Bovine lactoferrin promoter polymorphisms associate with reproductive performance and somatic cell count in Holstein-Friesian cattle in Ireland

F. O'Halloran[1], D.P. Berry[2], B. Bahar[3], D.J. Howard[4], T. Sweeney[3], L. Giblin[1]
[1]Teagasc, Moorepark Food Research Centre, Fermoy, Co.Cork, Ireland, [2]Teagasc, Moorepark Dairy Production Research Centre, Fermoy, Co.Cork, Ireland, [3]School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland, [4]Teagasc, Animal Production Research Centre, Mellows Campus, Athenry, Co.Galway, Ireland *Email: linda.giblin@teagasc.ie*

Introduction Lactoferrin plays an important role in the innate immune system, with well characterized anti-bacterial, anti-viral and immune modulatory properties. Lactoferrin gene expression is both constitutive and inducible and is species-, tissue- and cell-type specific. The objective of this study was to determine the allele and haplotype frequency of polymorphisms at positions -586, -190 and -28 of the bovine lactoferrin promoter in Holstein-Friesian sires and to quantify their association with performance.

Materials and methods Genomic DNA was isolated from semen straws of 848 Holstein-Friesian sires with progeny in Ireland. Genotyping analysis was performed by Sequenom® using the iPLEX Gold assay on a MassARRAY® Platform. Associations between genotypes and performance were quantified using weighted mixed models with genotyped individual included as a random effect and average expected relationships among individuals accounted for through a numerator relationship matrix; genotype was included as either a continuous or class fixed effect. The dependent variable was daughter yield deviation for milk production traits and de-regressed predicted transmitting ability for calving interval and functional survival.

Results The genotypes of Lf-586 and Lf-28 SNPs deviated (P<0.001) from Hardy-Weinberg equilibrium, with a marked deficiency in the TT and CC genotype respectively. The minor allele frequency of Lf-586, Lf-190 and Lf-28 was 0.14, 0.21 and 0.26, respectively. None of the three SNPs investigated were associated with milk, fat or protein yield or milk fat and protein concentration, although when included as a class effect in the model, Lf-28 was associated (P<0.05) with milk yield and protein yield. The strength of the association between the SNPs and the non-production traits differed by SNP and model fit (i.e. genotype included in the model as either a continuous variable or a class variable). The C to T allele substitution of the Lf-586 SNP associated with shorter calving interval (P=0.03) but increased somatic cell score (SCS) (P=0.06) (Table 1). Interestingly in a preliminary study, homozygous TT cows tended to have lower lactoferrin protein concentration in their milk over a lactation curve than their CC herdmates. In addition, transfection studies in mammary epithelial cells showed that a promoter variant which included the T allele had decreased transcriptional activity *in vitro* compared to a variant with the C allele. The G to A allele substitution of the Lf-190 SNP was associated with longer calving interval (P=0.01) and reduced functional survival (P=0.02) (Table 1). The CC genotype of Lf-28, which associated (P<0.10) with superior functional survival, also associated (P=0.02) with greater SCS. A multiple regression model that included all three SNPs indicated that the associations between both Lf-586 and Lf190 and calving interval were additive. The strength of the association with calving interval for Lf-586 (b=-0.98; SE=0.45) and Lf-190 (b=0.68; SE=0.31) was similar in strength to the univariate analyses. Five haplotypes were reconstructed (posterior means of the frequency in parenthesis) in the sequence of Lf-586, Lf-190 and Lf-28: CGA (53%), CAA (21%), TGC (13.5%), CGC (12%) and TGA (0.5%). Linkage disequilibrium (r^2) between Lf-586 and Lf-190, between Lf-586 and Lf-28 and between Lf-190 and Lf-28 was 0.04, 0.44, and 0.09 respectively. Association analysis indicated that sires with the $T_{-586}G_{-190}C_{-28}$ haplotype had improved genetic merit for calving interval and survival compared to $C_{-586}A_{-190}A_{-28}$ haplotype.

Table 1 Association between performance variables (somatic cell score, calving interval and functional survival) and (a) replacing a C allele with a T allele in the Lf-586 SNP (standard error in parenthesis), and (b) replacing a G allele to an A allele in the Lf-190 SNP (standard error in parenthesis)

	Lf-586		Lf-190	
Trait	C to T allele substitution	P-value	G to A allele substitution	P-value
SCS (log_e units*100)	1.64 (0.86)	0.06	0.22 (0.65)	0.75
Calving interval (days)	-0.72 (0.33)	0.03	0.68 (0.28)	0.01
Survival (%)	0.27 (0.18)	0.13	-0.39 (0.16)	0.02

Conclusions The C to T polymorphism at -586, which distorts a putative activating protein 2 (AP-2) binding site, was associated with shorter calving interval and higher SCS. The G to A polymorphism at -190, located in a putative selective promoter factor 1 (SP-1) transcription binding site, was also associated with longer calving interval and decreased functional survival and the association with calving interval was independent of the association with Lf-586. Based on the data we proposed a haplotype combination that associated with improved reproductive performance in the Holstein Friesian breed. We hypothesized that the observable phenotypic associations to lactoferrin promoter polymorphisms can potentially be explained by allele specific differences in constitutive or inducible levels of gene expression. The lack of a pleiotrophic effect of the promoter SNPs studied on both fertility and milk production traits strengthens the importance of these SNPs, or at least the lactoferrin promoter, in selection for improved fertility.

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