NUCLEOTIDE SEQUENCE AND PHENOTYPIC EFFECTS OF AN INDICINE HAPLOTYPE AT THE BOVINE GROWTH HORMONE (bGH) GENE

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SUMMARY

SSCP analysis of the bovine growth hormone (bGH) gene in Israel-Holstein cattle uncovered five intragenic haplotypes. Four, differed among themselves at two sites; while the fifth, denoted Haplotype *E*, differed from the others at six sites. A number of lines of evidence indicate that Haplotype of E is of indicine origin. Haplotype *E* had significant increasing effects on percent protein and kg protein/yr, and an almost significant reducing effect on milk somatic cell counts; but did not affect total milk, fat percent, or fertility. Sequencing of 1500 bp of bGH uncovered seven nucleotide differences uniquely distinguishing the putative indicine and taurine haplotypes. When extrapolated to the entire genome these nucleotide differences have major implications for QTL mapping and cattle genetic improvement.

Keywords: *Bos indicus, Bos taurus,* bovine growth hormone, milk protein percent, QTL mapping, marker assisted selection.

INTRODUCTION

SSCP analysis of the bovine growth hormone (bGH) gene in Israeli-Holstein cattle uncovered five intragenic haplotypes (Lagziel *et al.* 1996). Haplotypes *A-D* differed among themselves at two fragments while Haplotype *E* differed from the others at six fragments, one of which corresponded to the well known intron III *MspI* PCR-RFLP, at which Haplotype *E* carried the (-) allele (Hoj *et al.* 1993; Lee *et al.* 1993). A number of lines of evidence indicate that Haplotype *E* is of indicine origin: (i) The many differences between Haplotype *E* and the other haplotypes are consistent with the long evolutionary separation of *Bos indicus* and *Bos taurus* (Bradley *et al.* 1996; MacHugh *et al.* 1996); (ii) Among the parent animals of the International Bovine Reference Panel), animals of pure indicine origin carried only Haplotype *E* or closely related haplotypes, while animals having one direct taurine and one direct indicine ancestor, carried one copy of Haplotypes *A-D*, and one copy of Haplotype *E* or related haplotypes (Lagziel *et al.* 1996); (iii) The frequency of the diagnostic *Msp I* (-) bGH allele decreases with distance from the Indian subcontinent, being at highest frequency in the Brahman breed of India (S. Denise, pers. comm.), at moderate frequencies in breeds of Russia, the Ukraine and the Mediterranean basin (Lisovskii *et al.* 1996; Lagziel *et al.* 1997; S. Denise, pers. comm.) , and at low to zero frequency in the breeds of Northern Europe (Hoj *et al.* 1993; Lee *et al.* 1993; Lagziel *et al.* 1996; Yao *et al.* 1996; S. Denise, pers. comm.). The present study confirms previously reported positive effects of Haplotype *E* on milk protein percent (Lagziel et al. 1996) and presents comparative sequence data.

METHODS AND MATERIALS

Effects of Haplotype E on milk production and composition. Milk samples were collected from 500 daughters of the Israel-Holstein sire "Sho'eg" (heterozygous for Haplotypes *A* and *E*) and genotyped for the diagnostic *Msp I* PCR-RFLP. Genotyping of milk samples was based on Lipkin *et al.* (1993). PCR amplification was according to Lagziel *et al.*, (1996), using the following primer pair):

Bov3 5' ACA CCC AGG TTG CCT TCT GC
Bov4 5' GGA GAA GGG CGA GGA AGG AG

The PCR product was digested using *Msp I* . and separated on 2% agarose gels.

Comparative sequencing of Haplotypes *A-E* . The sampled individuals included parent animals of the International Bovine Reference Family Panel, four sires active in Israel-Holstein A.I. Centers, and two daughters of one of the sires. Based on the complete sequence of the bGH gene (Gordon *et al.* 1983), nine primer pairs were constructed to almost completely cover the gene sequence. Amplification was as in Lagziel *et al.* (1996), except for the primer pair Bov3 and Bov4 given above. PCR products were separated on 0.8% Agarose gels. Sequencing reaction was according to the sequenase protocol (USB, OH) or by automated dye-terminator cycle sequencing with AmpliTaq DNA polymerase.

RESULTS

Among the genotyped daughters of Sho'eg, Haplotype *E* had a significant increasing effect on percent protein and kg protein/yr, and an almost significant decreasing effect on milk somatic cell counts (MSSC); but did not have significant effects on total milk production, fat percent, or fertility (number of inseminations per pregnancy) (Table 1).

Table 1: Milk production and composition of 246 daughters of Sho'eg receiving Haplotype *E* relative to 227 daughters receiving Haplotype *A*

Trait	Deviation
milk, kg/yr	-2.8 kg
fat, kg/yr	+0.49
protein, kg/yr	+0.94*
% fat	+.006
% protein	+.011***
MSSC	-0.12+
Fertility	.005

+, P<.10; *, P<.05; ** , P<.01; ***, P<.001

Sequencing of a total of 1494 bp, showed seven unique sequence differences between Haplotype *E* and Haplotypes *A* - *D* (Table 2), distributed throughout the gene.

Table 2: Position and location of the sequence variants characterizing the various haplotypes at the bGH gene. Variants from the sequence reported in Gordon et al. (1983) are underlined

		Haplotype				
Fragment	Position	*A*	*B*	*C*	*D*	*E*
A	502	C	C	C	C	T
A	591	G	G	G	G	G
B	800	G	G	G	G	G
D	1547	C	C	C	C	CT
D	1548	G	G	G	G	C
E	1947	T	T	T	T	G
E	2017	T	C	T	C	C
F	2141	C	C	G	G	C
G	2291	A	A	A	A	C
H	2565	A	A	A	A	A
H	2567	G	G	G	G	T
I	2731	$(TC)_3$	$(TC)_3$	$(TC)_3$	$(TC)_3$	$(TC)_2$

DISCUSSION

The results of this study confirm the positive effect of Haplotype *E* on milk protein percentage (Lagziel *et al.* 1996), and show that this may be accompanied by a reduction in milk somatic cell counts, but not by a reduction in milk production or an increase in fat percent. Considering the low frequency of this allele in the improved dairy breeds of Northern Europe, it should be a highly useful candidate for marker assisted selection.

The finding of seven sequence differences, uniquely differentiating between taurine and indicine bGH haplotypes is consistent with long evolutionary separation of the two races (Bradley *et al.* 1996; MacHugh *et al.* 1996). Generalized over the entire bovine genome, this implies that in almost all cases it will be readily possible to identify a site which distinguishes between sequences of indicine or taurine origin. Considering that a typical gene, including regulatory regions comprises about 4,000 to 40,000 bp, the above figure implies a total of 16 to 160 nucleotide differences between the taurine and indicine versions of a given bovine gene; suggesting that many, perhaps most genes will have somewhat different functional attributes in their indicine as compared to their taurine versions. Taking unique markers and unique quantitative effects together, this means that the F2 or subsequent generations of a cross between taurine and indicine breeds can be analyzed as a cross between two inbred lines (Soller *et al.* 1976) or as an "Advanced Intercross Line" (Darvasi and Soller, 1996).

These designs provide much greater statistical power than the usual designs for animal populations, and are amenable to selective DNA pooling (Darvasi and Soller, 1994).

The Criollo cattle of South America were formed centuries ago by mixtures of taurine and indicine breeds; more recent synthetics include the Santa Gertrudis, Beefmaster, and Brangus in the United States, and the Girolanda and Ibage of Brazil. These breeds should be excellent candidates for taurine/indicine QTL mapping. Linkage disequilibrium will facilitate MAS and marker-assisted introgression (MAI) of selected taurine production alleles. Thus taurine/indicine QTL may provide a route to developing highly productive breeds adapted to tropical and subtropical regions.

Considering the wealth of expected functional differences between taurine and indicine genomes, we further propose that the indicine race comprises an immense genetic resource of favourable QTL alleles that can be introduced into the taurine breeds by MAI. In this context, basically taurine breeds, such as the Reggiana, Grey Ukrainian, Brown Carpathian, Charolais or Limousin, which apparently carry a significant indicine component, may provide useful populations for investigation of quantitative effects of indicine genes on a taurine background, and for subsequent MAS or MAI.

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