

Associations between leptin polymorphisms and performance traits in Holstein-Friesian dairy cattle

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Introduction Leptin modulates appetite, energy expenditure and the reproductive axis by signalling the status of body energy stores to the brain. Following parturition, cows enter negative energy balance (NEB), and mobilise body reserves in support of mammary milk synthesis. During early lactation NEB, adipose expression of leptin and circulating leptin concentrations are profoundly reduced. The reduction in leptin is likely involved in coordinating the neuroendocrine adaptations necessary to prioritise milk production over other physiological processes that are considered, temporarily at least, nonessential (e.g. reproduction, immune function). The aim of the present study was to quantify the associations between 9, novel and known, single nucleotide polymorphisms (SNPs) in the promoter and coding region of the leptin gene with performance traits in Holstein-Friesian dairy cattle in Ireland.

Materials and methods Genomic DNA was isolated from semen straws of 848 Holstein-Friesian sires with progeny in Ireland. For SNP discovery, ~3 kb of the regulatory region was amplified by polymerase chain reaction (PCR) from genomic DNA of 14 sires using six pairs of oligonucleotide primers designed based on the published sequence (GenBank Acc. No. AB070368). PCR amplicons were sequenced bi-directionally by Lark Technologies. 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; genotype was included as a continuous effect. The dependent variable was daughter yield deviation for milk production traits and de-regressed predicted transmitting ability for calving interval, functional survival, body condition score (BCS) and angularity. Weighting on the dependant variable was based on the reliability of the proof less the parental contribution. Multiple regression models were developed by backward elimination of non-significant ($P > 0.05$) SNPs.

Results All SNPs were segregating in this sample population and none deviated ($P \geq 0.05$) from Hardy-Weinberg equilibrium. Because of the strong linkage disequilibrium between phases among the SNPs LEP-1457, LEP-1609 and LEP-580, only LEP-1457 was retained for the association analysis. The T allele in LEP-2470 was associated ($P < 0.05$) with reduced milk protein concentration and showed a tendency to associate with increased ($P < 0.10$) milk yield (Table 1). The G allele of LEP-1239 was associated with reduced milk fat and protein concentration and tended to be associated with increase somatic cell score (SCS). LEP-963 demonstrated an association with milk fat %, milk protein % and a tendency to associate with milk yield. The T allele of Try7Phe, as well as being associated with protein yield (Table 1) was associated with reduced angularity (allelic substitution effect = -0.74 standard deviation units; SE = 0.23) and tended to be associated with BCS. However the minor allele frequency for TryPhe was 7% with no homozygous TT animals present in the data set. When a multiple regression model was developed the inclusion of the most significant SNP negated the inclusion of any other SNP indicating that the significance of several SNPs in the univariate analyses was an artefact of linkage with a possible causative mutation or mutations. There was no association between LEP-1457 and calving interval or survival.

Table 1 Allele substitution effect (standard error in parenthesis) between seven SNPs and milk performance traits

SNP	Allele substitution	Milk yield (kg)	Fat yield (kg)	Protein yield (kg)	Fat (%*100)	percentProtein (%*100)	percentSCS (units*100)
LEP-2470	C → T	23.61 (14.29) [†]	0.05 (0.51)	0.20 (0.40)	-1.72 (1.08)	-1.14 (0.52)*	0.06 (0.96)
LEP-1457	A → G	-7.73 (9.16)	0.16 (0.32)	0.00 (0.25)	0.72 (0.69)	0.45 (0.33)	-0.97 (0.61)
LEP-1239	C → G	14.57 (9.87)	-0.38 (0.35)	0.03 (0.27)	-1.59 (0.73)*	-0.80 (0.35)*	1.06 (0.65) [†]
LEP-963	C → T	-16.29 (9.62) [†]	0.27 (0.34)	-0.15 (0.27)	1.54 (0.72)*	0.73 (0.35)*	-0.79 (0.64)
Tyr7Phe	A → T	-39.19 (22.70) [†]	-1.11 (0.80)	-1.25 (0.63)*	0.56 (1.70)	-0.07 (0.83)	-1.52 (1.53)
Arg25Cys	C → T	-13.32 (9.61)	0.36 (0.34)	-0.07 (0.27)	1.52 (0.72)*	0.71 (0.35)*	-1.00 (0.64)
Ala80Val	C → T	7.97 (10.55)	-0.12 (0.38)	0.21 (0.29)	-0.73 (0.79)	-0.09 (0.39)	1.05 (0.70)

Significance of difference from zero [†] = $P < 0.10$; * = $P < 0.05$

Conclusions In summary, 3 SNPs in the promoter region and one SNP in the coding region significantly associated with milk production traits. All 3 of the promoter SNPs lie within putative transcription factor binding sites. In addition, Tyr7Phe associated with angularity. Previous reports have found associations between both leptin polymorphisms and milk production traits. Interestingly none of the SNPs, including LEP-1457, associated with calving interval in our data set even though previous work had found an association with LEP-1457 and first postpartum luteal activity (Leifers *et al.* 2005).

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