# Mapping of the Melatonin Receptor 1a (MTNR1A) Gene in Pigs, Sheep and Cattle

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### Summary and Implications

Human and sheep Melatonin receptor 1a (MTNR1A) gene information was used to clone a portion of the coding region of this gene in pigs, and to identify polymorphisms of the gene for its assignment to both the genetic linkage and physical maps. MTNR1A maps to pig chromosome 17, establishing a new region of conserved synteny between this chromosome and human chromosome 4. Furthermore, we have assigned MTNR1A to bovine chromosome 27 and sheep chromosome 26. The addition of genes like MTNR1A to livestock genome maps allows questions about evolutionary events and the genetic basis for quantitative traits in livestock to be addressed.

### Introduction

Extensive efforts in the area of livestock genetics have produced comprehensive genetic linkage maps in the pig (Archibald et al. 1995; Rohrer et al. 1996). As regions of chromosomes are found to be conserved between human and livestock genomes, this information may help to identify genes in regions thought to affect quantitative traits. It is this potential that is directing livestock genome mapping in the direction of comparative genome mapping.

The hormone Melatonin is secreted by the pineal gland and known to help regulate circadian rhythms and reproduction changes in seasonally reproductive mammals. These effects are exerted through the binding of Melatonin receptors located in the brain. Reppert et al. (1994) cloned the cDNA for a high affinity Melatonin receptor 1a (MTNR1A) in human and sheep. The high sequence conservation of frog, sheep and human MTNR1A genes would suggest a strong likelihood of conservation in other species.

MTNR1A was recently mapped to human chromosome 4q35.1 and mouse chromosome 8 (Slaugenhaupt et al. 1995). Previous work has shown human chromosome 4 genes are conserved on pig chromosome 8, with a rearranged order (Ellegren et al. 1993). We were interested in mapping MTNR1A in the pig to see if its location would agree with this prior work. We also wanted to map this gene in sheep and cattle to further define previously established synteny in these species (see Table 3). It was therefore our objective to use Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and Southern blot hybridization analysis to map MTNR1A in pigs, sheep and cattle.

### Materials and Methods

PCR primers were designed from coding region of the human and sheep sequence. A 520 base pair (bp) product was purified and sequenced. This fragment was used to detect potential polymorphisms on membranes containing American and Chinese breeds and PiGMaP DNA (Archibald et al. 1995). Physical mapping data was obtained by using the pig x rodent somatic cell hybrid panel (Yerle et al. 1996) and a pig YAC library (Rogel-Gaillard et al. 1996). This approach determines where a gene is physically located by performing PCR experiments with whole and fragmented pig chromosomes in a present/not present PCR strategy.

A portion of the sheep and cattle MTNR1A gene was produced from genomic DNA using primers designed from sheep sequence (Reppert et al. 1994). In each species, an 824 bp product was produced and purified for sequencing. These products were cut with several enzymes to identify an RFLP, and the sheep AgResearch International Mapping Flock (IMF; Crawford et al. 1995) and the International Bovine Reference Panel (IBRP; Barendse et al. 1994) were genotyped.

Porcine and bovine linkage analyses were performed using the software package CRIMAP version 2.4 (Green et al. 1990) with data from PiGMaP ResPig Database (Archibald et al. 1995) and the Cattle Genotypic Database (Barendse et al. 1994). Pairwise linkage analysis was performed for all loci with a LOD score of 3.0 considered significant. The ovine two-point linkage analysis was performed with custom-built software built into the sheep genotype database (Crawford et al. 1995).

### Results and Discussion

The sequencing of the pig, sheep and cattle PCR products confirmed that we had the MNTR1A gene. A comparison of the percent identity at the protein level across species is shown in Table 1. Three polymorphic fragments at 4.3, 4.2, and 3.9 kilo bases (kb) were detected in the PiGMaP families (Archibald et al. 1995). Alleles at 3.8 and 2.9 kb were observed in additional breeds of pig. Allele frequencies were calculated for all breeds and are shown in Table 2. Results of the two-point linkage analysis produced a significant LOD score with microsatellite S0296 previously mapped to the PiGMaP chromosome 17 map (Archibald et al. 1995). The best multipoint map orders the genes on chromosome 17 as (cM shown in brackets): MTNR1A-[10.3]-S0296-[23.8]-ENDO-[6.3]-S0292-[21.1]-S0204-[7.0]-S0014. Physical mapping results confirmed the location on pig chromosome 17, and further localized the gene to the short arm at 17q1.2 (Fig 1.).

Two polymorphic fragments of 286 bp and 236 bp were identified in the sheep gene product. Results of the two-point linkage analysis produced significant LOD scores between MTNR1A and microsatellites BM6526 and OarJMP23 on sheep chromosome 26. The best map places MTNR1A between CSSM43 and BM6526 with a decrease in overall map length from 61.3 cM to 56.6 cM.

Two polymorphic alleles of 394 and 205 bp were identified in the cattle gene fragment. Results of the two-point linkage analysis produced significant LOD scores between MTNR1A and the following loci on chromosome 27: INRA16, INRABERN191, RM209, BM3507, TGLA179, CSSM36 and CSSM43. The best multipoint map places MTNR1A between RM209 and TGLA179.

The high level of sequence conservation across species made MTNR1A a good gene for comparative mapping. We were interested in the MTNR1A gene due to its location on human and mouse genome maps. Previous comparative mapping information has shown conserved synteny between human chromosome 4 and pig chromosome 8. We were initially surprised at the linkage of the porcine MTNR1A gene to loci on chromosome 17, since no homology between human chromosome 4 and pig chromosome 17 has been previously reported. Our result, however, has been twice confirmed with strong physical mapping data. Two possible explanations for this new information exist. The first is that we have identified a pig gene similar, but not identical to, MTNR1A. The other is that in the occurrence of the rearrangement of pig chromosome 8, the very end of the chromosome broke off and is now part of chromosome 17. This piece could be too small to be detected by some mapping techniques. This new information presents the question of what genes define the breakpoint on chromosome 8, and may be answered with additional mapping of other genes conserved between human chromosome 4 and mouse chromosome 8 (Table 3).

In cattle, the mapping location of MTNR1A is on chromosome 27. This result is in agreement with previous mapping information shown on the human and bovine comparative map (Womack and Kata, 1995). The mapping of MTNR1A to chromosome 26 in sheep adds to the syntenic conservation of cattle chromosome 27 and sheep chromosome 26 (Crawford et al. 1995).

The mapping of the Melatonin receptor 1A gene in these species has provided intriguing new information of the homology between genes on the end of human chromosome 4 and livestock chromosomes, and suggests that other human chromosome 4 genes may reside on these chromosomes. There are several genes on the short arm of human chromosome 4 that are not mapped in pigs, sheep and cattle. Once the order of these loci are determined, then questions about the genetic basis for quantitative traits in livestock and the evolution of each species in this region can be addressed.


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| | Pig | Sheep | Human1a | Human1b |
|---|---|---|---|---|
| **Pig** | --- | 83 | 84 | 63 |
| **Cattle** | 84 | 97 | 84 | 60 |

**Table 1. A comparison of MTNR1A percent protein identity across species.** Amino acid identity is calculated for a portion of the MTNR1A gene. Melatonin receptor 1b sequence published by Reppert et al. (1995).

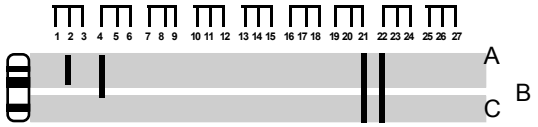


**Figure 1. Physical mapping results in the pig.** Results of PCR experiments with the porcine somatic cell hybrid panel localized MTNR1A to chromosome 17q1.1-q1.4 region.

**Table 2. Allele frequencies of the *Taq* I RFLP in several breeds of pig.**

| | | Frequency of each allele | | | | |
|---|---|---|---|---|---|---|
| Breed | Number | 4.3 kb | 4.2 kb | 3.9 kb | 3.8 kb | 2.9 kb |
| Meishan | 14 | .04 | .04 | .93 | 0 | 0 |
| Minzhu | 3 | 0 | 0 | .67 | 0 | .33 |
| Wild Boar | 2 | 0 | 0 | 1.0 | 0 | 0 |
| Large White | 11 | .23 | 0 | .77 | 0 | 0 |
| Yorkshire | 8 | .19 | 0 | .81 | 0 | 0 |
| Chester White | 8 | .38 | 0 | .38 | .25 | 0 |
| Hampshire | 11 | 0 | .23 | .73 | .04 | 0 |
| Landrace | 11 | 0 | 0 | .86 | 0 | .14 |
| Duroc | 10 | 0 | 0 | .65 | .35 | 0 |

**Table 3. The mapping assignments of distal HSA4q loci in the mouse, pig, sheep and cattle.**

| Locus Symbol | Human[a] | Mouse[b] | Pig[c] | Sheep[d] | Cattle[e] |
|---|---|---|---|---|---|
| IF | 4q24-25 | 3 | - | 6 | 6 |
| IL2 | 4q26-27 | 3 | 8 | - | 17 |
| FGF2 | 4q25-27 | 3 | - | 17 | - |
| FGG | 4q28 | 3 | 8 | 17 | 17 |
| UCP | 4q28-31 | 8 | - | 17 | - |
| MLR | 4q31 | - | - | - | 17 |
| TDO2 | 4q31 | - | - | - | - |
| KLK3 | 4q34-35 | 8 | - | - | - |
| F11 | 4q35 | - | - | - | 17 |
| ANT1 | 4q35 | 8 | - | - | 27 |
| MTNR1A | 4q35.1 | 8 | 17 [f] | 26 [f] | 27 [f] |

[a] Human Genome Database
[b] Mouse Genome Database
[c] PIGBASE
[d] SHEEPBASE
[e] BOVBASE
[f] Assigned from this paper