



CSN1S1 gene: allele frequency, and the relationship with milk production traits in three Iranian indigenous cattle and Holstein breeds

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Introduction

- αs1 casein is one of the major proteins in milk of mammals with two common genetic variants of B and C
- “C” variant has a Gly at position 192, while the “B” variant has Glu, difference at position 148 can be detected by PCR-SSCP
- Allele C was found associated with higher protein and casein contents in milk, while the B variant was found with higher total milk production
- CSN1S1\*B is almost fixed in Holstein, as a result of long-term selection for high milk production
- Five allele has been reported for CSN1S1-promoter with the most frequency of allele ‘2’
- Significant allele substitution effect was found for milk fat and protein yields, allele 2 or 3, and for protein content; allele ‘4’

The objectives

- The aim of this study was to:
- estimate the allelic frequency in polymorphic site of exon 17 and promoter of αs1-casein gene in three Iranian native and Holstein cattle breeds
  - association between polymorphic sites in coding/promoter regions with milk production traits

Materials and methods

- **Samples:** 406 genomic DNA from three Iranian native *Bos indicus* Mazandarani (n = 97) and Golpaygani (n = 112), and one *Bos taurus* Sarabi (n = 87) cattle and Holstein (n = 110) (Fig. 1)
- **DNA extraction:** from whole blood by Miller et al. (1988) procedure
- **Amplification and Primers**
- A 216bp fragment of upstream of αs1-casein was amplified by Cy5 labeled primers
- Alleles 1, 2 and 3+4 were directly assigned by ALF instrument and AlleleLocator software
- SSCP was used to distinguish allele 3 and 4
- A 265bp fragment of exon 17, was amplified to genotype C and B alleles
- Fragments were subjected to SSCP analysis and alleles were designated manually compared to reference samples
- **Statistical analysis:** Variance analysis was performed using statistical package JMP 4.0.4
- **Model:**  $Y_{ijkl} = M + S_i + H_j + CG_k + \alpha(DIM_{ijkl} - ) + e_{ijkl}$ , where  $Y_{ijkl}$  is the milk, fat production or fat percent measured on each of  $ijkl$ th animal,  $M$  is the overall population mean,  $S_i$  is the effect of  $i$ th season,  $H_j$  is the effect of  $j$ th herd,  $CG_k$  is the fixed effect associated with the  $k$ th combined genotypes of promoter and coding region,  $\alpha$  is the linear regression coefficient of days-in-milk on milk production, and  $e_{ijklmn}$  is the random residual effect

Results

- **Frequencies:** Four variants of promoter were found in all breeds in different frequencies with allele ‘2’ being predominant and allele 4 the least frequent in all breeds
- The allele frequencies of CSN1S1\*B variant were 0.53, 0.66, 0.4 and 0.99 for Mazandarani, Sarabi, Golpaygani and Holstein, respectively
- Chi-Square test showed significant differences between indigenous breeds and Holstein
- Intragenic haplotypes:** Allele B of the coding region was found in combination with all four promoter allele
- Allele 4 of the promoter was not found in any cow having the exon 17 allele C in all breeds except Mazandarani

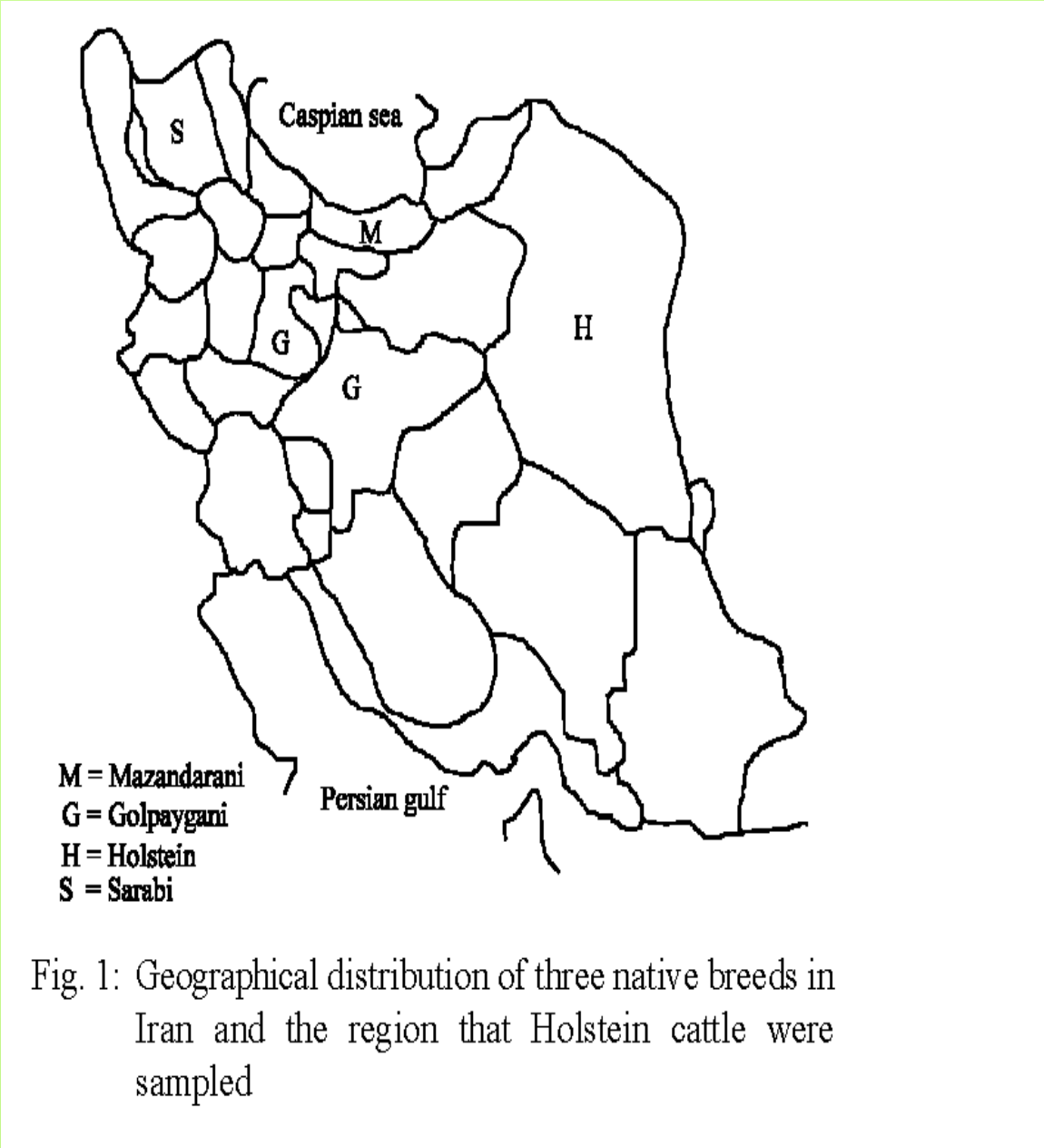


Fig. 1: Geographical distribution of three native breeds in Iran and the region that Holstein cattle were sampled

Table 1- Effect of combined genotype on milk production traits in Holstein and Golpaygani breeds

| Variables          | P values of traits    |                       |                       |
|--------------------|-----------------------|-----------------------|-----------------------|
|                    | Milk yield            | Fat yield             | % Fat                 |
| Holstein: Season   | 0.0773 <sup>ns</sup>  | 0.1087 <sup>ns</sup>  | 0.6155 <sup>ns</sup>  |
| Herd               | 0.1189 <sup>ns</sup>  | 0.0024 <sup>**</sup>  | <0.0001 <sup>**</sup> |
| Days in milk (d)   | <0.0001 <sup>**</sup> | <0.0001 <sup>**</sup> | 0.1763 <sup>ns</sup>  |
| Combined genotype  | 0.5920 <sup>ns</sup>  | 0.5503 <sup>ns</sup>  | <b>0.0488 *</b>       |
| R <sup>2</sup>     | 0.58                  | 0.64                  | 0.56                  |
| Golpaygani: Season | 0.1805 <sup>ns</sup>  | 0.4536 <sup>ns</sup>  | 0.8529 <sup>ns</sup>  |
| Days in milk (d)   | 0.0004 <sup>**</sup>  | 0.0774 <sup>ns</sup>  | 0.93 <sup>ns</sup>    |
| Combined genotype  | 0.4776 <sup>ns</sup>  | 0.7072 <sup>ns</sup>  | 0.9449 <sup>ns</sup>  |
| R <sup>2</sup>     | 0.7                   | 0.87                  | 0.61                  |

Table 2- Effect of combined genotype on milk and fat percentage of milk in Holstein

| Level                    | BC/23   | BB/14   | BB/24   | BB/23   | BB/33   | BB/22   | BB/12   | BB/34    |
|--------------------------|---------|---------|---------|---------|---------|---------|---------|----------|
| Least Sq Mean (%F)       | 3.61    | 3.43    | 3.35    | 2.98    | 2.95    | 2.945   | 2. 50   | -        |
| Least Sq Mean (milk, kg) | 7259.73 | 9395.42 | 7156.43 | 8543.71 | 8860.81 | 8054.78 | 9261.12 | 8236.673 |

Results:

- **Statistical analysis:** Statistical analysis performed for Holstein and one of indigenous breed (Golpaygani)
- Combined genotypes of CSN1S1 promoter and exon 17 had no significant effects on milk and fat yields in Golpaygani breed
- Significant difference between combined genotypes was observed for fat percentage in Holstein (P<0.05) (Table 1)
- BC/23 genotype yielded the highest fat percentage (P<0.05) in Holstein but it had no significant effect on Golpaygani (Table 2)
- BC/22 genotype had no significant effect on milk production of Golpaygani, although they tended to produce higher milk than the other genotypes

Conclusion

- Differences of allelic frequencies and milk production traits between breeds might be due to differences in origin of breeds or selection plans applied to Holstein population to improve milk production and non-selection program in indigenous breeds.
- There was only one combination with BC genotype which suggests that C variant affect on fat percentage. There was not any homozygous CC for CSN1S1 to show whether C variant would be more advantageous for fat content.
- In order to know more about indigenous breeds and their potential favorable characters in Iran, it is suggested to establish registration and recording programs.

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Fig. 1- Mazandarani Breed; Fig. 2- Sarabi Breed; Fig. 3- Golpaygani Breed