Assignment¹ of the bovine *PTGFR* and *HSD3B1* genes to bovine chromosome 3 with somatic and radiation hybrid panel mapping

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¹To our knowledge this is the first time *PTGFR* has been mapped in cattle. This is a more precise localization of *HSD3B1* previously mapped to BTA3q12 by Gautier et al., 2001.

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Rationale and significance

The prostaglandin F receptor gene (*PTGFR*) encodes a protein called FP which is a member of the seven-transmembrane domain receptor superfamily of G-protein coupled receptors (Zaragoza et al., 2004). This protein is a receptor for prostaglandin F₂-alpha which is known to be implicated in reproductive processes such as ovulation, luteolysis and parturition, smooth muscle contraction, calcium mobilization and other physiological processes (Betz et al., 1999). The bovine receptor gene structure has been characterized, as well as the gene from human, mouse and rat, and consists of three exons and two introns (Ezashi et al., 1997; Zaragoza et al., 2004). The human gene has been localized to a very small region on HSA1 (q31.1) (Thomas et al., 2003). The hydroxyl-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1 (*HSD3B1*) enzyme is involved in the biosynthesis of all classes of hormonal steroids including aldosterone, cortisol and testosterone. This enzyme is expressed in the placenta,

adrenals, testes, and in peripheral tissues such as muscle and adipose tissue (Simard et al., 1996). In humans, the gene contains 4 exons and 3 introns and is located on HSA1p13 (Berube et al., 1989; Simard et al., 1996). The human intron-exon junctions are also in agreement with the sequence of the bovine gene (Zhao et al., 1989). Congenital deficiency of *HSD3B1* activity causes a severe depletion of steroid formation by the adrenals and gonads and is frequently lethal in infants. Because of their important implications in reproductive functions we report here the assignment of the bovine *PTGFR* and *HSD3B1* genes. *PTGFR* was mapped for the first time. *HSD3B1* was previously assigned to BTA3 by Gautier et al. (2001), by analysis on the INRA hamster-bovine somatic cell hybrid panel. In this report, physical mapping of *HSD3B1* was also performed by PCR typing on a different bovine-hamster somatic cell panel with a different PCR primer pair, followed by RH mapping.

Materials and methods

Polymerase chain reaction (PCR) primers were used to amplify a region of about 390 bp and 260 bp of the bovine *PTGFR* and *HSD3B1* genes, respectively. The primer sequences (*PTGFR* – Forward: 5'-CTG GAA AAA TCA GCC CTC AC-3' and Reverse: 5'-AAG AAG CCA CAA TCA ATC CC-3' and *HSD3B1* – Forward: 5'-AGC ACC ATA GAA GTG GCT GG-3' and Reverse: 5'-CAG GAT GCC GTT GTT ATT CA-3') were designed from *Bos taurus* mRNA sequences (GenBank accession numbers NM_181025 and NM_174343, respectively) using Primer3 (http://www.genome.wi.mit.edu/genome_software/other/primer3.html). The PCR reactions were performed in 10-μl reaction mixtures

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Table 1. Segregation of *PTGFR* and *HSD3B1* with bovine chromosome 3 in somatic cell hybrids

Reference markers/ new markers	UWCA7/		HUI246/		BM2924/	
	<i>PTGFR</i>	<i>HSD3B1</i>	<i>PTGFR</i>	<i>HSD3B1</i>	<i>PTGFR</i>	<i>HSD3B1</i>
+/+	4	4	4	4	4	4
-/-	27	27	26	26	25	25
+/-	0	0	0	0	0	0
-/+	0	0	0	0	0	0
% concordant	100	100	100	100	100	100
% discordant	0	0	0	0	0	0

containing 50 ng of template DNA; 1.5 mM MgCl₂; 10 mM Tris-HCl; 50 mM KCl; 0.2 mM dGTP, dTTP, dATP and dCTP; 10 pmol each sense and antisense primer and 0.5 U of Taq DNA polymerase (Ampli-Taq Gold; PE Applied Biosystems, Foster City, CA, USA). PCR conditions included 95°C for 10 min; 35 cycles of 95°C for 30 s, 63°C (*HSD3B1*) and 65°C (*PTGFR*) for 30 s and 72°C for 30 s; with a final extension cycle of 72°C for 7 min. PCR products were visualized on 2% agarose gels in 1.0× TBE buffer and stained with ethidium bromide. Initial bovine chromosomal assignment for both genes was obtained by PCR analysis using a somatic hybrid cell panel of 31 hamster/bovine and mouse/bovine hybrid somatic cell lines described previously (Womack and Moll, 1986). A whole-genome radiation hybrid (WGRH5000) panel (Womack et al., 1997) composed of 90 selected radiation hybrid cell lines was used to determine the specific chromosomal location for both genes. PCR results were scored as present (1), absent (0), or ambiguous (2). All PCRs were performed twice and scored independently. RHMPPER 1.22 software (Slonim et al., 1997) was used in assignment of PCR product to a chromosome and identification of the nearest marker in the bovine WGRH5000 map (Everts-van der Wind et al., 2004). Minimum LOD score accepted for linkage was 10.

Results

Somatic hybrid panel

As shown in Table 1, a bovine-specific band was detected in four of the 31 hybrid cell lines for both new markers. The data vector obtained for *PTGFR* and for *HSD3B1* was: 10000 00000 01100 00000 00000 000001 (1 = present in the cell line; 0 = not present in the cell line). Both genes were assigned on BTA3 with significant concordant value of 100% to the previously mapped reference markers UWCA7, HUI246 and BM2924 (Table 1).

Radiation hybrid panel

RH mapping confirmed the assignment of *PTGFR* and *HSD3B1* genes to BTA3. All PCRs were performed twice and scored independently resulting in the following vectors:

HSD3B1: 01000 00000 00000 10110 10010 01010 00011 00001 00000 00000 10011 00010 10000 00100 00000 00000 00000 10000

PTGFR: 00000 00000 00000 00000 00010 00000 00010 00000 00000 00000 00001 00000 00000 00101 00100 00000 10000 01001

A retention frequency (RF) of 0.10 (*PTGFR*) and 0.20 (*HSD3B1*) was calculated based on nine and eighteen bovine-specific PCR bands, respectively. The RF value observed on

PTGFR is a little lower than the published RF of 0.18 for BTA3 (Band et al., 2000). Two-point LOD scores showed that *PTGFR* is linked to *DPYD* (dihydropyrimidine dehydrogenase) gene with a LOD score of 12.0 for a θ value of 0.16 and *HSD3B1* is linked to *SAG* (S-antigen; retina and pineal gland – arrestin) with a LOD score of 18.2 for a θ value of 0.05. The gene *DPYD* is located in RH linkage group 1, position 138.2 and *SAG* is located in RH linkage group 2, position 112.2 of the second-generation cattle WGRH5000 map (Everts-van der Wind et al., 2004).

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