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Identification of novel SNPs and evaluation of allelic frequencies of Myo D gene in Pakistani cattle breeds

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Abstract

Research on association of candidate genes and their associations with meat characteristics involves many cattle breeds for improving the quality of meat. Present research was performed to identify the Novel SNPs and evaluation of Allelic frequencies of MyoD gene in coding and non-coding regions of the 04 Pakistani cattle breeds. PCR –SSCP technique was employed for the screening of novel SNPs and Genotyping of the bovine samples. The PCR product were separated on agarose gel and photographed under UV-illumination. Hereafter, the PCR product were purified and sequenced on ABI310 genetic analyzer. The sequence was aligned by using MEGA software. We have sequence selected samples of each breed, which include 04 samples of Red Sindhi (RDS), 05 samples of Dhani (DH), 02 samples of Cholistani (CH) and 02, samples of Thari (TH) breed. Overall four sequence variants were observed, one in the coding (exon 2) region (which not only caused the change of Nucleotide c.679G>A and codon GAC>AAC but also altered the amino acid sequence as p.Asp227Asn) and the other three variants were in non-coding (intronic) region (Intronic region T>A, Intronic C>G and Intronic T>A that only altered the Nucleotide but did not cause any change in the codon sequence). In addition, X^2 test showed that genotypes in three cattle breeds (RDS, DH, CH is not in Hardy - Weinberg equilibrium (P> 0.05), while, TH breeds were in Hardy-Weinberg equilibrium (P> 0.05), which may be due to the long process of intensive commercial breeding selection.

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Introduction

Myogenic determination gene (MyoD-1) gene belongs to MyoD Gene family having four family members, MyoD1, MyoG, MyF5 and MyF-6 gene and all the genes do share their common homology with the region known as Basic helix Loop helix. (BHLH) and the members of the gene family performs their key role in muscle formation, development and differentiation of muscle fibers and therefore, it is assumed as important gene for QTLs as reported by (Bhuiyan, M.S.A., *et al.*, 2009). Single nucleotide Polymorphism can be defined as the presence of altered nucleotide in the sequence of DNA molecule in any specific population (Risch, N. J., 2000) and Gray *et al.*, 2000).

Whereas, microsatellite markers to choose from genomic localization of QTL wise linkage map and associated with economic traits. (Biochard *et al.*, 2003; Casas *et al.*, 2003; Ashwell *et al.*, 2004; Iharah *et al.*, 2004; Snelling *et al.*, 2005: Hu *et al.*, 2007). However, microsatellite markers are labour-intensive and specific laboratories, and does not provide information on potential gene QTL but SNPS exist more frequently in humans and in mice, at least one SNP exists in every 1000bp of human genome and 1 SNP per 500bp of cattle genome (Lindblad - Tao Li *et al.*, 2000; Heaton *et al.*, 2001).

In addition, the location of candidate genes from the marker-assisted selection of animals is extremely important and quantitative characteristics of the relationship between better yields (He *et al.*, 2007; Dario *et al.*, 2008). So far, genome-wide association study applied to a panel of SNP markers across the genome panel to provide uniform distribution, however, recent work has shown that, in the context of gene SNP analysis provides high power, even if a single nucleotide polymorphism itself causes no functional changes (Jorgenson and Wittee, 2006). Myogenic determination gene has been mapped to chromosome number 15 for tenderness and carcass traits (Rexroad *et al.*, 2001; Casas *et al.*, 2003) and reported polymorphism and the quantity and quality of pork traits associated with the gene (Te PAS *et al.*, 1999; Cieslak *et al.*, 2000; Carmo *et al.*, 2005; Wyszynska-Koko *et al.*, 2006, 2006, *et al.*, Werner *et al.*, 2007; Humpolicek *et al.*, 2007).

Bhuiyan *et al.*, 2009, reported the relationship between SNP of MyoD gene family and meat quality and quantitative traits. However, little work has been reported concerning the role of SNP Identification and control of QTL traits in Pakistan. Therefore, the present study was to determine the frequency of novel SNP allele in cattle breeds of Pakistan and to assess its allelic frequencies in Pakistani cattle breeds.

Materials and methods

DNA extraction

The experiment was carried out on 554 cattle animals belonging to four different breeds at the age of 1.5 to 2 years from different cattle farm centres around the Sukkur division. The Red Sindh cattle's (RS n=369) were selected from Ghousala farm at Gambat, district Khairpur, Dhani cattle's (DN n=64) blood samples were obtained from Naheed Kazi cattle farm, District Khairpur, Cholistani cattle's (CL n=53) blood samples were gathered from Village Rasool Bux Ujjan, District Khairpur and finally the Thari cattle (Th n=64) samples were taken from Mubeen Ahmed Khan Phulpoto cattle farm, district Khairpur, All the samples were stored at -20C and DNA was extracted from leucocytes.

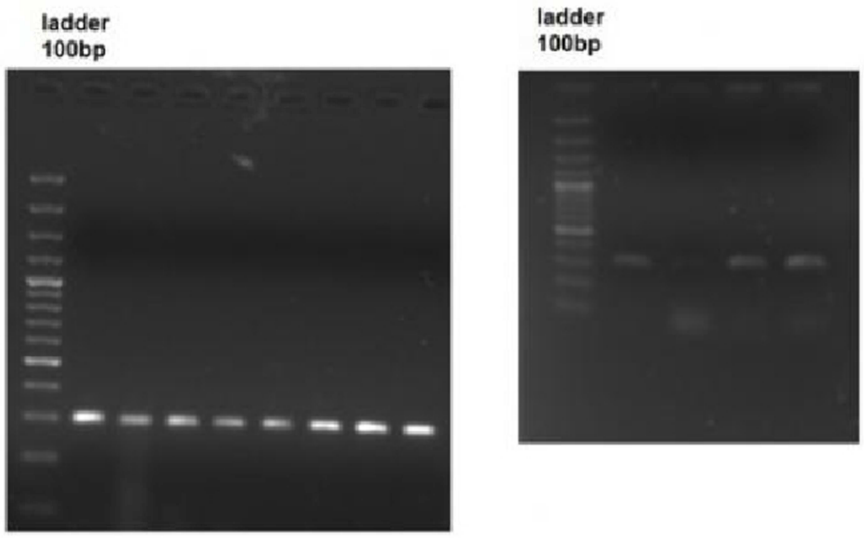
PCR–SSCP analysis

Following primers were used for amplification of MyoD gene sequence. Information about the primers is given below in table 1.

Table 1. Oligonucleotide primers pair used to amplify Exon2 of MyoD gene.

Gene Accession no.	Oligonucleotide sequence (5′ to 3′)	Amplicon size (bp)	Annealing temp. (°C)
MyoD1 gene NC-007313	F: GCTCTGTTCCTATTGGCCTC	472	62
MyoD1 gene NC-007313	R: GATCCAGGTCCTCGAAGAAG	472	62
MyoD1 gene NC-007313	F: AAGTCAACGAGGCCTTCGAG	468	60
MyoD1 gene NC-007313	R: CGTATCCTTCCTGTGCATCC	468	60

PCR reaction was performed under standard protocol as described by Zhang *et al.*, 2007. Soon afterwards, the PCR reaction the PCR product was quantified on 2% horizontal gel electrophoresis and the gel was stained with 200ng/ml ethidium bromide. Finally, The PCR product were purified and sequenced on ABI310 genetic analyzer. The photos of Agarose gel electrophoresis are shown in joint Fig. 1 & 2. Fig. no.1 & 2. Showing Agarose gel electrophoresis of the PCR product of MyOD gene.



MYOD1 Exon 2 PCR Product run on 2%(Agarose Gels

PCR product was confirmed genotyping using a single stranded confirmation polymorphism, the following reaction (4ul in the PCR product by 95% formamide, 25 mmol EDTA, 0.025% and 0.025% xylene-Cyanole bromophenol blue) of DNA denaturing solution 8ul mixing, it was incubated at 98℃ 10 minutes. Ice and freezing.

The denatured DNA was pipetted into a 10% polyacrylamide gel for 120 volt 12 hours, and finally gels was stained and visualized with Silver nitrate and Sodium hydroxide (Zhang *et al.*, 2007).

Statistical analysis

Allele and genotype frequencies of each cattle breed were determined by X^2 test and demographic indicators, such as gene homozygous, gene heterozygosity and effective number of alleles, polymorphism information content and other values were determined based on the calculation methods of Nei & Roychoudhury 1974 and Nei and Li 1979.

Results

Identification of Single Nucleotide Polymorphism

We have sequence selected samples of each breed, which include 04 samples of Red Sindhi (RDS), 05 samples of Dhani (DH), 02 samples of Cholistani (CH) and 02, samples of Thari (TH) breed. Overall four sequence variants were observed, one in the coding (exon 2) region (which not only caused the change of Nucleotide c.679 G>A and codon GAC>AAC but also altered the amino acid sequence as p. Asp227Asn) and the other three variants were in non-coding (Intronic) region (Intronic region>A, Intronic C>G and Intronic T>A that only altered the Nucleotide but did not cause any change in the codon sequence) as shown in table no. 2.

Table 2. Sequence Variants identified in exon 2 of MYOD1 Gene.

S. No.	Exon	Nucleotide Change	Codon change	Amino acid Change
1	2	c.679G>A	GAC>AAC	p. Asp227Asn
2	Intronic	T>A	Nil	Nil
3	Intronic	C>G	Nil	Nil
4	Intronic	T>A	Nil	Nil

Evaluation of Allelic, Genotypic frequencies of different Cattle breeds

In addition, the single nucleotide polymorphism allele frequency is then determined by the X^2 test calculations. The same test also revealed that among 03 kinds of cattle genotypes (RDS, DH, CH is not in Hardy-Weinberg equilibrium (P> 0.05), while, TH breeds were in Hardy-Weinberg equilibrium (P> 0.05). Single nucleotide polymorphisms (c.679G> A) allele frequencies of polymorphisms showed it is not evenly distributed as shown in Table 3.

Data shown here indicates that the allele frequency range MYOD1-A gene allele from 0.5827 (RS= Red Sindhi cattle) among four different breeds to 0.9434 (Cholistani cattle= CH) is present in significant differences in allele frequencies between RS, CL and DH cattle breeds ($X^2$0.05 $X^2$0.01) groups, which indicates that these three species are in Hardy-Weinberg equilibrium. CC genotype frequency from Dhani cattle breeding range of 0.0566 to 0.334 respectively in the RS varieties than the AA genotype, which ranged from 0.498 to 0.943 CH cattle is relatively low. This observation is possible because of random genetic drift occurs due to C allele frequencies. However, TH cattle breeds genotypes in Hardy-Weinberg equilibrium does not agree and showed significant differences in allele frequencies, this observation is possible because the cattle breed ID TH breed possess more drift and selection. All four genotype frequencies between cattle breeds diversity was moderate (0.25 < PIC value <0.5). Other genetic index, gene heterozygosity (HE), effective number of alleles (NE) and polymorphic information content (PIC) varied from 0.106 to 0.486, 1.11 to 1.946 and 0.101~0.38 respectively as in table 4.

Table 3. Allelic and Genotype frequencies (%) of the MyoD gene for SNP in cattle Population.

Breed	Gene SNP (c.679G>A) Genotype			Total	Allelic frequencies A C HW Value		
Breed	AA	AC	CC	Total	Allelic frequencies 2 X A C (HW)		
RS	0.4986 (184)	0.168(62)	0.333(12)	369	0.582	0.417	158.07
DH	0.7813 (50)	0.000(0)	0.218(14)	64	0.781	0.218	64.00
CH	0.943 (50)	0.000(0)	0.0566(3)	53	0.943	0.0556	36.8
TH	0.708 (34)	0.187(9)	0.104(5)	68	0.802	0.197	8.05

Note: HW= Hardy-Weinberg equilibrium X2 0.01 = 9.210, X2 0.05=5.991.

Table 4 Other genetic indices of MyoD gene loci

Breed	Gene homozygosity	Gene heterozygosity	Effective allele number	PIC
RS	0.513	0.486	1.946	0.368
DH	0.658	0.341	1.519	0.283
CH	0.893	0.106	1.119	0.1011
TH	0.682	0.317	1.465	0.267

Note: PIC value have been classified in three levels: Low polymorphism (PIC value < 0.25), medium polymorphism (0.25<PIC <0.5) and high polymorphism (PIC > 0.5).

As per the general trend, PIC values have been divided into three levels: low polymorphism (PIC value <0.25), the polymorphisms (0.25 <PIC < 0.5) and highly polymorphic (PIC> 0.5), while taking into account the above-mentioned PIC classification, all Bosindicus breeds showed a high level and then we recommend high MYOD1-a allele frequencies can be used for characterize the Bosindicus.

Discussion

In beef cattle breeding, the main consideration is to speed up the growth rate. QTL mapping and identification of candidate genes that affect growth traits will greatly enhance the progress toward this goal (Li *et al.*, 2004). Candidate genetic polymorphisms and their association with economic traits have been performed to determine the genetic basis of productive traits and to develop marker-assisted selection.

Study of association of candidate gene for meat traits provides intensive information of genetics of the traits (O' vilo *et al.*, 2006) and variations in these genes is very important to be studies because they contribute towards the socioeconomic development of any country and molecular assisted selection of the genes.

Growth rate and lean meat content are two important economic traits in meat producing animals. Mammalian meat production is related to the amount of muscle fibers in the muscle, a strictly embryonic process that is regulated by the MYOD gene family (Olson, 1990). The MYOD gene family consists of four structural and functionally related genes: MYOD1 (MYF3), MYOG (myogenin), MYF5 and MYF6 (herculin). All four genes are composed of three exons and share the same homology in the region encoding basic helix-loop-helix (bHLH) domains (Fujisawa-Sehara *et al.*, 1990). A number of novel mutations have been described to control the characteristic of the meat quantity and meat quality in a study on many breeds of pig (Cieslak *et al.*, 2002; Uryl *et al.*, 2002 and other features of muscle tissues (Klosowska and fielder, 2003; Klosowska *et al.*, 2001, 2004). Nevertheless, there have been very few studies on the MyoD1 gene in cattle. Our results of the present study shows the similarity of positive associations of the Novel SNPs in Pakistani cattle breeds with their genotypic and allelic frequencies as those of the results of Bhuiyan (Bhuiyan M.S.A. *et al.*, 2009; Camous *at el.*, 2011 and Fubiao song *et al.*, 2012, however differs from the findings of Li gao *et al.*, 2011 in reference to the allele and genotypic frequencies of MyoD gene.

We concluded a strong association of the c.679G>A novel SNP with allelic and genotypic frequencies and the corresponding genotypes of MyoD1 gene polymorphisms in the Pakistani indigenous cattle breeds. This result is consistent with previous studies on other bovine and porcine populations.

Conclusion

Identification of Novel SNPs and evaluation of Allelic frequencies of MyoD gene in Pakistani indigenous cattle breeds were studies in 04 cattle breeds and high-level polymorphism level was observed. Moreover, results of our study suggests that this SNP (c.679G>A) could be used as part marker panels for breed composition and population admixture analyses due to marked differences in allelic and genetic frequencies among these native Pakistani cattle breeds.

References

Adoligbe C, Zan L, Farougou S, Wang H, Ujjan JA. 2012. Bovine GDF10 gene polymorphism analysis and its association with body measurement traits in Chinese indigenous cattle. Molecular biology reports **39,** 4067-4075.

Ashwell MS, Heyen DW, Sonstegard TS, Van Tassell CP, Da Y, Van Raden PM, Lewin HA. 2004. Detection of quantitative trait loci affecting milk production, health, and reproductive traits in Holstein cattle. Journal of dairy science **87,** 468-475.

Bhuiyan MSA, Kim NK, Cho YM, Yoon D, Kim KS, Jeon JT, Lee JH. 2009. Identification of SNPs in MYOD gene family and their associations with carcass traits in cattle. Livestock Science **126,** 292-297.

Boichard D, Grohs C, Bourgeois F, Cerqueira F, Faugeras R, Neau A, Levéziel H. 2003. Detection of genes influencing economic traits in three French dairy cattle breeds. Genetics Selection Evolution **35,** 77-102.

Carmo FMDS, Guimarães SEF, Lopes PS, Pires AV, Guimarães MFM, Silva MVGBD, Gomide LADM. 2005. Association of MYF5 gene allelic variants with production traits in pigs. Genetics and Molecular Biology **28,** 363-369.

Casas E, Shackelford SD, Keele JW, Koohmaraie M, Smith TPL, Stone RT. 2003. Detection of quantitative trait loci for growth and carcass composition in cattle. Journal of Animal Science **81,** 2976-2983.

Cieślak BD, Kapelański W, Blicharski T. 2000. Restriction fragment length polymorphisms in myogenin and myf3 genes and their influence on lean meat content in pigs. Journal of Animal Breeding and Genetics **117,** 43-55.

Cieslak D, Kuryl J, Kapelanski W, Pierzchala M, Grajewska S, Bocian M. 2002. A relationship between genotypes at MYOG, MYF3 and MYF5 loci and carcass meat and fat deposition traits in pigs. Animal Science Papers and Reports **20,** 79-92.

Dario C, Selvaggi M, Carnicella D, Bufano G. 2009. STAT5A/AvaI polymorphism in Podolica bulls and its effect on growth performance traits. Livestock Science **123,** 83-87.

Gao L, Ujan JA, Zan L, Xue M, Camus A. 2013. A novel polymorphism of resistin gene and its association with meat quality traits in Chinese Bostaurus. African Journal of Biotechnology **10,** 1252-12256.

Gray IC, Campbell DA, Spurr NK. 2000. Single nucleotide polymorphisms as tools in human genetics. Human molecular genetics **9,** 2403-2408.

Heaton MP, Grosse WM, Kappes SM, Keele JW, Chitko-McKown CG, Cundiff LV, Laegreid WW. 2001. Estimation of DNA sequence diversity in bovine cytokine genes. Mammalian Genome **12,** 32-37.

Hu ZL, Fritz ER, Reecy JM. 2007. Animal QTLdb: a livestock QTL database tool set for positional QTL information mining and beyond. Nucleic Acids Research **35** (Suppl 1), D604-D609.

Humpolíček P, Urban T, Tvrdoň Z. 2007. Relation of porcine myogenin gene PCR/RFLP MspI and reproduction traits of the Czech Large White sows. Livestock Science **110,** 288-291.

Ihara N, Takasuga A, Mizoshita K, Takeda H, Sugimoto M, Mizoguchi Y, Snelling WM. 2004. A comprehensive genetic map of the cattle genome based on 3802 microsatellites. Genome Research **14,** 1987-1998.

Jorgenson E, Witte JS. 2006. A gene-centric approach to genome-wide association studies. Nature Reviews Genetics **7,** 885-891.

Khatib H, Zaitoun I, Wiebelhaus-Finger J, Chang YM, Rosa GJM. 2007. The association of bovine PPARGC1A and OPN genes with milk composition in two independent Holstein cattle populations. Journal of dairy science **90,** 2966-2970.

Klosowska D, Kuryl J, Cieslak D, Elminowska-Wenda G, Walasik K, Kapelanski W. 2001. The relationship between polymorphisms in porcine MYOG, Myf-3 and Myf-5 genes and microstructural characterises of *Longisimus* muscle-a preliminary study. In International congress OF meat Science and Technology **47,** pp. 142-143. Japan Society for Meat Science and Technology.

Klosowska D, Kuryl J, Elminowska-Wenda G, Kapelanski W, Walasik K, Pierzchala M, Bogucka J. 2004. A relationship between the PCR-RFLP polymorphism in porcine Myog, MYOD1 and MYF5 genes and microstructural characteristics of *M. longissimus lumborum* in Pietrain x (Polish Large White x Polish Landrace) crosses. Czech Journal of Animal Science-UZPI (Czech Republic).

Kuryl J, Kapelanski W, Cieslak D, Pierzchala M, Grajewska S, Bocian M. 2002. Are polymorphisms in non-coding regions of porcine MyoD genes suitable for predicting meat and fat deposition in the carcass. Animal Science Papers and Reports **20,** 245-254.

Li C, Basarab J, Snelling WM, Benkel B, Murdoch B, Hansen C, Moore SS. 2004. Assessment of positional candidate genes 5 and 1 for growth on bovine chromosome 5 in commercial lines of Journal of Animal Science **82(1),** 1-7.

Lindblad-Toh K, Winchester E, Daly MJ, Wang DG, Hirschhorn JN, Laviolette JP, Shah N. 2000. Large-scale discovery and genotyping of single-nucleotide *polymorphisms* in the mouse. Nature genetics **24,** 381-386.

Nei M, Li WH. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences **76,** 5269-5273.

Nei M, Roychoudhury AK. 1974. Sampling variances of heterozygosity and genetic distance. Genetics **76,** 379-390.

Olson Eric N. 1990. "MyoD family: a paradigm for development." Genes & development **4,** 1454-1461.

Ovilo C, Fernández A, Rodríguez MC, Nieto M, Silió L. 2006. Association of MC4R gene variants with growth, fatness, carcass composition and meat and fat quality traits in heavy pigs. Meat Science **73,** 42-47.

Rexroad Iii, CE, Bennett GL, Stone RT, Keele JW, Fahrenkrug SC, Freking BA, Smith TP. 2001. Comparative mapping of BTA15 and HSA11 including a region containing a QTL for meat tenderness. Mammalian Genome **12,** 561-565.

Risch NJ. 2000. Searching for genetic determinants in the new millennium. Nature **405,** 847-856.

Snelling WM, Casas E, Stone RT, Keele JW, Harhay GP, Bennett GL, Smith TP. 2005. Linkage mapping bovine EST-based SNP. BMC genomics **6,** 1.

Te Pas MF, Soumillion A, Harders FL, Verburg FJ, Van den Bosch TJGalesloot, P, Meuwissen TH. 1999. Influences of myogenin genotypes on birth weight, growth rate, carcass weight, backfat thickness, and lean weight of pigs. Journal of Animal Science **77,** 2352-2356.

Tian WQ, Wang HC, Song FB, Zan LS, Wang H, Wang HB, Ujan JA. 2011. Association between a single nucleotide polymorphism in the bovine chemerin gene and carcass traits in Qinchuan cattle. Genetics and Molecular Research **10,** 2833-2840.

Verner J, Humpolíček P, Knoll A. 2007. Impact of MYOD family genes on pork traits in Large White and Landrace pigs. Journal of Animal Breeding and Genetics **124,** 81-85.

Wyszyńska-Koko J, Pierzchała M, Flisikowski K, Kamyczek M, Różycki M, Kurył J. 2006. Polymorphisms in coding and regulatory regions of the porcineMYF6 and MYOG genes and expression of theMYF6 gene in *M. longissimus dorsi* versus productive traits in pigs. Journal of applied genetics **47,** 131-138.

Zhang C, Wang Y, Chen H, Lan X, Lei C. 2007. Enhance the efficiency of single-strand conformation polymorphism analysis by short polyacrylamide gel and modified silver staining. Analytical biochemistry **365,** 286-287.