX individuals and are often lethal in XY. A typical example is represented by human patients carrying only one X chromosome (Turner syndrome). The patients carrying only one X chromosome develop a female phenotype, but they show some health problems [Reindollar, 2011]. Girls who are mosaics for Turner syndrome may be fertile, but this is a rare condition. Assisted reproduction techniques can help some women with Turner syndrome to become pregnant.

In cattle, the X monosomy condition seems to be very rare, with only one case being described to date [Prakash et al., 1995]. In this work, we report the molecular characterization of a young cow carrying a large deletion of the p arm in the mother-derived X chromosome. This abnormality deletes around 40 Mb of the X chromosome sequence. However, despite the large number of genes deleted, none seem programmed to escape inactivation.

Material and Methods

Subject Description

A young cow from the Marchigiana breed underwent routine cytogenetic analysis as required for all female subjects selected to enter a reproduction center. At the time when the analysis was performed, the cow was 10 months old and had a normal, clearly visible external phenotype corresponding to the Marchigiana standard. The cow is still alive and is currently pregnant.

Cytogenetic Analyses

Peripheral blood lymphocyte cultures to obtain normal and BrdU-treated cells (R-banding), as well as the CBA-banding procedure, were performed following the protocols reported by Iannuzzi and Di Berardino [2008].

FISH Analyses

Six BACs (table 1) were selected taking in account the data available on the BovMap database (<http://locus.jouy.inra.fr/cgi-bin/bovmap/intro2.pl>), considering their physical position and the data obtained from banding experiments. They were grown overnight at 37°C in LB Broth (LB) supplemented with chloramphenicol, and DNA was extracted according to the methods described at the CHORI web site (<http://bacpac.chori.org/>). The FISH procedure was performed as reported in De Lorenzi et al. [2007]. FISH analyses with telomere probes were performed as reported in De Lorenzi et al. [2010].

Table 1. BACs used

BAC	Ends	Genome position (BTAX, BosTau6)
629C12	CR813468/CR813467	6,358,384–6,236,804
143C10	CR827881/CR827880	18,253,539–18,142,038
585E05	CR810928/CR810927	25,787,625–25,908,703
238E09	CR795318/CR795317	33,785,256–33,910,100
577H05	CR810536/CR810535	145,824,973–145,955,589

Array Analyses

Whole-genome array-CGH, using a SurePrint G3 Bovine CGH Microarray 180k (Agilent Technologies, Santa Clara, Calif., USA) with a resolution of about 11 kb, was performed on our subject to determine the genomic size of the deletion and exclude any concurrent microdeletion/microduplication elsewhere in the genome. Array-CGH was performed as reported in De Lorenzi et al. [2010]. DNA extracted from a normal male subject was used as control.

Analyses of Anomaly Origin

DNA from the subject, as well as from its mother and father, was extracted using the UltraClean BloodSpin DNA Isolation Kit (MoBio) following the supplier's indications. Several VNTRs and SNPs specific to the genomic region involved in the deletion were examined, but only one yielded an informative result: SNP rs29024121 (NCBI SNP database). The genomic region was amplified by PCR using the following primers: forward: 5'-TGG-ACTACACAGACACCCTACAA-3' and reverse 5'-GGGGCC-TTGATAAGGATTTT-3'. PCR products were then directly sequenced.

Results

Analysis of Giemsa-stained metaphases revealed the presence of only one X chromosome together with the presence of a subtelocentric chromosome (fig. 1a). This analysis was performed on more than 200 metaphases to exclude chimera chromosome constitution (99% confidence for the presence of another cell line with a proportion above 3% [Hook, 1977]). The parents were analysed and found to have a normal karyotype establishing a 'de novo' origin of this anomaly.

FISH analysis, performed with cattle BACs mapping on Xq (fig. 1b, c), confirmed that the subtelocentric chromosome represents an abnormal X chromosome. Subsequent FISH analysis with 4 cattle BACs specific to the Xp chromosome region (fig. 1d–f) detected the presence of a genomic deletion involving almost the whole p arm.

Fig. 1. FISH results on cattle. The figure reports the results obtained by FISH. Normal X chromosomes are indicated by green arrows whereas deleted X chromosomes are indicated with pink arrows. White arrows indicate X centromeres. **a** Giemsa stained metaphase. **b–e** FISH results obtained with the BACs reported in table 1. **f** FISH results with the telomeric probes (green signals).

Given that the deletion involves the Xp telomere, we performed FISH analysis with telomeric probes. The results showed that the telomere is present and, interestingly, the signal observed on the Xdel p arm is always much stronger than in the normal Xp (fig. 1g). C-banding in Xdel was as in normal X (fig. 2a–d). Abnormal X was late replicating in all 30 examined cells, as revealed by the RBA-banding technique (fig. 2e–h).

In order to define in greater detail the genome region involved in the deletion, we performed CGH array analyses, which showed that the deletion involves the Xp arm from the telomere to bp 39.449.125–39.467.501, referring

to the BosTau6 cattle genome assembly (fig. 3). Considering the reference DNA was from a normal male subject, the deletion region appears balanced, whereas the normal X appears duplicated. At the same time, at the q end of the X chromosome, the balanced region corresponds to a unique PAR region present on the cattle genome. Finally, from the analysis of an SNP mapped to the deleted region (SNP rs29024121), it emerged that the only normal (i.e. nondeleted) X chromosome present derives from the father. Hence, the deletion has a maternal origin (fig. 4).

Fig. 2. CBA- and RBA-banding. Details of CBA- (left) and RBA- (right) banding patterns of cow metaphase plates at the different degree of chromosome contraction. **a** Note the negative C-band in both abnormal (large arrow) and normal (small arrow) X chromosomes. Though pale, a very small heterochromatic band can be seen in both normal and abnormal Xp-prox. **b** RBA-banding showing the abnormal (and late replicating) X (large arrow) as well as the normal (and early replicating) X (small arrow). Arrows indicate the centromere in all details.

Discussion

The young cow in question was found to be morphologically normal and pregnant. The 39.5-Mb deletion removes around 141 RefSeq genes from the genome, and it would be interesting to ascertain whether some of these genes are programmed to escape the inactivation process.

From an evolutionary point of view, the deleted part of BTAX corresponds to human Xq24–28 [Goldammer et al., 2009]. More precisely, the first gene present on the cattle Xp telomere is *SLC6A14* (BTAX 733 kb), whereas the last gene in the Xpdel is *GAB3* (BTAX 39,064 kb). These 2 genes are located on HSAX 115,567 kb and 153,906 kb, respectively, and appear to be part of a single evolutionary conserved genome fragment, although some inversions and small discrepancies are present (for online supplementary fig. 1, see www.karger.com/doi/10.1159/000342189). The next step is to verify whether some of the genes in the deleted region escape the inactivation process. In human females, a deleted X chromosome is usually inactivated in most cells of the adult. This is caused by a skewed (nonrandom) X inactivation probably due to a powerful selection mechanism during embryogenesis that ensures a less harmful gene imbalance. The phenotype, in cases with complete skewing of X inactivation, was either normal, consistent

Fig. 3. Array-CGH results. BTAX array-CGH profiles. The whole X chromosome is reported on the left whereas the 2 main regions are enlarged on the right. The upper region (**a**) reports the origin of the deletion, whereas the lower region (**b**) reports the PAR boundary localization (the control DNA was from XY subjects).

Fig. 4. Parental origin of the anomaly. The sequencing results for the genome region surrounding the rs29024121 SNP is displayed. **a** Mother of the subject. **b** Father of the subject. **c** Subject carrying Xp deletion. SNP is yellow boxed. The mother (normal XX) is C/C, the father (XY) is T, and the deleted subject (only one p arm carrying the SNP is present) is T.

with a single gene disorder or consistent with the classical Turner syndrome. In such cases, haploinsufficiency of the genes located in the deleted region, and which normally escape X inactivation, may explain the pathological phenotype.

In search of these genes on the human genome [Carrel and Willard, 2005], we can observe that at least 4 genes in the HSAX region homologue to the BTAX deleted region

clearly escape the inactivation process: EST F03810, Plexin B3 (*PLXNB3*), Arginine vasopressin receptor 2 (*AVPR2*) and the inhibitor of kappaB kinase gamma (*IKBK*G alias *NEMO*). None of these genes are present in the current genome assembly (BosTau6). Searching for these genes by using bioinformatic procedures reveals that EST F03810 shows no homology within the cattle genome, whereas *PLXNB3*, *AVPR2* and *IKBK*G show homology within the cattle genome region 39.8/40.5 Mb, hence, after the deletions (online suppl. fig. 1). Given this result, it probably holds that no genes programmed to escape the inactivation process are found in the deleted region. This observation probably explains why the cow in question was morphologically normal and pregnant.

FISH analysis with pan-telomeric (or simple telomeric) probes (TTAGGG/AATCCC)_n confirm the presence of telomeric sequences on the short arm of both normal and deleted chromosome X, although, on the latter, hybridization signals were much more intense than those detected on the homologue. Since telomeres stabilize linear chromosomes against chromosome degradation, fusion and incomplete replication, simple terminal-deleted chromosomes must restore the lost telomere and stabilize themselves and do it essentially through 2 mechanisms: (a) ‘healing’ of the truncated chromosome sequences by the addition, mediated by telomerase, of a new telomeric sequence at the breakpoint [Vermeesch et al., 1998; Var-

ley et al., 2000]; (b) ‘capturing’ of a telomere from another chromosome with formation of a reciprocal translocation [Kostiner et al., 2002; Ballif et al., 2004]. In our subject, whole-genome array-CGH with a resolution of about 11 kb failed to identify any concurrent telomeric microduplication elsewhere in the genome, excluding ‘capturing’ of a telomere from another chromosome. We suggest that the more intense TTAGGG/AATCCC signal on the deleted chromosome X could be attributed to the presence of larger number of telomeric repeats added by telomere healing, a mechanism involved in the repair and stabilization of this terminal deletion. Regarding the origin of the deletion, it may be noted that in humans the large majority of de novo deletions involving the X chromosome is of paternal origin [James et al., 1998; Lachlan et al., 2006].

In this study, we described a very rare case of BTAX chromosome anomaly. We report the findings of CGH array analysis and genomic analysis, and suggest a parental origin for the anomaly.

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