Short Communication

Single nucleotide polymorphisms in five adipokine genes in dairy cattle populations

K.A. Nephawe[1#] and D.M. Spurlock[2]
[1] Agricultural Research Council, Private Bag X2, Irene 0062, South Africa
[2] Department of Animal Science, Iowa State University, Ames, 50011, USA

Abstract

Genetic diversity in candidate genes for fitness and production traits was explored in three populations of dairy cattle. The study focused on adipokines, including *leptin (LEP)*, *tumor necrosis factor alpha (TNF)*, *interleukin-8 (IL8)* and *interleukin-10 (IL10)* as candidate genes. The three populations of interest included young Jersey and Holstein (modern Holstein) sires, and Holstein sires born prior to 1970 (traditional Holstein). Pools of DNA representing each sire group were used as template to generate PCR products for sequencing and identification of single nucleotide polymorphisms (SNP). Sequences of PCR products were assembled and SNPs identified using Sequencher 4.5 software. One SNP representing each gene and a previously reported SNP in *LEP* were selected for genotyping across all bulls. A multiplexed genotyping assay was developed using the ABI PRISM SNaPshot Multiplex Kit. Allele and genotypic frequencies were determined for each sire group, and genotypic frequencies were in agreement with Hardy-Weinberg equilibrium. Allele frequencies were compared among sire groups using the chi-square test. A significant difference between Jersey and Holstein was observed for all genes, and modern and traditional Holstein groups differed for the previously described *LEP* and *IL10* SNP. Although allele frequency differences between modern and traditional Holsteins may reflect the effect of selection pressure for production traits on these genomic regions, random genetic drift or sampling bias could also have contributed to the observed differences.

Keywords: Fitness traits, genetic markers, adipokines, dairy cattle
[#] Corresponding author. E-Mail: nephawek@lda.gov.za
[#] Current Address: Limpopo Department of Agriculture, Private Bag X9487, Polokwane 0700, South Africa

Fitness traits such as fertility, health and survival are important concerns of the livestock industry worldwide. Genetic improvement of these traits through traditional breeding methods is challenging because of difficulties in collecting accurate phenotypic data, and/or low heritability of the traits. However, these characteristics make fitness traits good candidates for improvement by marker assisted selection (MAS).

One approach to enhance development of genetic markers is the candidate gene approach. Using this approach, a particular gene is hypothesized to be involved in the regulation of a trait of interest based on the gene's known biological function. Sequences of these candidate genes are then evaluated to identify variation among animals, and associations between candidate gene polymorphisms and trait phenotypes are evaluated (Rothschild & Soller, 1997). The current study hypothesized that adipokines are important regulators of the partitioning of energy among the immune system, fitness and production traits in dairy cattle.

The objectives of the research described herein were to identify polymorphisms in genes encoding adipokines, and to determine whether differences in allele frequencies exist among dairy breeds and sire groups. Adipokines are proteins secreted by adipose tissue, and are being increasingly recognized as critical regulators of energy balance, lipid metabolism and other biological processes (Trayhurn & Wood, 2004). Many adipokines, including *tumor necrosis factor alpha (TNF)*, *interleukin-8 (IL8)*, and *interleukin-10 (IL10)*, are important regulators of immune function. *Leptin (LEP)*, also an adipokine, is the hormone product of the obese gene and plays a central role in the regulation of appetite, energy partitioning and body composition (Houseknecht *et al.*, 1998; Baile *et al.*, 2000).

Three populations of dairy cattle (traditional Holsteins, modern Holsteins and modern Jerseys) were chosen to represent differences in selection emphasis. Jersey (n = 19) and modern Holstein (n = 18) were each represented by young sires currently under evaluation by a U.S. artificial insemination (AI) organization. These groups were chosen to represent two dairy breeds that have undergone long-term selection for production traits, with a greater emphasis on component traits in Jersey compared to Holstein sires. Traditional Holsteins (n = 16) were represented by Holstein bulls from the National Animal Germplasm Program (NAGP; www.ars-grin.gov/animal/) born prior to 1970. This group was chosen to represent the Holstein breed before intense selection pressure for production traits that has occurred since widespread use of AI. In all groups, bulls that shared sires or maternal grandsires were avoided. DNA was extracted from semen provided by Genex, CRI or the NAGP using standard protocols.

The project focused on genetic variation in genes that encode adipokines, including *LEP*, *TNF*, *IL8* and *IL10***.** Primers were designed to amplify regions of each candidate gene by PCR, and pools of DNA representing each sire group were used as template to generate PCR products for sequencing and identification of single nucleotide polymorphisms (SNP). Each reaction contained 25 ng DNA, 10 mM each dNTP, 50 mM $MgSO_4$, 10 µM each primer, 1X High Fidelty PCR Buffer, Platinum Taq High Fidelity enzyme (Invitrogen). Cycling conditions included denaturation at 94 °C, followed by 32 cycles of 94 °C for 30 seconds, 54 °C for 30 seconds, and 68 °C for 105 seconds. Sequences of PCR products were assembled and SNP identified using Sequencher 4.5 software (GeneCodes Corporation).

One SNP representing each gene and a previously reported SNP in *LEP* (Buchanan *et al.*, 2002) were selected for genotyping across all bulls. A multiplexed genotyping assay was developed using the ABI PRISM SNaPshot Multiplex Kit (Applied Biosystems). Manufacturer's suggested protocols were followed to design SNP primers and process SNaPshot reactions using a 3100 Genetic Analyzer (Applied Biosystems). Genotypes were determined using GeneScan 3.0 software (Applied Biosystems). The SNP, PCR and genotyping primers are presented in Table 1.

Allele and genotypic frequencies were determined for each sire group, and genotypic frequencies were in agreement with Hardy-Weinberg equilibrium (Falconer & Mackay, 1996). Allele frequencies were tabulated and compared among sire groups by chi-square test using statistical analysis software (SAS, 1985). Significant differences ($P < 0.05$) in allele frequency among groups were found for all SNP (Table 1). For the *LEP* SNP initially described by Buchanan *et al*. (2002), traditional Holstein had a higher ($P < 0.05$) frequency of the C allele compared to the other two groups. For IL-10, allelic frequencies differed ($P < 0.05$) among all three groups, with the highest frequency of the A allele found in modern Holstein, and the lowest in Jersey. For the remaining SNP (*LEP*, *TNF* and *IL8*), allele frequency in Jersey was different ($P < 0.05$) from the two Holstein groups, but modern and traditional Holstein did not differ from each other.

Buchanan *et al.* (2003) studied the *LEP* SNP and observed that animals homozygous for the T allele produced more milk (1.5 kg/d *vs*. CC animals) and had higher somatic cell count linear scores, without significantly affecting milk fat or protein percent over the entire lactation. They concluded that milk yield advantage observed in cows homozygous for the T allele, could represent a major economic advantage to dairy producers. Thus the current study indicates that both the modern Holsteins and Jerseys have a higher frequency of the favourable alleles as compared to the traditional Holsteins.

Genetic polymorphisms were identified in the *LEP*, *TNF*, *IL8* and *IL10* genes, and significant differences in allele frequencies were observed among the three groups of dairy sires. The three populations studied were chosen because they represented differences in selection emphasis placed on production traits. Thus, the observed differences in allele frequency may suggest that the genomic regions studied are associated with the differing selection goals. Differences in the *LEP* and *IL10* allele frequencies between modern and traditional Holstein are of particular interest because they may reflect the intense selection pressure for increased milk and production traits that has occurred in the Holstein breed since the 1970's. This *LEP* SNP has previously been associated with milk and protein yield in dairy populations (Buchanan *et al.*, 2003), and results from the current study make *IL10* a particularly interesting candidate gene for future investigations. Nevertheless, it should be recognized that the observed differences in allele frequencies may also result from random genetic drift or sampling bias.

Table 1 Description of polymorphisms and allele frequencies in three breed groups of dairy bulls. The Jersey (n = 19) and Modern Holstein (n = 18) groups include young sires currently being evaluated by an artificial insemination organization. The Traditional Holstein group (n = 16) includes bulls born prior to 1970

Gene[1]	Polymorphism[2]	Primer Sequence (5' to 3')	Genotyping Primer (5' to 3')	Allele Frequency[3]		
				Jersey	Modern Holstein	Traditional Holstein
LEP	U50365:g.2857A>G	F: GGTGAGACTTCCTGGAGAAT R: CAACATGTCCTGTAGTGACC	CCTTACTTCTTGTGCCCA	A: 0.45[a]	0.17[b]	0.13[b]
LEP	U50365:g.1180C>T[4]	F: ATCGACGATGTGCCACGTGTGG R: GGACCTCTGTGACTCCTTCTGG	$(T)_{22}$GGTGTCATCCTGGACCTTGC	C: 0.42[a]	0.53[a]	0.72[b]
TNF	AF011926:g.2180A>G	F: CTTGCTCTCTCTCACATACC R: TACCGGCTTGTTACTTGAGG	$(T)_{16}$ATCAACAGCCCTCTGGTTCA	A: 0.18[a]	0.39[b]	0.44[b]
IL8	AY849380:g.1181C>T	F: GAATCTTAGTTTGCTTGCCCG R: ATGGTTCCTTGAGTACAGGC	$(T)_{7}$ATGGGGTCGCTAAGAGT	C: 0[a]	0.53[b]	0.53[b]
IL10	ENSBTAG00000006685:g.4132487A>G	F: GTTGCTCATACTCTCTCC R: CTCATGGCTTTGTAGACACC	$(C)_{33}$TGATGAAAACACATTAG	A: 0.03[a]	0.39[b]	0.25[c]

[1]Gene symbols are abbreviated as: *Leptin (LEP), Tumor necrosis factor alpha (TNF), Interleukin 8 (IL8) and Interleukin 10 (IL10)*

[2]Polymorphisms are described according to guidelines of the Human Genome Variation Society (www.genomic.unimelb.edu.au/mdi/mutnomen/). Accessions for *LEP*, *TNF* and *IL8* are from the GenBank database (www.ncbi.nlm.nih.gov/Genbank/index.html); accession for *IL10* is from the Ensembl database (www.ensembl.org), release 40. All web sites were accessed September, 2006

[3]Frequencies of the designated allele of the bi-allelic polymorphism are given. Different superscripts among sire groups or breeds indicate significant differences in allele frequency ($P < 0.05$)

[4]This polymorphism was initially described by Buchanan *et al.* (2002)

The current study identified novel SNP within four important adipokine candidate genes, defined differences in allele frequency between Jerseys and Holsteins for each gene, and identified *IL10* as a particularly interesting novel candidate gene for production traits in Holsteins. These genes would be given a high priority for future research and in studying the association between the SNPs with fitness and production traits in dairy cattle.

References

Baile, C.A., Della-Fera, M.A. & Martin, R.J., 2000. Regulation of metabolism and body fat mass by leptin. Ann. Rev. Nutr. 20, 105-127.

Buchanan, F.C., Fitzsimmons, C.J., Van Kessel, A.G., Thue, T.D., Winkelman-Sim, D.C. & Shumutz, S.M., 2002. Association on a missence mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. Genet. Sel. Evol. 34, 105-116.

Buchanan, F.C., Van Kessel, A.G., Waldner, C., Christensen, D.A., Laarveld, B. & Schmutz, S.M., 2003. Hot topic: an association between a leptin single nucleotide polymorphism and milk and protein yield. J. Dairy Sci. 86, 3164-3166.

Falconer, D.S. & Mackay, T.F.C., 1996. Introduction to Quantitative Genetics, Longman Group Ltd, Essex, U.K.

Houseknecht, K.L., Baile, C.A., Matteri, R.L. & Spurlock, M.E., 1998. The biology of leptin: A review. J. Anim. Sci. 76, 1405-1420.

Rothschild, M.F. & Soller, M., 1997. Candidate gene analysis to detect genes controlling traits of economic importance in domestic livestock. Probe 8, 13–22.

SAS, 1985. Statistical Analysis Systems user's guide (5th ed.). SAS Institute Inc., Raleigh, North Carolina, USA.

Trayhurn, P. & Wood, I.S., 2004. Adipokines: inflammation and pleiotropic role of white adipose tissue. Br. J. Nutr. 92, 347-355.