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Short Communication

Genetic Polymorphisms of *IGF-1* Gene in Pakistani Marecha Camel

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ABSTRACT

Insulin-like growth factor-1 (IGF-1) is secreted peptide growth factor, that involved in a variety of physiological processes such as somatic growth, metabolism, tissue repair, lactation and reproduction. It controls differentiation and maintenance of different function in various and specific tissues. The liver is the primary source of IGF-1 and considered as candidate genes for growth because they play a significant role in development and growth regulation. IGF-1 control protein metabolism and is extremely conserved region amongst species. IGF-1 have not been studied before in camel. The DNA samples of Marecha camel were collected from the Camel Breeding and Research Station at Rakhmani Bhakkar, Pakistan. Four polymorphic sites were detected in the *IGF-1* gene. A significant finding was the occurrence of a T→C polymorphism in exon 5 that causes a substitution of an amino acid from Cysteine to Arginine.That Cys/Arg polymorphism may serve as authoritative genetic marker for selective improvement of growth traits in camel. These results suggest that Marecha camel has genetic variability in the IGF-1 and may be helpful for preservation strategies and future selection programs.

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Authors' Contribution
AN designed the study. SS carried out the genomic work. GY and GA collected the sample. SS, AN, MJ and MYZ analyzed the data and wrote the manuscript. AN and ASH revised the manuscript.

Key words
Polymorphism, IGF-1, *Camelus dromedarius*, Marecha

Insulin-like growth factor 1 (IGF-1) play significant physiological roles in development, metabolism, growth regulation and lactation (Lucy, 2008). IGF-1 stimulates production, differentiation and protein metabolism and is vital for the function of various organs. It controls differentiation by maintaining differentiated function in various tissues and in some specific cell (Werner *et al*., 1994). IGF-1 also influence on mitogenic and anabolic of growth hormone in different tissues Laron (2001). Liver is the main source of IGF-1 although it is also produced in a tissue by specific manner (Etherton, 2004). IGF-1 has been related with fertility and reproductive measurements such as first calving age, ovarian cyclicity, conception rate, ovulation development of pre-implantation embryo (Velazquez *et al*., 2008). This shws strong genetic effect of *IGF-1* gene on both transcriptional and translational level (Wang *et al*., 2003). IGF-1 also controls the genetic variation of traits such as average daily weight gain, body size, live and carcass weight, food conservation efficiency, fat deposition and milk production (Johnston *et al*., 2001).Several studies have exposed positive association of IGF1 serum concentration and liver IGF1 mRNA expression with feed efficiency, body gain and protein synthesis (Hayden *et al*., 1993; Stick *et al*., 1998). IGF1 is also significant for growth in domestic livestock animals. Growth is controlled by complex system among which somatotropic axis plays significant role. GH and *IGF-1* genes control somatotropic axis and responsible for the postnatal growth.IGF-1 mainly mediates function of GH that acts on the growth of muscles and bones (Sellier, 2000) Candidate genes have biological effect on physiology and development of trait as such genes instruct structural protein in biochemical and regulatory pathway by influencing on expression of traits (Byrne *et al*., 1996) and can be verified by QTLs (Yao *et al*., 1996) .

The insulin-like growth factor 1 (*IGF-1*) gene is considered as a candidate for growth and muscle development as it plays significant role in development and growth regulation (Breier, 1999). The main objective of current study was to identify novel polymorphisms in IGF-1 which can be used as marker assisted selection (MAS) for future camel selection and breeding plans.

Material and methods

Blood samples (n=105) from the Marecha camel were collected from the Camel Breeding and Research Station (CBRS), Rakhmahni, Bhakkar. These blood samples (10 mL) were collected aseptically from jugular vein of the

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camels into tubes containing ethylenediamine tetra-acetic Acid (EDTA) used as anticoagulant agent.

DNA was isolated from white blood cells (WBCs) suspended in the preserved samples via the standard phenol-chloroform-isoamyl alcohol DNA extraction protocol (Sambrook *et al*., 2001). Each DNA sample was quantified in order to check quality and quantity required for further processing by PCR. Agarose gel electrophoresis (AGE) and nano spectrophotometer methods were used for the quantification of DNA. The exact concentration of DNA was measured by NanoDrop (Thermo Scientific, Dubuque, USA) (Table I).

Table: List of primers for IGF-1 genes.

Primer	Sequence (5' to 3')
IGFF1	TTTCCTGTCTACAGTGTCTG
IGFR1	CCAAAAGTTCATATCCATGC
IGFF2	TCACTCACACCTCCTGTTGC
IGFR2	GGGCAGTCATTCCGTTCTT
IGFF3	AGCCTCCCAATTAAGCCAAT
IGFR3	CCAGGACTTGAAAGGTCACAT
IGFF4	TGGGGGTGTCAGAGATGAGT
IGFR4	GCTCCAGCAGGCCTACTTTT
IGFF5	AGTCTGAGTGAGGTCTGGCT
IGFR5	GGGTCAACAGCAATCTACCA

The *IGF-1* gene of *Camelus dromedarius* has five exons and four introns. For amplification, five primers were designed by using software Primer3 (http://frodo.wi.mit.edu/) from NCBI database (accession number NW_011591632). Primer sequences were optimized by using OligoTM primer design software.

DNA samples were amplified following optimization of primers. Each reaction mixture consisted of 1.5 µl of the DNA solution (50 ng/µl), 2.5 µl of 10x PCR buffer, 2.0 µL of 2.5 mM dNTPs, 2.0 µL of 2.5 mM/µL, 0.4 µL of 0.5 5 U/µL *Taq* Polymerase, 0.5 µL of each primer (10 pmol/µL) and 14.1 µL deionized water making up a final volume of 25 µl.

The thermal cycler was programmed for 5 min initial denaturation at 95°C, followed by 30 sec denaturation at 94°C, 30 sec primer annealing at 37°C, 45 sec extension at 72°C for 35 cycles and then final extension at 72°C for 10 min at annealing temperature of 65-55°C. PCR products were examined on a gel documentation system after running on the gel. The gel results were analyzed against a ladder of 50 bp. Sequencing of PCR products were performed using an ABI Prism 3100 Genetic Analyzer (Applied Biosystem Inc. Foster City,CA).

All the sequences were aligned with the help of NCBI online software blast-2-sequence (http/www.ncbi.nlm.nih.com). polymorphisms were identified from the aligned sequences. BioEdit translate tool was used to analyse polymorphisms to look for changes in codons and hence amino acids and protein sequence.

Protein structure was retrieved from mRNA sequence of genes from NCBI Gene bank database and induced mutation by using BioEdit ver. 7.0 software. Then sequence of mRNA back translated into protein through the Lasergene 7.1 software. For designing three-dimensional structure of IGF-1, we used SWISS-MODEL available on the ExPASy website (Bahrami *et al*., 2012).

Results and discussion

The present study was designed to discover polymorphisms in *IGF-1* gene as it is important and significant gene associated with growth. The aim of present study was to determine genetic diversity in the camel population through identification of polymorphisms, to know whether this gene could be used for marker assisted selection in future camel breeding programs.

Sequence analysis revealed four polymorphic sites in amplified exonic and intronic region of *IGF-1* gene. Out of these four SNPs, three were identified in intronic and one in exonic region (Table II).

These polymorphisms were further analyzed for their codon change and translated to their corresponding amino acids (Fig. 1). Nucleotide change at P67161 is from T→C. This nucleotide changes the amino acid codon from TGC→CGC indicating an amino acid substitution from Cystine acid (C) to Arginine (A) (Fig. 2).

IGF-1 is a polypeptide composed of 70 amino acids and molecular weight 7.5 kDa (Daughaday *et al*., 1989). IGF-1 amino acid sequence is identical in cattle, dog, pig and human (Nixon *et al*., 1999). IGF1 nucleotide sequence is about 70-90 kb in humans, goat, pigs, rats and chicken (Kajimoto *et al*., 1991). The bovine IGF1 gene was located on chromosome 5 (Grosse *et al*., 1999). Exon number varies between species such as goats, sheep and pig have 1-6 exons (Mikawa *et al*., 1995) and rats and humans 1-5 (Rotwein *et al*., 1986; Shimatsu *et al*., 1987).

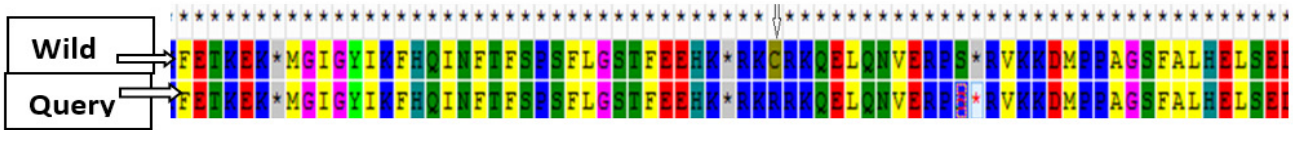

Fig. 1. Cysteine/Arginine polymorphism in *IGF-1* gene product.

Table II. Identified polymorphisms in insulin like growth factor 1 gene.

Sr. No.	Nucleotide position*	Mutation	Codon change	Region of mutation	Amino acid substitution	Change type	Allele frequency		Major allele frequency
1	53561	C>T	-	Intronic	-	-	0.7045	0.2955	0.7045
2	53571	T>A	-	Intronic	-		0.7523	0.2477	0.7523
3	67020	T>A	-	Intronic	-		0.6519	0.3481	0.6519
4	67161	T>C	TGC>CGC	Exonic	Cys > Arg	Non-synonymous	0.6075	0.3925	0.6075

*Accession Number: NW_011591632

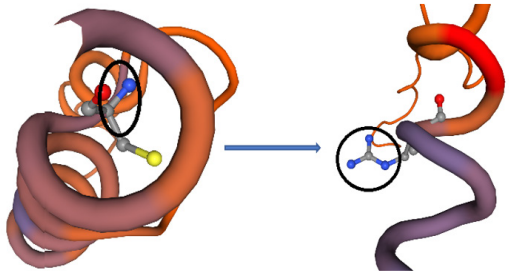

Fig. 2. The changes in amino acids from Cysteine to Arginine in *IGF-1* gene.

Identification of polymorphisms in Marecha individuals was main purpose of our work. The complete sequence of coding region was sequenced in indigenous camel breed. Five primers were used to flanked *IGF-1* gene consist of 211bp from exon 1,157bp from exon2, 181bp from exon 3,51bp from exon 4 and 733bp from exon 5. Amplified fragment of 636bp, 377bp, 962bp, 490bp and 489bp were obtained. This amplified sequence of IGF-1 was compared with Gene bank reference sequence (NW_011591632). All identified SNPs of cameline *IGF-1* gene in current study are novel and not reported previously. Out of 4 SNPs, three SNPs in intronic region at nucleotide position 53561 and 53571 and 67020 and one SNP in coding region of exon-5 at nucleotide position 67161 were observed. Three mutations were transition and one was transversion. Deletion and insertion polymorphism were not detected in sequence region. SNP identify in coding region was non- synonymous. The nucleotide substitution at 67,161 exposed Cystine (C) to Arginine (R) polymorphism. As Cystine is an amino acid of non-acidic nature whereas Arginine is basic. The different nature of both amino acids is a clear indication that the nature of final protein product of the gene was changed. This polymorphic site is very important as the nature of substituted amino acids changed due to the substituted nucleotide. These SNPs may serve as a powerful genetic resource for the development of DNA markers for association studies

IGF-1 polymorphism widely studied and the association between SNPs and production trait has been found in farm animals. IGF-1 is considered as good candidate marker for growth and meat production in livestock. High IGF-1 concentration was reported in blood of Simmental which is beef breed than dairy Holstein cows. Growth rate show correlation with concentration of IGF-1 in both breeds (Schlee *et al*., 1994). During evolution of species, IGF-1 has been conserved and its activity connected with reproductive pattern. It plays very significant role in feed consumption and metabolism in ruminants. In Nellore and Canchim breeds, significant correlation between body weight and SNPs has been studied by Curi *et al*. (2005). SNP in promoter region of (IGF1/SnaBI) Charolias cattle breed has been found to be associated with growth trait and can be helpful for genetic evaluation of this breed through MAS strategies (Rosa *et al*., 2010). Mullen *et al*. (2011) studied 5 promoter, intronic and regulatory region of IGF-1 to explore its significant association with growth, fertility and development in Holstein Friesian dairy cattle. Sharma *et al*. (2015) described significant association of 18 SNPs with body weight in Sirohi and Jamunapari goat breeds. These SNPs can be used as candidate genes in selection and genetic improvement of animals. Khadem *et al*. (2010) found significant association of mutation g.295T>C in the *IGF-1* gene with egg laying which caused amino acid change from cysteine to arginine in Jinghai yellow chickens. This changed mutation can be used as marker-assisted selection for improvement of chicken genetics in future. These findings will be helpful in giving more awareness about potential of this gene in marker assisted selection for growth in a camel breeding program.

Conclusion

The polymorphism identified in *IGF-1* gene showed high level of genetic variability and further studies could be initiated to identify their potential as genetic markers associated with growth performance traits and selection in camel breeding program.

Statement of conflict of interest

The authors declare no conflict of interest.

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