

Associations between single nucleotide polymorphisms in the leptin gene with body measurements and IGF-I in UK dairy heifers

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Introduction Appropriate growth of dairy heifers is important to the dairy industry as it affects their future fertility and longevity. Growth is controlled by a complex series of interactions involving the somatotrophic axis, including growth hormone (GH) and insulin like growth factor-I (IGF-I). Leptin, produced primarily by adipose tissue, plays a key role in energy homeostasis and has also been implicated in controlling bone growth through both direct and indirect actions (Gat-Yablonski and Phillip, 2008). Growth can be analysed by measuring size traits such as weight and height. Juvenile height is a useful indicator of skeletal development and is also related to adult stature. These traits can be affected by single nucleotide polymorphisms (SNPs) in the effective genes. This study investigated the associations between four SNPs with size traits and circulating IGF-I concentrations. The SNPs included were: UASMS1 and UASMS2 (both located in the leptin gene promoter region, Nkrumah *et al.*, 2005); Exon2FB (located in exon 2 of the leptin gene, Buchanan *et al.*, 2002) and BGHR (located on exon 8 of the bovine growth hormone receptor; Blott *et al.*, 2003).

Materials and methods Holstein-Friesian heifers (n = 449), born between August 2003 and October 2004 on 18 commercial UK dairy farms and 1 primarily research farm (with 3 groups) were recruited for this study. Each heifer was measured aged approximately 1 month (28 ± 0.8d), 6 months (184 ± 0.8d, pre-pubertal) and 15 months (452 ± 3d, post pubertal at start of service period). Weight, height at withers (HT), crown rump length (CRL) and heart girth diameter were recorded. Blood samples were collected. Whole blood was spotted onto Whatman FTA cards (Whatman International Ltd, Maidstone, UK) for subsequent DNA extraction. Genotyping was performed by Orchid Cellmark (Abingdon, Oxford, UK). Plasma was harvested for measurement of IGF-I by OCTEIA IGF-I plate kits (Immunodiagnostic Systems Ltd, Tyne and Wear, UK). Mixed model association analysis was used to determine any significant associations between SNP genotypes and the individual traits measured at 1, 6 and 15 months. Animals were grouped according to their herd (1 to 21), year (2003 or 2004) and season (1 = March to May, 2 = June to August, 3 = September to November and 4 = December to February) of birth. Age was fitted as a 4th-order polynomial. SNP was fitted as a fixed effect with 3 levels. All known pedigree information for the preceding 3 generations for each heifer was included (n=2251 animals) and a heifer permanent environmental effect was fitted to account for repeated measurements on each animal. Analyses were performed with ASREML v2.0.

Results The phenotypic measurements of the heifers at each age are summarized in Table 1 and the genotype frequencies are reported in Table 2. Allele frequencies of all SNPs were distributed according to the Hardy-Weinberg equilibrium expected values. UASMS1 and Exon2FB were in close linkage disequilibrium ($r^2 = 0.98$).

Table 1 Phenotypic measures, mean ± SE (n = 449)

| Age | 1 month | 6 months | 15 months |
|---------------|----------|-----------|-----------|
| Weight (kg) | 56 ± 0.7 | 175 ± 1.7 | 373 ± 2.4 |
| HT (cm) | 80 ± 0.2 | 104 ± 0.3 | 126 ± 0.3 |
| CRL (cm) | 94 ± 0.4 | 135 ± 0.5 | 169 ± 0.5 |
| Girth (cm) | 89 ± 0.4 | 131 ± 0.4 | 174 ± 0.5 |
| IGF-I (ng/ml) | 42 ± 1.3 | 86 ± 1.8 | 105 ± 1.5 |

Table 2 SNP genotype frequencies

| SNP | CC | CT | TT |
|---------|------|------|------|
| UASMS1 | 0.17 | 0.48 | 0.35 |
| UASMS2 | 0.74 | 0.24 | 0.02 |
| Exon2FB | 0.35 | 0.48 | 0.17 |
| | TT | TA | AA |
| BGHR | 0.65 | 0.31 | 0.04 |

The only significant associations of SNPs with the traits measured related to HT. UASMS1 had a significant association with HT ($P < 0.05$) throughout the study period. Animals with a TT allele were on average 0.85cm and 1.10cm taller at 1 and 15 months respectively than CC or CT animals. Exon2FB and BGHR were also weakly associated with HT: Exon 2FB, CC > CT = TT, $P < 0.05$ at 15 months only; BGHR, TT = AT > AA, $P < 0.05$ at 6 months only. No associations were found with UASMS2.

Conclusion Leptin SNPs have previously reported associations with milk production, body condition score (BCS), fertility, energy balance and protein yield in adult dairy cows. For example, cows carrying the CT genotype of Exon2FB had a lower BCS in early lactation, whereas homozygous CC cows produced less milk (Chebel *et al.*, 2008). We show here that leptin SNPs are also associated with juvenile height measurements. The tallest heifers carried the T allele of UASMS1 and the C allele of Exon2FB. This provides further evidence that leptin SNPs are informative for marker assisted selection, as they relate to both growth and productivity.

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