Single nucleotide polymorphisms in the bovine Neuropeptide Y5 Receptor gene and their predicted role in physico-chemical characteristics of the receptor protein

T Alam[1], B Bahar[1], S Waters[2], M McGee[2], T Sweeney[1]
[1]Cell & Molecular Biology Lab, Veterinary Science Centre, University College Dublin, Dublin, Dublin, Ireland
[2]Grange Beef Research Centre, Dunsany, Co Meath, Ireland
Email: tanweerbio@gmail.com

Introduction In Ireland, enteric fermentation by ruminants contributes ~14% of the total greenhouse gas emissions. For a 'sustainable beef production system' there is an increasing demand to select livestock species with high feed efficiency and consequently a reduction in enteric methane emissions (Hegarty *et al.,* 2007). Neuropeptide Y (NPY) is a neurotransmitter that regulates appetite and energy homeostasis in animals and humans. The physiological functions of neuropeptide are mediated through a number of membrane bound G-protein coupled receptor (GPCR) molecules. Among the various types of GPCRs, neuropeptide Y5 receptor (NPY5R) is an important molecule which plays vital roles in the feed intake behaviour of animals (Kalra *et al.,* 2007). Single nucleotide polymorphisms (SNP) in the bovine *NPY5R* gene are likely to influence feed intake behaviour in cattle and may act as a potential genetic marker for selection of animals with high feed energy utilization efficiency. The aim of this research was to identify SNPs in the bovine *NPY5R* gene and predict the alteration of functionality of the mutated receptor.

Materials and methods Genomic DNA was extracted from blood samples (n=73) of beef cattle: Aberdeen Angus (9); Aubrac (1); Belgian Blue (1); Blonde d'Aquitaine (1); Charolais (11); Hereford (6); Limousin (15); Parthenais (3); Salers (3); Shorthorn (1); Simmental (14); *Bos Indicus* (8). The samples were sourced from the performance trials conducted by the Irish Cattle Breeding Federation, Tully (Co. Kildare) and Teagasc Grange Beef Research Centre (Co. Meath) and *Bos indicus* from India. Two sets of PCR primers were designed to amplify a total length of 2.1 kb of the bovine *NPY5R* gene (GeneID: 781872). Sequencing of the PCR products were performed in both forward and reverse directions. SNPs were identified by multiple sequence alignment, using Molecular Evolutionary Genetics Analysis (MEGA) software. Physico-Chemical properties of the mutated receptor protein were determined using ProtParam software (Gasteiger *et al*., 2005).

Results Based on the alignment of the 2.1 kb sequence, a total of 18 SNPs were identified (Table 1). Of these SNPs, 4 were non-synonymous and 10 were synonymous. Of the total 17 SNPs, 4 were present in the regulatory region (5′ UTRs) and 13 in the exonic region which corresponds to the seven transmembrane domain of the receptor molecule. Interestingly, one SNP (G/A) causes an amino acid substitution (M67I) in the first intracellular loop of the receptor molecule. While another two SNPs (C/T, C/T) cause amino acid substitutions at positions 312 and 313, one SNP (C/T) introduces a stop codon that occurs in the third intracellular loop of the 7 transmembrane domain of the Y5 receptor molecule. This stop codon is likely to cause a premature termination of the polypeptide leading to a truncated Y5 receptor protein. The predicted changes in the physico-chemical properties of the Y5 receptor protein (Table 2) suggest important physiological consequences due to the presence of this SNP.

Table 1 Alleles and functions of the SNPs identified.

SNPs	Function	SNPs	Function
T/C	5′ UTR	A/C	Synonymous
G/T	5′ UTR	C/T	Synonymous
T/G	5′ UTR	C/T	Leu→Phe
C/T	5′ UTR	C/T	Pro→Leu
C/T	Synonymous	C/T	Arg→Stop codon
G/A	Met→Ile	C/T	Synonymous
C/T	Synonymous	T/C	Synonymous
G/A	Synonymous	A/G	Synonymous
C/T	Synonymous		

Table 2 Properties of wild and truncated Y5 receptor protein

Properties	Wild type Y5 receptor	Truncated Y5 receptor
Number of amino acids	446	364
Molecular weight	50775	41282
Theoretical pI	9.19	9.31
Extinction coefficient (M^{-1} cm^{-1})	56225	46005
Formula	$C_{2303}H_{3628}N_{610}O_{620}S_{31}$	$C_{1864}H_{2950}N_{498}O_{515}S_{22}$

Conclusion There is high degree of genetic variation (1SNP/123 base) present in the bovine *NPY5R* gene. The SNPs identified in the regulatory and exonic regions of the bovine *NPY5R* gene, specifically those causing amino acid change and premature termination of the Y5 receptor protein and leading to alteration in the physico-chemical properties are likely to play vital physiological roles in the neuropeptide Y mediated energy homeostasis in cattle. Hence, genetic associations of the SNPs identified, with the feed intake traits of the animals is currently being investigated.

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References

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