Associations between SNPs in the bovine GnRH receptor gene and breeding values for fertility traits in dairy cattle

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Introduction Subfertility is an increasingly important problem in dairy cattle. An unfavourable genetic correlation between fertility and milk yield (Royal *et al.*, 2002) means that losses in breeding values for the former accompany gains in the latter. We have investigated whether single nucleotide polymorphisms (SNPs) in the GnRH receptor (GnRHr) are associated with fertility in dairy cattle, using predicted transmitting abilities (PTAs) for fertility traits available through the UK fertility index (Wall *et al.*, 2003).

Materials and methods Using a panel of 54 sires, DNA was isolated from semen by phenol-chloroform extraction, and from whole blood using a modification of the Puregene DNA Purification Kit (Gentra) based on sequential precipitation of protein and DNA with a phenol/chloroform extraction step. The coding sequence of bovine GnRHr (NW_001495209.1 | Bt6_WGA699_4) spanning 3 exons and a fragment of the GnRHr gene promoter region were amplified from 100 ng aliquots of genomic DNA in 35 PCR cycles with high fidelity DNA polymerase (Accuzyme Mix, Bioline). PCR products were purified and sequenced (Beckman CEQ8000 Sequencer). A total of 481 bulls of known parentage were subsequently genotyped by primer extension; however not all were genotyped at every locus. The 7 SNPs found in the GnRHr gene were analysed to determine the minimum number of segregating haplotypes (Schouten *et al.*, 2005). Genotype associations with PTAs for calving interval (CI), days to first service after calving (DFS), non-return rate at 56 days after first insemination (NR56), number of inseminations required to achieve conception (CINS), 305 day milk yield (305 MY), and an index of milk yield (Profit Index, PIN) were tested by REML in GENSTAT. PTAs for CI, DFS, NR56, CINS and PIN were calculated in 2004; values for CI, NR56 and 305 MY only were also derived in January 2009 (data available for 399 of the same bulls). The multivariate linear mixed model fitted was: $y_{ijkl} = \mu_i + \alpha_{ij} + \beta_{ik} + \varepsilon_{ijkl}$, where y_{ijkl} is the value of trait i for son l of sire j inheriting haplotype k; α_{ij} is the effect of sire j for trait i; β_{ik} is the effect of haplotype k for trait i; and ε_{ijkl} is the multivariate residual error. As PTAs were not selected for reliability, they were deregressed to account for variation in reliabilities of estimates.

Results Seven SNPs were identified in the GnRHr gene. Relative to the translation initiation codon these were at -331 (A>G), -108 (T>C), +206 (G>A), +260 (C>T), +341 (C>T), +383 (C>T) and +410 (C>T), the most frequent allele being given first. All the SNPs in exon 1 were silent. The SNPs at +206 and +383, and those at -108, +260, +341 and +410 formed two groups with complete linkage disequilibrium within groups but incomplete linkage disequilibrium between groups. Therefore, -331, -108 and +206 were selected as tag SNPs for haplotype and association analysis. The following 5 haplotypes were sufficient to explain the genotypes (maximum likelihood estimates of frequencies in parentheses): ATG (0.692), ATA (0.013), GCG (0.145), ACA (0.101) and ACG (0.048), SNPs being referred to by the most 5' position in each group (i.e. -331A>G, -108T>C and 206G>A), and identified by the nucleotides at each position (i.e. A-T-G). All loci were in Hardy-Weinberg equilibrium. The -108T>C alteration was associated with beneficial effects on fertility (Table 1) after accounting for effects of sire (fitted as a random effect), particularly with CI and DFS (reductions in PTAs of 0.43 ± 0.203 and 0.43 ± 0.130 days, p<0.03 and <0.001 respectively). The association with DFS (but not with CI) remained significant (p<0.01) when sire and PIN were both accounted for, suggesting that the association with fertility was not mediated solely through milk yield. The association with CI remained significant after deregression with (p=0.01) or without (p<0.05) accounting for 305 MY. There were no significant associations with other fertility traits, and no effects of the SNPs at positions -331A>G or +206G>A.

Table 1 Estimated effects of the linked allelic substitutions (α) at -108/+260/+341/+410 on fertility breeding values.

Model	CI (days)			DFS (days)			NR56 (%)			CINS (number)		
Benefit:	Decrease			Decrease			Increase			Decrease		
	α	s.e.	P	α	s.e.	P	α	s.e.	P	A	s.e.	P
Sire+SNP	-0.86	0.40	<0.03	-0.86	0.26	<0.001	-0.11	0.23	NS	-0.000	0.005	NS
Sire+PIN+SNP	-0.67	0.44	NS	-0.58	0.27	<0.01	-0.10	0.24	NS	-0.002	0.006	NS

Table shows analysis of genotypes of 406 bulls using REML (multivariate linear mixed model) with sires accounted for as a random effect. P indicates the significance of the Wald test. Note PTAs have been doubled to show effects on breeding values.

Conclusions Selection against the GnRHr ATG and ATA haplotypes will improve fertility.

References
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