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# Brief Report
# Chromosomal assignment of the ovine *hairless* (*hr*) gene by fluorescence *in situ* hybridization

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Congenital hypotrichosis in mammalian species consists of partial or complete absence of a hair coat at birth. Affected individuals having a partial hair coat at birth may loose it subsequently. Tactile hairs and eyelashes are generally present. Cases of hypotrichosis have been found in cattle (Mohr and Wriedt 1928; Julian 1963; Bracho et al. 1984; Drögemüller et al. 2002), and in sheep (Nel 1964; Dolling and Brooker 1966; Lazovskii 1983; Mackie and McIntyre 1992). Several genes have been found responsible for hypotrichosis in various species (Pantelouris 1968; Cachon-Gonzalez et al. 1994). In humans, mice, and rats, the *hairless* (*hr*) gene is often responsible for this disorder and is highly homologous suggesting high conservation among mammals (Ahmad et al. 1998; Panteleyev et al. 1999). The *hr* gene codes for a protein which is a transcriptional co-repressor for thyroid hormone receptors (Potter et al. 2001).

More recently, hypotrichosis was described in Sicilian dairy sheep belonging to the Valle del Belice breed (Finocchiaro et al. 2003). Because the hypotrichotic phenotype is an undesirable defect for farmers, affected lambs are slaughtered soon after birth. Therefore information on the population frequency of the disorder is missing. The disorder was found under recessive genetic control (Finocchiaro et al. 2003). Hence the hypotrichotic phenotype is observed only when the allele is present in the population with reasonable frequency, so that homozygous carriers appear. Often the tendency towards mating related animals results in the appearance of the disorder. The *hr* gene was chosen as a functional candidate gene for the hypotrichotic disorder in the Valle del Belice dairy sheep (Finocchiaro et al. 2003). After sequencing the *hr* gene in Valle del Belice carriers, a C→T point mutation was found in exon 3 at 1312 bp resulting in a TAG stop codon. Successively a PCR-SSCP test was developed (Finocchiaro et al. 2003). All affected animals, genotyped with the PCR-SSCP test turned out to be homozygous carriers of the mutation, whereas heterozygous carriers and non-carriers were not affected. The *hr* gene therefore was found to be the gene responsible for the congenital hypotrichosis in Valle del Belice dairy sheep.

The aim of this paper was to physically map the ovine *hr* gene using fluorescence *in situ* hybridization (FISH).

The probe for the FISH was directly labelled by PCR in the presence of digoxigenin 11-dUTP (Roche Diagnostics) with the primer sequences and PCR procedure, as reported in Richard et al. (1994). The primer sequences were 5′-GCCCAGCTGCCAGCCCGCAA (HR3Forward) and 5′-ACAGTGGCTGGGAGTAGTGGGC (HR4a Reverse).

The PCR reaction was as follows: initial denaturation at 92°C for 2 min, 35 cycles with denaturation at 94°C for 1 min, annealing at 65°C for 40 s and elongation at 72°C for 5 min, followed by a final denaturation at 72°C for 5 min. Chromosome preparations were arranged from fibroblast cultures by standard procedures (Mezzelani et al. 1995), except for the hypotonic treatment, with 0.02 M KCl, at 37°C for 13 min. After a few days at −20°C, the slides were

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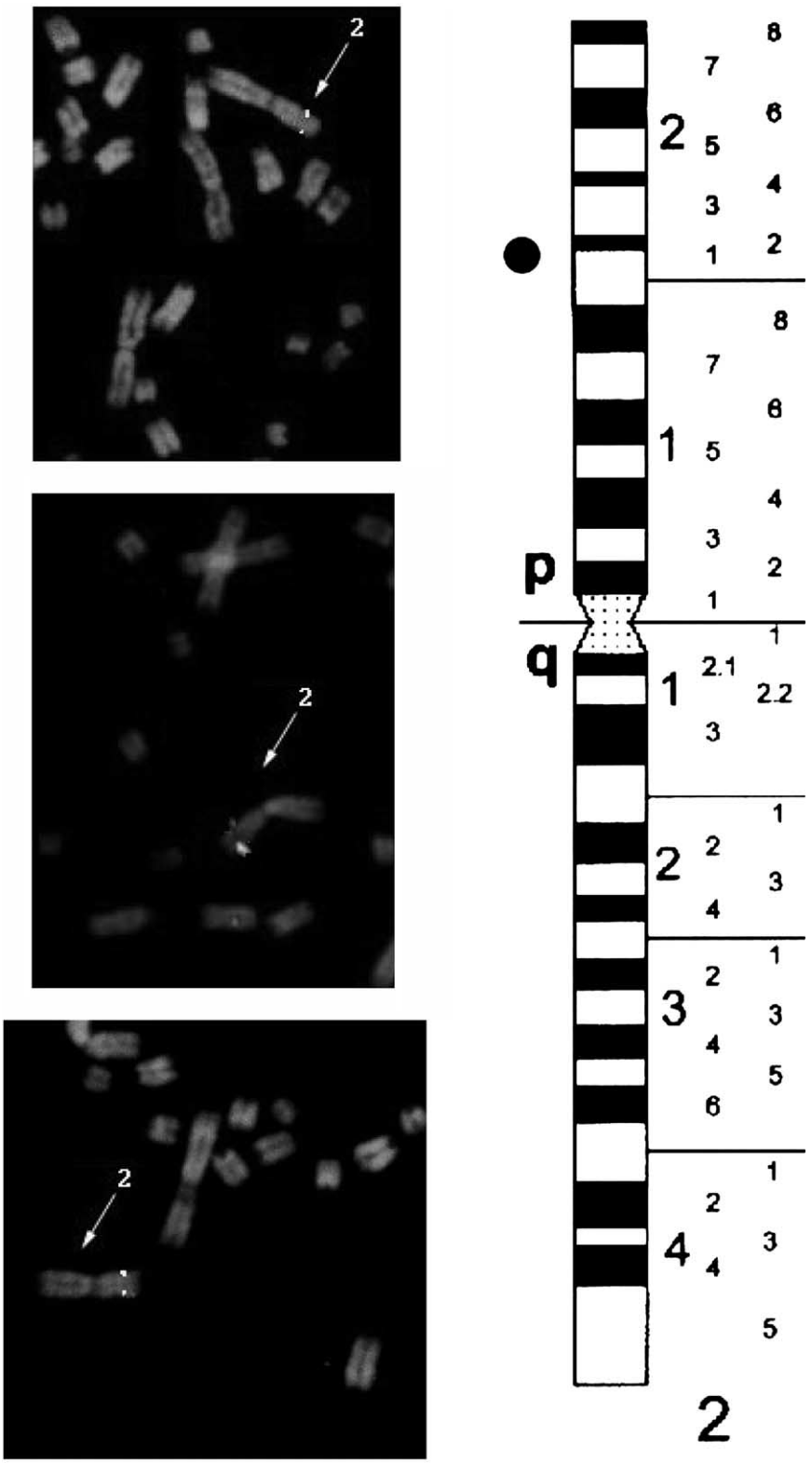


**Fig. 1.** Chromosomal assignment to OAR 2p21-22 for the ovine *hairless* (*hr*) gene using FISH with labelling of the mutation.

stained with 0.005% Quinacrine Mustard for 10 s (QFQ-banding), and well-spread metaphases, with distinctive banding, were imaged on a Leitz Aristoplan microscope connected to a CCD-camera (Photometrics) controlled by an Apple Macintosh Quadra 950 computer. The probe was then hybridized in situ to metaphase chromosomes at a final concentration of 0.5 ng $ml^{-1}$ (5 ng $slide^{-1}$) in the presence of 3 mg of Cot-1 DNA (Sigma-Aldrich). The probes were detected via antidigoxigenin-rhodamine (Roche Diagnostics). DAPI was used to counterstain the chromosomes. Digitized images were taken separately for each fluorochrome and merged with the computer programs IPLab Spectrum MultiProbe (Signal Analytics) and Gene Join (Office of Cooperative Research, Yale University). Chromosome identification and banding followed the latest chromosome standard nomenclature (ISCNDB 2000, Cribiu et al. 2001).

## RESULTS AND DISCUSSION

The results of the hybridization of the *hr* gene on ovine chromosomes are shown in Fig. 1. Fifty metaphase spreads were examined, with 50% showing a specific signal on at least one of the homologous chromosomes.

A preliminary study on an internet data bank (http://www.informatics.jax.org) showed that the regions of the *hr* gene on murine chromosome 14 and human chromosome 8 present homology with ovine chromosome 2. Using FISH labelling the mutation the chromosomal assignment for the ovine *hr* gene was OAR 2p21-22. The regional localization of the probe was established by determining the FLcen values on sheep chromosomes ($0.58 \pm 0.07$), as described by Lichter et al. (1990). This result confirmed the most likely location of the *hr* gene on ovine chromosome 2.

In human, the hairless gene has been localised on chromosome 8p21.2 by means of a radiation hybrid panel (Cichon et al. 1998). In pig, the HR gene has been localised on chromosome 14 by means of linkage mapping (Fernandez et al. 2003). In cattle, a gene similar to hairless (accession number XR_028305) has been recently predicted on chromosome 8 by automated computational analysis derived from an annotated genomic sequence (NW_001495463) using the gene prediction method GNOMON.

As reported in ISCNDB 2000, the International System for Chromosome Nomenclature of Domestic Bovids the bovine chromosome 8 is homologous to ovine chromosome 2p and to human chromosomes 8p and 9 (Cribiu et al. 2001). Comparative status of porcine, ovine, bovine and human chromosomes obtained by chromosome painting and gene mapping revealed the correspondence among SSC 14q1.2-q1.4, OAR 2, BTA 8q2.1-q2.3 and HAS 8p (Fronicke and Wienberg 2001).

Furthermore, we used the Oxford grid comparative-mapping tool available on the http://oxgrid.angis.org.au/sheep/ website, which is able to move from a region of interest on a particular chromosome of a species to the orthologous regions of any other species, linking up with relevant sequence data when available. In this way, the comparitive predictions of orthologues between sheep and cattle and between sheep and human showed the correspondence among a region of sheep chromosome 2 with a part of bovine chromosome 8 and of human chromosome 8. In particular, two reference loci in this region are LPL and BMP1. Using the Entrez Gene tool, the LPL gene is localized on OAR 2, on HSA8p21.3, on BTA8q and on SSC 14q12-q14, whereas BMP1 is mapped on HSA8p21 and BTA8q21.

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