

Supplement of Arch. Anim. Breed., 63, 345–354, 2020
<https://doi.org/10.5194/aab-63-345-2020-supplement>
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Supplement of

Polymorphism and molecular characteristics of the *CSN1S2* gene in river and swamp buffalo

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Table S1. The functional effect of non-synonymous substitutions on buffalo α_{S2} -CN

| SNP | Substitution | PROVEAN score | Prediction (cutoff=-2.5) |
|----------|--------------|---------------|--------------------------|
| c.234C>A | p.Glu63Asp | -0.301 | Neutral |
| c.382A>G | p.Lys113Glu | -1.621 | Neutral |
| c.391G>A | p.Ala116Thr | -1.693 | Neutral |
| c.484T>A | p.Phe147Ile | -2.350 | Neutral |
| c.568G>A | p.Ala175Thr | 1.936 | Neutral |
| c.580T>C | p.Tyr179His | -3.981 | Deleterious |
| c.587A>G | p.Lys181Arg | -2.072 | Neutral |
| c.642C>G | p.Asn199Lys | -2.602 | Deleterious |

Table S2. Amino acid composition of mature peptide of buffalo α_{S2} -CN variant A

| Amino acids | Number | Frequency (%) | Amino acids | Number | Frequency |
|-------------|--------|---------------|-------------|--------|-----------|
| Ala (A) | 8 | 3.9 | Lys (K) | 23 | 11.1 |
| Arg (R) | 5 | 2.4 | Met (M) | 4 | 1.9 |
| Asn (N) | 13 | 6.3 | Phe (F) | 6 | 2.9 |
| Asp (D) | 5 | 2.4 | Pro (P) | 10 | 4.8 |
| Cys (C) | 2 | 1.0 | Ser (S) | 17 | 8.2 |
| Gln (Q) | 16 | 7.7 | Thr (T) | 16 | 7.7 |
| Glu (E) | 23 | 11.1 | Trp (W) | 3 | 1.4 |
| Gly (G) | 2 | 1.0 | Tyr (Y) | 13 | 6.3 |
| His (H) | 5 | 2.4 | Val (V) | 13 | 6.3 |
| Ile (I) | 11 | 5.3 | Pyl (O) | 0 | 0.0 |
| Leu (L) | 12 | 5.8 | Sec (U) | 0 | 0.0 |

| | | Percent Identity | | | | | | | | | | | | | | | | | |
|------------|----|------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|----|---------------------|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | | |
| Divergence | 1 | █ | 99.5 | 99.5 | 99.5 | 99.1 | 99.1 | 98.6 | 98.6 | 98.6 | 98.6 | 98.6 | 95.9 | 95.5 | 94.6 | 94.4 | 95.5 | 1 | Buffalo A |
| | 2 | 0.5 | █ | 99.1 | 99.1 | 98.6 | 99.5 | 99.1 | 99.1 | 99.1 | 99.1 | 98.1 | 95.5 | 95.0 | 94.1 | 93.9 | 95.0 | 2 | Buffalo B |
| | 3 | 0.5 | 0.9 | █ | 99.1 | 99.5 | 98.6 | 98.2 | 98.2 | 98.2 | 98.2 | 98.1 | 95.5 | 95.0 | 94.1 | 93.9 | 95.0 | 3 | Buffalo C |
| | 4 | 0.5 | 0.9 | 0.9 | █ | 99.5 | 98.6 | 98.2 | 98.2 | 98.2 | 98.2 | 98.1 | 95.5 | 95.0 | 94.1 | 93.9 | 95.0 | 4 | Buffalo D |
| | 5 | 0.9 | 1.4 | 0.5 | 0.5 | █ | 98.2 | 97.7 | 97.7 | 97.7 | 97.7 | 97.7 | 95.0 | 94.6 | 93.7 | 93.4 | 94.6 | 5 | Buffalo E |
| | 6 | 0.9 | 0.5 | 1.4 | 1.4 | 1.8 | █ | 99.5 | 99.5 | 99.5 | 99.5 | 97.7 | 95.0 | 94.6 | 93.7 | 93.4 | 94.6 | 6 | Buffalo F |
| | 7 | 1.4 | 0.9 | 1.8 | 1.8 | 2.3 | 0.5 | █ | 99.1 | 99.1 | 99.1 | 97.2 | 94.6 | 94.1 | 93.2 | 93.0 | 94.1 | 7 | Buffalo G |
| | 8 | 1.4 | 0.9 | 1.8 | 1.8 | 2.3 | 0.5 | 0.9 | █ | 99.1 | 99.1 | 97.2 | 94.6 | 94.1 | 93.2 | 93.0 | 94.1 | 8 | Buffalo H |
| | 9 | 1.4 | 0.9 | 1.8 | 1.8 | 2.3 | 0.5 | 0.9 | 0.9 | █ | 99.1 | 97.2 | 95.5 | 95.0 | 94.1 | 93.9 | 95.0 | 9 | Buffalo I |
| | 10 | 1.4 | 0.9 | 1.8 | 1.8 | 2.3 | 0.5 | 0.9 | 0.9 | 0.9 | █ | 97.2 | 94.6 | 94.1 | 93.2 | 93.0 | 94.1 | 10 | Buffalo J |
| | 11 | 0.0 | 0.5 | 0.5 | 0.5 | 0.9 | 0.9 | 1.4 | 1.4 | 1.4 | 1.4 | █ | 94.8 | 94.4 | 93.9 | 93.0 | 94.4 | 11 | Buffalo K |
| | 12 | 4.2 | 4.7 | 4.7 | 4.7 | 5.1 | 5.1 | 5.6 | 5.6 | 4.7 | 5.6 | 3.9 | █ | 99.5 | 98.6 | 98.6 | 99.5 | 12 | α_{S2} -CN A |
| | 13 | 4.7 | 5.1 | 5.1 | 5.1 | 5.6 | 5.6 | 6.1 | 6.1 | 5.1 | 6.1 | 4.4 | 0.5 | █ | 98.2 | 98.1 | 99.1 | 13 | α_{S2} -CN B |
| | 14 | 5.6 | 6.1 | 6.1 | 6.1 | 6.6 | 6.6 | 7.1 | 7.1 | 6.1 | 7.1 | 4.9 | 1.4 | 1.8 | █ | 97.2 | 98.2 | 14 | α_{S2} -CN C |
| | 15 | 4.4 | 4.9 | 4.9 | 4.9 | 5.4 | 5.4 | 5.9 | 5.9 | 4.9 | 5.9 | 4.0 | 0.0 | 0.5 | 1.4 | █ | 98.1 | 15 | α_{S2} -CN D |
| | 16 | 4.7 | 5.1 | 5.1 | 5.1 | 5.6 | 5.6 | 6.1 | 6.1 | 5.1 | 6.1 | 4.4 | 0.5 | 0.9 | 1.8 | 0.5 | █ | 16 | α_{S2} -CN E |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | | |

Figure S1. Percent identity/divergence among the variant sequences of buffalo α_{S2} -CN and *Bos* genus α_{S2} -CN. Data above the diagonal line represents the percent identity and below the diagonal line represents the sequence divergence.

10 20 30 40 50 60 70 80
Buffalo A KHTMEHVSSEESIIISQETYSQEKNAIHPSKENCSTFCKEVIRNANEEYSIGSSSEESAEVATEEVKITVDDKHYQK
 hhhhhccccchhhhhhhhhhccccceeecccchhhhhhhhhhhcchhheccccchhhhhcchhhheehhhhhhhh
Cattle A KNTMEHVSSEESIIISQETYSQEKNAIINPSKENCSTFCKEVVNRNANEEYSIGSSSEESAEVATEEVKITVDDKHYQK
 hhhhhccccchhhhhhhhhhccccceeecccchhhhhhhhhhhhhcchhheccccchhhhhcchhhheehhhhhhhh
 90 100 110 120 130 140 150 160
Buffalo A ALNEINQFYQKFPQYLQYLYQGPIVLNPWDQVKRNAVPIPTLNRQLSTSEENSKKTVDMESTEVTCKTKLTEEDKNR
 hhhhhhhhhhhhhhhhhhccccceeeccccccccccccccccchhccccchccccccccccccccccchhhhhhh
Cattle A ALNEINQFYQKFPQYLQYLYQGPIVLNPWDQVKRNAVPIPTLNRQLSTSEENSKKTVDMESTEVTCKTKLTEEEKNR
 hhhhhhhhhhhhhhhhhhccccceeeccccccccccccccccchcchccccccccccccccccccccchhhhhhhh
 170 180 190 200
Buffalo A LNFLKKISQHYQKFAWPQYLKTQYQKAMKPWTQPKTNVIPYVRYL
 hhhhhhhhhhhhhccccchhhhhccccctccccccccccccce
Cattle A LNFLKKISQRYQKFAWPQYLKTQYQKAMKPWTQPKTNVIPYVRYL
 hhhhhhhhhhhhccccchhhhhccccctccccccccccccce

Figure S2. Predicted secondary structure of mature peptide of α_{S2} -CN variant A in buffalo and cattle.

Alpha helices, extended strands, beta turns, and random coils are indicated with the h, e, t, and c, respectively.