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Table 1 Lod scores.

Markers ECA6q	Z	θ (%)
COR070 – TKY028	12.3	3.9
ETHEC1 – COR070	12.4	2.5
ETHEC1 – TKY028	13.6	1.4
Maximized likelihoods for locus order		
COR070 – ETHEC1 – TKY028	27.1	2.5/1.4
ETHEC1 – COR070 – TKY028	26.3	2.5/3.9
COR070 – TKY028 – ETHEC1	28.1	3.9/1.4

Polymorphisms in the equine WNT1 gene allow linkage mapping to ECA6q

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WNT1 also termed *INT1* belongs to a family of oncogenes and is a homologue of the Drosophila *wingless* gene. These genes are secreted glycoproteins involved in a variety of developmental processes[1]. *WNT1* maps to human chromosome 12q13, a region sharing homology with horse chromosome 6q – e.g. locus *Pmel17/gp100* HSA 12q13–q14 maps to ECA6q23[2].

Using cross-species techniques, we amplified and sequenced partial putative intron 1, exon 2, intron 2 and partial exon 3 of equine *WNT1* (GenBank accession no. AY156994). Homology between horse and human *WNT1* fragments were confirmed up on size and protein coding sequence identity. Two SNPs and a microsatellite were detected:

SNP1, *WNTE2*, in putative exon 2
(pos. 388 – G-T; GenBank AY156994)
SNP2, *WNTI2*, in putative intron 2
(pos. 767 – G-A; GenBank AY156994)
MS, *ETHEC1*, in putative intron 2
(pos. 811–840; GenBank AY156994).

The following primer pairs were used:

WNT1 fragments:
WNT15'F1 GCAACAACCAAAGTCGCCGCAACTG
(GenBank X03072, pos. 410–434)
WNT1E2F1 CACGAACCTGCTTACAGACTCCA
(GenBank X03072, pos. 1306–1328)
WNT1E2R1 CTCGGTTGACGATCTTGCCGAAG
(GenBank X03072, pos. 1535–1513)
WNT1E3R1 GTGCAGGATTCGATGGAACCTTC
(GenBank X03072, pos. 2334–2312)

Microsatellite *ETHEC1*:
ETHEC1F GTTCTGAGTCGTGGACGCTCGC
(GenBank AY156994, pos. 737–758)
ETHEC1R GTCTCAGCGATGCAAGCCTTGC
(GenBank AY156994, pos. 897–876)

Amplification was performed with 2 mM $MgCl_2$, 0.2 mM dNTP's, 2.5 units of *Taq* polymerase GoldStar Red (Eurogentec, Seraing, Belgium) or a *Taq* polymerase from Amersham-Pharmacia Biotech (Piscataway, NJ, USA), 100 pmol of each primer, 1 μl of genomic DNA (200–400 ng/μl based on blood or hair samples) in a 25-μl reaction containing the accessory buffer system. After an initial denaturation step (95 °C for 5 min), PCR proceeded at 95 °C for 30 s, 56–58 °C for 30 s and 72 °C for 45 s for 35 cycles, followed by an extension at 72 °C for 7 min in a PTC-100 thermocycler (MJ Research, Waltham, MA, USA).

Polymorphism screening was carried out in four individuals of the Swiss Franches Montagnes horse breed (*FM*). *WNT1*-PCR fragments were used for direct sequencing on an ABI 377 (Applied Biosystems, Foster City, CA, USA) following standard protocols and manufacturer's instructions. Microsatellite forward primers were 5'-labelled with a fluorescence tag, in order to perform fragment length analysis of the obtained PCR product with the mentioned sequencer and the GeneScan software package 3.1 (Applied Biosystems). The analysis followed standard protocols and manufacturer's instructions. Microsatellites *ETHEC1*, *COR070* and *TKY028* – the latter two from ECA6q[3] – were typed in a pedigree containing 188 *FM*-horses. Marker genotypes and pedigree data were analysed for possible linkage with the *LINKAGE* package at http://www.hgmp.mrc.ac.uk/(Rockerfeller University, NY, USA).

The following results were obtained: microsatellite *ETHEC1* revealed six alleles in the tested horses with an estimated frequency of 0.01, 0.34, 0.21, 0.19, 0.06, 0.19 using *ILINK*. *ETHEC1* showed significant linkage with microsatellites *COR070* (eight alleles with a frequency of 0.03, 0.14, 0.04, 0.04, 0.24, 0.41, 0.03, 0.07) and *TKY028* (eight alleles with a frequency of 0.14, 0.02, 0.21, 0.07, 0.28, 0.18, 0.08, 0.02) – (Table 1). Thus, the linkage results indicate that *ETHEC1* maps to horse chromosome 6q close to the two mentioned markers. The most likely order using *ILINK* again was found *COR070 – TKY028 – ETHEC1* (Table 1). *ETHEC1* was detected in putative intron 2 of the equine *WNT1* gene. As a consequence, the horse *WNT1* gene is considered to map to this horse chromosome region. The SNPs *WNTE2* and *WNTI2* create a *FauI* and a *Bst*4CI cutting site, respectively. SNP *WNTE2* does not change the amino acid sequence encoded by putative *WNT1* exon 2. The variation and frequency of the two SNPs were not further analysed.

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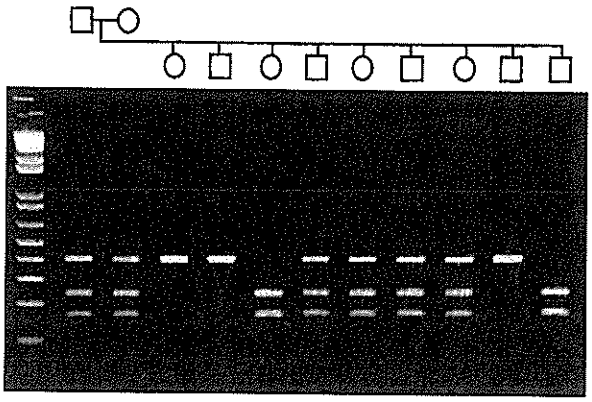

Figure 1 Photograph of an ethidium bromide-stained 2% agarose gel of an embryo transfer family from a Charolais sire and Limousin dam both heterozygous for a single nucleotide polymorphism in pro-opiomelanocortin. The uncut allele is 390 bp and the cut allele includes two fragments of 233 and 157 bp. The left lane shows a 1-kb plus DNA ladder (Invitrogen, Burlington, ON, USA).

Linkage mapping of POMC to bovine chromosome 11

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Source/description: Pro-opiomelanocortin (*POMC*) is a prohormone that is cleaved post-translationally to produce many peptides, such as α-melanocyte-stimulating hormone, which interact with specific receptors including melanocortin receptor 4[1,2]. Mutations in the human *POMC* gene have been described between individuals of weight extremes[3] and a fat mass quantitative trait loci (QTL) has been located on human chromosome 2[4]. *POMC* is therefore intimately involved with appetite and obesity and was considered here as a candidate gene for growth and fat.

Primer sequences: Forward: CGT GCA TCC GGG CCT GCA AGC Reverse: GTC AGC TCC CTC TTG AAT TCG AG

PCR and restriction digest conditions: The 20 μl PCR reaction contained 100 ng of bovine genomic DNA, 0.2 μM of each primer, deoxynucleotide triphosphates (dNTPs) (200 μM), Tris–HCl pH 8.8 (45 mM) $(NH_4)_2SO_4$ (11 mM), $MgCl_2$ (4.5 mM), β-2 mercaptoethanol (6.7 mM), ethylenediaminetetraacetic acid (4.5 mM), spermidine (0.25 mM), dimethyl sulphoxide (10%) and 0.65 U *Taq* DNA polymerase (Invitrogen, Burlington, ON, USA). The cycling protocol was 2 min at 94 °C, 35 cycles of 95 °C for 1 min, 52 °C for 45 s, 72 °C for 1 min, with a final extension at 72 °C for 4 min. A 2-h digestion with *Bts*I (New England Biolabs, Mississauga, ON, USA) was carried out in a 37 °C waterbath. The digested PCR-products were separated on a 2% agarose gel.

Polymorphism: A SNP was detected at nt 154 of the PCR-product (GenBank no. J00021, nt 254) where a cytosine was substituted with a thymine (amino acid remains serine). The mutation introduced a *Bts*I restriction site generating two fragments of 233 and 157-bp, from the 390-bp PCR-product. The 233 and 157 bp allele occurred with a frequency of 50% in 20 unrelated cattle.

Mendelian inheritance: Segregation was consistent with codominant inheritance and was found in 19 families screened. One of these families is shown in Fig. 1.

Chromosomal location: The Canadian Beef Cattle Reference Herd[5] was used for linkage mapping. As part of a larger QTL study, 162 microsatellites, eight of which were on cattle chromosome (BTA) 11, were used to genotype 14 grandparents, 20 parents and 136 offspring. *POMC* was mapped 2 cM from *BM8118* (LOD = 23.84) and 15 cM from *BM746* (LOD = 4.88). The most likely order using the 'BUILD' function of CRI-MAP (version 2.4, St Louis, MO, USA) was *BMS2569-RM096-INRA111-ILSTS036-BM8118-POMC-BM746-ILSTS045-HEL13*. *POMC* maps to human chromosome 2p23[6], and has been assigned to bovine chromosome 11 by synteny mapping[7], therefore the linkage assignment presented here supports previously published mapping data.

Comments: Pro-opiomelanocortin was a candidate gene for carcass weight and average daily gain (post-weaning) as a result of QTL peaks which were identified through previous research (F. C. Buchanan, D. C. Winkelman-Sim and S. M. Schmutz, unpublished). The location of *POMC* was consistent with the QTLs, however, no non-conservative substitutions were found in the coding regions of this gene.

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