Assignment\(^1\) of the ovine uroporphyrinogen decarboxylase (UROD) gene to chromosome 1p34→p36 by fluorescence in situ hybridization

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1 To our knowledge this is the first time this gene has been mapped in sheep.

Rationale and significance

Heme biosynthesis is a complex multistep process, taking place partly in the cytosol and partly in the mitochondria. A dysfunction of one or more enzymes in the biochemical pathway results in the accumulation of intermediates of the porphyrin synthesis and to the onset of porphyrias. Porphyrias are mainly hereditary; however, they can also be induced by exogenous factors. Depending on which enzyme of the heme biosynthesis is defective, different types of porphyrias can be discriminated (Phillips et al., 2001). Porphyrina cutanea tarda (PCT), caused by a defect of the uroporphyrinogen decarboxylase, is a chronic hepatic porphyria and most often the form of porphyrias in man (Egger et al., 2002). Uroporphyrinogen decarboxylase (UROD) catalyzes the synthesis of copro-porphyrinogen III from uroporphyrinogen III by a stepwise decarboxylation of acetic acids. The clinical signs of PCT are caused by the reduced UROD activity that results in an increase of uro- and heptacarboxyporphyrin in the liver (Elder, 1998). These and other metabolites are then excreted in the urine and faeces (Poh-Fitzpatrick, 1998). The presence of porphyrins in the urine leads to a brown discoloration. The highly carboxylated uro- and heptacarboxyporphyrin are mainly found in the urine, whereas porphyrins with 4, 5, or 6 carboxyl groups are less abundant (Poh-Fitzpatrick, 1993). In the liver, deposition of porphyrins results in an inflammation of hepatocytes, fibrosis and hemosiderosis. Once the storage capacity of the liver is exhausted, porphyrins are also deposited in the skin resulting in a severe photosensitivity. Especially in animals, those that are exposed to sunlight develop blisters, hyperkeratose, acanthose and other defects of the skin at hairless areas.

The human UROD gene has been mapped to HSA1p34 and the murine ortholog to chromosome 4 (51.4 cM) (MacLellan et al., 1985; Dubart et al., 1986). The UROD gene harbours 10 exons spanning a region of approximately 3 kb. Molecular analyses and identification of UROD gene mutations in livestock have never been described so far. We have isolated and characterized the ovine UROD gene and report here its chromosomal location.

Materials and methods

An ovine genomic λEMBL3 library (CLONTECH Laboratories GmbH, Heidelberg, Germany) was propagated in E. coli K803. Screening of the library was done by PCR using primers UD-\(^\beta\)-fwd (5’-GCC ATT AAG AGA GC-3’) and UD-\(^\beta\)-rev (5’-ACC AGA GCA TCA GTG AG-3’) derived...
from exon 5 and exon 6 of the human UROD gene sequence (EMBL Database acc. no. U30787), according to standard protocols (Ausubel et al., 1993). Isolated recombinant phages were purified and restricted with different restriction enzymes for subcloning and DNA sequencing. Restriction fragments were subcloned into pGEM®4-Z (Promega) and used for transformation of E. coli XL1-blue (Stratagene). Plasmid DNA was prepared using the Qiagen plasmid kit (Qiagen Plasmid Purification Kit, Qiagen). DNA sequencing was done using the Thermo Sequenase Fluorescent Labelled Cycle Sequencing Kit (Amersham Pharmacia Biotech) and IRD800 labelled standard universal and reverse sequencing primers. Reaction products were separated in 6% polyacrylamide sequencing gels on a LI-COR 4000L automated DNA sequencer at 1,500 V, 35 mA and 31 W for 12–14 h. Analysis of the DNA sequences was done with the BaseImageIR V2.3 software and then further processed using the Sequencher™ 4.1 Software (Gene Codes Corporation, Ann Arbor, MI).

Sheep metaphase chromosome spreads were prepared from phytohemagglutinin (PHA)-stimulated peripheral lymphocytes. Phage lambda-DNA was labeled with digoxigenin-dUTP (Roche) by nick translation. To block repetitive sequences a 10-fold excess of sheep Cot-1 DNA was added. The denatured probe was allowed to pre-anneal for 20 min at 37°C before hybridization. Final probe concentration was 50 ng/μl. Hybridization to metaphase chromosomes was performed at 37 °C for 72 h. Digoxigenin was detected by a monoclonal mouse anti-digoxigenin antibody and a FITC-conjugated sheep anti-mouse IgG antiserum (Sigma). Chromosomes were counterstained with DAPI and mounted in Vectashield (Vector Laboratories, UK). Digital Images were acquired by a peltier-cooled B/W CCD camera (Axio-cam, Zeiss, Germany) attached to an epifluorescence microscope (Axioplan 2, Zeiss, Germany). The chromosomal position of FISH signals was assessed according to the standardized sheep karyotype (Ansari et al., 1999) by comparison with the G-band-like DAPI staining pattern and by measuring the relative fractional length from the telomere of the q arm to the hybridization signal (Flqter).

Probe name(s): λUD1
Probe type: Ovine genomic λEMBL3 phage
Insert size: app. 16 kb
Vector: λEMBL3
Proof of authenticity: Sequence accession no. OAR295031
Gene reference: UROD

Results

Mapping data:
Most precise location: 1p34→p36
Number of chromosomes examined: 13
Flqter: 0.93 ± 0.003

References