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Short Communication

Polymorphic Status of *PRKAA2* Gene in Pakistani Buffaloes

Waqas Ahmad Khan[1], Tanveer Hussain[2*], Masroor Ellahi Babar[2], Asif Nadeem[1], Ali Raza Awan[1], Ambrin Fatima[3] and Rashid Saif[2]

[1]*Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, 54000, Pakistan; Department of Biotechnology, University of Sargodha, Sargodha, Pakistan*
[2]*Department of Molecular Biology, Virtual University of Pakistan, Lahore, Pakistan*
[3]*Health Biotechnology Division, National Institute of Biotechnology and Genetic Engineering, Faisalabad, Pakistan*
*For correspondence: tanveer.hussain@vu.edu.pk

Abstract

This study was designed to find single nucleotide polymorphism (SNPs) in coding and non-coding region of PRKAA2 gene in Nili-Ravi and Kundi buffaloes. The PRKAA2 gene (AMPKα2) of 100 animals from both buffalo breeds were sequenced for SNPs identification. A total number of 43 inter and intra-generic SNPs were found. Out of which 17 SNPs were detected among buffalo breeds (intra-generic). All SNPs were intronic and may have a role in gene regulation and splicing patterns. These SNPs might be associated with commercially important production traits. This is the first study to identify novel SNPs that are linked to energy metabolism and production traits of buffalo. © 2016 Friends Science Publishers

Keywords: AMPK; PRKAA2; Buffalo; SNP; Pakistan

Introduction

AMPK has a vital role in maintaining energy balance in all eukaryotes and regulates different aspects of cellular function (Hardie, 2011). Indirectly, AMPK also promotes feeding and feeding behavior of the cell (Hardie *et al*., 2012). AMPK α2 or PRKAA2 is activated by leptin kindles during the fatty acid oxidation in muscle. Activation of PRKAA2 by drugs like 5-aminoamidazole-4-carboxamide 1-β-D-ribofuranoside restrains protein synthesis in rat skeletal muscle (Bolster, 2002). Variations in hypothalamic PRKAA2 regulate food intake as well as body weight gain (Minokoshi, 2004). The variants in PRKAA2 are significantly associated with serum lipoproteins in normal Caucasian females and showed significant associations between variants of AMPK genes subunit and weight gain activated by clozapine and olanzapine (Souza, 2012). Involvement of this gene was also reported in the regulation of lipids, glucose metabolism and protein synthesis (Zhang, 2011). Importance of this energy-status sensor has driven scientists to work on the structure-function relationship studies of its isoforms, especially the constitution of γ regulatory subunit. Similar roles of subject gene are anticipated in mammalians species based on high conservation status of this gene in eukaryotes. In current study, sequencing and SNPs analysis of PRKAA2 gene in two Pakistani buffalo breeds, Nili-Ravi and Kundi was carried out to find out novel SNPs in the coding and non-coding region of PRKAA2 gene.

Materials and Methods

Blood samples from 100 pure Nili-Ravi and Kundi buffaloes were collected in tubes containing EDTA and DNA was extracted by the standard organic method (Maryam *et al.,* 2012). The extracted DNA was quantified by agarose gel electrophoresis and spectrophotometry. The genomic DNA was amplified by PCR (polymerase chain reaction) using primers designed by EPIC approach (exon primed intron crossing) from cattle PRKAA2 gene sequence available at GenBank accession number AC_000160 (Table 1). Both sense and antisense strands of the amplified DNA was sequenced by using Big Dye terminator cycle sequencing chemistry v 3.1 (Applied Biosystems, USA) and electrophoresis was done by an automated DNA sequencer (ABI Prism 3130xL Genetic Analyzer, Applied Biosystems, USA). Sequences were analyzed manually through ChromasLite software 1.45 (http://www.technelysium.com.au/chromas.html). The sequences were BLAST against reference GenBank sequences using Blast2Sequences bioinformatics tool (Tatusova and Madden, 1999) followed by the identification of novel SNPs detection.

Results

In the current study, a total of 43 inter and intra-generic SNPs were detected in PRKAA2 gene after alignment of

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Table 1: List of primers used to amplify different regions of PRKAA2 gene

Primer name	Primer sequence (5'-3')	Product size	Primer location
PRKAA2F1	TAGTTGGAGAGCATCAAT TGACAG	1039	Int1, Ex2
PRKAA2R1	CTTGGTCAAGTTGCTGATACTC		
PRKAA2.UPSEQ1	GTTAAAGATTATCTGCCA		
PRKAA2.UPSEQ2	TCTAAGTTTGTAACTTGCTTTATC		
PRKAA2F2	CACTTCCTGGCAGACTCAGATG	830	Ex2, Int2
PRKAA2R2	ACAGTTTGATAATATGAGGATGAC		
PRKAA2F3	GTACCTGGTTGACAACAGAAGC	1448	Ex8, Int8, Ex9
PRKAA2R3	CAA AGA GCC CGT GAG AGA GC		
PRKAA2.DNSEQ1	GGATTTTATGCCCATGGCCA		
PRKAA2.DNSEQ2	TCCTCCAAAGGTGTGCTTTC		
PRKAA2.DNSEQ3	GAATCCTTCTCAACATACTGT		
PRKAA2.DNSEQ4	ATACTAGATCCTTGGACGTG		

Table 2: Genotypic and allelic frequencies of PRKAA2 gene

Genotypes		Locus	Allelic variant frequency		
Name	Alleles		Nili-Ravi (100)	Kundi (100)	All breeds (200)
NK1A	TT	41506	0.25	0.30	0.275
NK1B	TC		0.50	0.45	0.475
NK1C	CC		0.25	0.25	0.250
NK2A	CC	41538	0.30	0.35	0.325
NK2B	AA		0.70	0.75	0.725
NK3A	AA	42087	0.90	0.85	0.875
NK3B	GG		0.10	0.15	0.125
NK4A	TT	42647	0.24	0.35	0.295
NK4B	CC		0.76	0.65	0.705
NK5A	GG	42679	0.64	0.72	0.68
NK5B	GC		0.10	0.12	0.11
NK5C	CC		0.24	0.18	0.21
NK6A	AA	42707	0.55	0.64	0.595
NK6B	AG		0.25	0.18	0.215
NK6C	GG		0.20	0.18	0.19
NK7A	CC	42828	0.12	0.12	0.12
NK7B	CT		0.24	0.18	0.21
NK7C	TT		0.64	0.70	0.67
NK8A	AA	42215	0.75	1.0	0.875
NK8B	CC		0.25	0	0.125
NK9A	AA	42635	0.60	0.75	0.675
NK9B	TT		0.40	0.25	0.325
NK10A	TT	70442	0.24	1.0	0.62
NK10B	TG		0.28	0	0.14
NK10C	GG		0.48	0	0.24
NK11A	GG	70470	0.42	0.38	0.40
NK11B	GA		0.30	0.16	0.23
NK11C	AA		0.28	0.46	0.37
NK12A	AA	70471	0.68	0.85	0.765
NK12B	TT		0.32	0.15	0.235
NK13A	AA	70556	0.40	0.24	0.32
NK13B	AG		0.48	0.55	0.515
NK13C	GG		0.12	0.21	0.165
NK14A	CC	70841	0.85	0.94	0.895
NK14B	TT		0.15	0.06	0.105
NK15A	GG	71337	0.65	I.0	0.825
NK15B	TT		0.35	0	0.175
NK16A	TT	71499	0.85	0.70	0.775
NK16B	GG		0.15	0.30	0.225
NK17A	CC	71554	0.75	1.0	0.875
NK17B	CT		0.10	0	0.05
NK17C	TT		0.15	0	0.075

cattle PRKAA2 gene sequence against buffalo RKAA2 sequences. Among those 17 SNPs showed variation among Nili-Ravi and Kundi buffalos (intra-generic). The intra-generic SNPs are detected in intron 1, 2 & 8. The exon 2, 8 and 9 are conserved regions in all buffalo breeds. Two SNPs are found in first intron at locus 41506 T→C and 41538 C→A. Intron 2 showed seven genetic variations at loci 42087 A→G, 42647 T→C, 42679 G→C, 42707 A→G, 42828 C→T, 42215 A→C and 42635 A→T. The SNP at 42215 A→C is only present in Nili-Ravi buffalo. Eight

SNPs were found in intron 8 at 70442 T→G, 70470 G→A, 70471 A→T, 70556 A→G, 70841 C→T, 71337 G→T, 71499 T→G and 71554 C→T. The SNPs at 70442 T→G, 71337 G→T and 71554 C→T are only found in Nili-Ravi buffalo (Table 2).

Discussion

It has been observed that PRKAA2, the gene encodes α2 catalytic subunit is more conserved than PRKAB1, the gene encodes the β1 regulatory subunit, which is in accordance with the functions of the different AMPK subunits (Zhang, 2011). The occurrence of intra-generic variation only on intronic region (1, 2, 8) and conservation of coding region (exon 2, 8, 9) in Nili-Ravi and Kundi buffalo is according to previous findings about PRKAA2 gene. These intronic SNPs does not directly code protein but they may have a regulatory effect of AMPK gene and protein expression by upsetting gene regulation and splicing pattern (Chamary *et al.*, 2006). The SNPs in PRKAA2 gene found in this study are new and have not been reported previously. More studies along with functional analysis are needed to fully explain how these polymorphisms may affect PRKAA2 activity and/or probably act as candidate markers associated with energy metabolism in buffalos followed by association with economically important traits.

Conclusion

The Nili-Ravi and Kundi buffalo is very important genetic resource of Pakistan. This is the first study on molecular characterization and identification of genetic variants of PRKAA2 gene in these buffalo breeds. The findings of this study related to genetic variability of PRKAA2 gene of Nili-Ravi and Kundi buffalo might have association with production traits like feed intake, feeding behavior and growth etc.

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