Placement of Prolactin on Pig Chromosome 7 by Linkage and Physical Mapping

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Summary and Implications

The study of genes which code for functional protein products have important implications in comparative genetic mapping and perhaps to the swine industry. The identification of a polymorphism, or change at the DNA level, is used to assign these genes to their corresponding chromosomes by the statistical method of two-point linkage analysis. A polymorphism was detected in the Prolactin gene (PRL) and was used to linkage map PRL to pig chromosome 7. To confirm this location, PRL also was genetically physically mapped using a pig x rodent somatic cell hybrid panel. These results confirm that the short arm of pig 7 contains another of the genes on human chromosome 6.

Introduction

PRL is a hormone primarily secreted by the anterior pituitary in response to other hormones, such as estrogen. PRL has numerous actions in mammalian tissue and is essential for reproductive success. The role of PRL in the synthesis of milk proteins has been well characterized (Kelly et al., 1991). PRL also is thought to play a role in the maintenance of pregnancy by acting on copora lutea cells and possibly initiating transcription of genes such as progesterone (Yuan and Lucy, 1996).

In prior work, PRL had been weakly mapped to pig chromosome 7 using a Southern-Restriction Fragment Length Polymorphism (RFLP). Comparative mapping between species allows us to make use of marker-rich genomes, like the human genome map. A rearrangement of the loci on human chromosome 6 to pig chromosomes 1 and 7 has been demonstrated, but through the placement of a limited number of markers. Since PRL is mapped to human chromosome 6, a more precise localization on pig chromosome 7 could be helpful in defining the rearrangement.

Materials and Methods

Sequence information from human genomic DNA (Truong et al., 1984) and porcine cDNA (Schulz-Aellen et al., 1989) was compared to locate likely intron-exon boundaries in the porcine cDNA. Pig specific primers were designed to span an intron, or non-coding region. This intron region was targeted with the expectation that the likelihood of a polymorphism is greater in introns than in the highly conserved coding regions. The primers amplified a 2.8 kb product. This product was purified and sequenced to confirm that it was indeed PRL. Grandparent animals from the three generation European Gene Mapping Reference (EGM) families were screened to identify an RFLP. Two polymorphic sites were found using the restriction enzyme BstUI.

Individuals from the EGM family were genotyped and analyzed using the software package CRIMAP version 2.4 (Green et al., 1990) with data from PiGMaP ResPig Database (Archibald et al., 1995). Pairwise linkage analysis was performed for all loci with LOD scores of three or greater being considered significant. A multipoint analysis was then performed to construct a multipoint map. The pig PRL primers also were used to physically map this gene in a pig x rodent somatic cell hybrid panel (Yerle et al., 1996). This panel allows markers to be physically assigned to cytogenetic regions by a positive-negative PCR amplification strategy.

Results and Discussion

Both of the BstUI sites in theamplified fragment were found to be polymorphic. When both sites are present, digestion results in fragments sizes of approximately 1350, 1020, and 410 base pairs. The genotypes were observed to be inherited in an autosomal Mendelian pattern. The two polymorphic sites were reported as one haplotype to give more power to the two-point linkage analysis. This analysis produced significant LOD scores between PRL and several loci on pig chromosome 7. The best multipoint map places PRL on the p-arm between S0064 and S0013 (Fig. 1). The more accurate placement of PRL on pig chromosome 7 allows this locus to be placed in the framework map and strengthens the order of the existing markers.

To physically map PRL, the primers were used in amplification reactions with DNA isolated from a somatic cell hybrid panel. The expected product was seen in clones 7, 10, 11, 16, 19, 21, 23, 24, 25, and 27. The data was submitted via the INRA Cellular Genetics Laboratory web-page and localized PRL to 7p.11.5-12 with 100% concordance (Fig. 2). This physical map location confirmed the genetic linkage placement.

DNA samples from unrelated animals from 7 breeds were also genotyped. The allele frequencies for the PRL BstUI haplotypes were calculated within breeds (Table 1).

Implications

Previous comparative mapping research has revealed several similarities between species, as well as differences. Genes located on human chromosome 6 have been mapped to pig chromosomes 1 and 7. Since PRL is mapped to

human chromosome 6, a better defined location of PRL in the pig genome was needed. Both the physical and genetic linkage data presented here for PRL strengthens the evidence of a rearrangement of some of the genes on human chromosome 6 to pig chromosome 7. The mapping of other genes from human chromosome 6 will further elucidate the rearrangement and breakpoint locations on this pig chromosome.

Acknowledgments

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Figure 1. Pig chromosome 7 multipoint map.

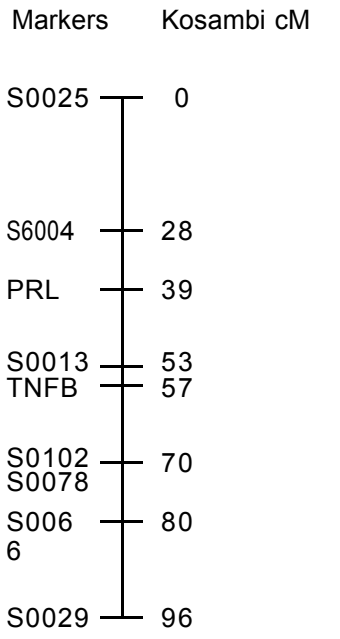

Figure 2. Somatic cell clones which contain portions of pig chromosome 7 (Location indicated by arrow on left).

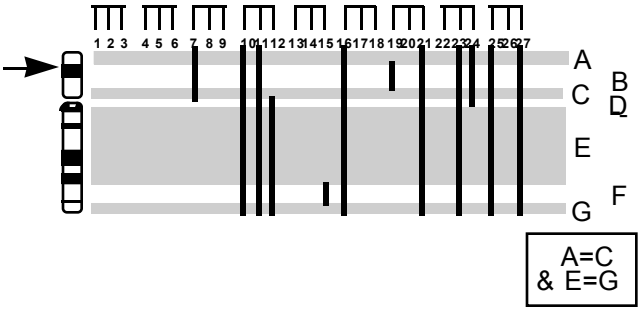

Table 1. The haplotype frequencies of PRL BstUI in seven breeds of pigs.

Breed	n	1	2	3
Chester White	9	0.83	0.17	0
Duroc	10	1.0	0	0
Hampshire	11	1.0	0	0
Landrace	10	1.0	0	0
Large White	11	0.41	0.23	0.36
Yorkshire	10	0.60	0.25	0.15
Meishan	9	0.78	0.11	0.11