

Influence of the genotype of the bovine osteopontin gene on its promoter activity: study of the basal activity of six different haplotypes

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The important role of OsteoPontiN (OPN) in immunity is reflected in the protection it provides. OPN is synthesized and secreted by a variety of cells and tissues, including the mammary gland. It is now recognized as an important proinflammatory cytokine with beneficial pleiotropic functions, including pathogen clearance.

In cattle, OPN increased during mammary gland infection. We identified DNA polymorphisms (SNPs) in the OPN gene (*SPP1*) which are associated with the mammary health status (somatic cell score) of lactating cows. In order to verify whether the genotype of the regulating sequence influences gene synthesis, the activity of the following promoter haplotypes was compared side-by-side *in vitro*: H1-GCG, H2-GTA, H3-ACG, H10-GCA, H11-GTG, and H12-ACA alleles. Each promoter was fused to transcribe the reporting luciferase gene, which produced an amount of luciferase proportional to the intensity of the promoter. It was measured in two biological systems: macrophage (BOMAC) and mammary epithelial (MAC-T) cell lines.

The basal activity of the H1 and H10 alleles was greater than that of the other alleles (H2, H3 and H11) ($P < 0.004$); H12 was found to be almost as active as H10 ($P = 0.108$) in BOMAC but remained weaker than H1 ($P = 0.014$). The general profiles of the activity of H1 vs. H10, H2 vs. H11, and H3 vs. H12 were highly similar, especially in the bovine mammary epithelial cells. More specifically, the two SNPs located above the transcriptional starting site have functional roles, because the presence of some genotypes almost doubled gene expression.

Implications: In this study, we demonstrated that the SNPs in the *SPP1* gene found to be associated with the somatic cell score are not only positional, but also functional markers and may contribute directly to the somatic cell trait. Whereas a positional marker is linked by association, a functional marker has an increased value because of its dual role. This genomic milestone, moreover, plays a direct function by modulating gene expression, which in turn influences the character. The factors that affect the function of these SNPs are under investigation.