



Aberrant promoter methylation of the *CD4* gene in peripheral blood cells of mastitic dairy cows

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ABSTRACT. Bovine mastitis is the most common and costly disease of dairy cattle. Cluster of differentiation 4 (*CD4*) is closely related to the immune response in mastitis. We quantified promoter CpG methylation levels of the *CD4* gene in Chinese Holsteins with clinical mastitis (CM) and in healthy controls; these levels were quantitatively detected with bisulfite pyrosequencing assays and confirmed by cloning sequencing. We found that the bovine *CD4* promoter had 16% more methyl groups in the cows with CM ($75.0 \pm 5.8\%$) compared to the controls ($59.0 \pm 8.5\%$). The decreased expression level of *CD4* in CM cows may be downregulated by the increased DNA methylation levels in the *CD4* promoter. Two-dimensional hierarchical clustering analyses showed large differences in promoter *CD4* methylation between mastitic and healthy cows; the dendrogram clearly distinguished the cows with clinical mastitis from healthy controls based on methylation levels. The DNA methylation level of the *CD4* gene was strongly influenced by mastitis status in all comparisons. We suggest that

the DNA methylation level of the *CD4* promoter can be used as a molecular marker for clinical mastitis in dairy cows.

Key words: *CD4*; Promoter DNA methylation; Cow; Clinical mastitis; Peripheral blood cell