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Comparative Study of Genetic Polymorphism of Fat Mass and Obesity Associated Gene in Three Indigenous Goat Breeds of District Khairpur, Sindh, Pakistan

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Abstract

Obesity has become much more common in recent decades. This is due to the so-called "obesity environment," in which high-calorie foods and beverages are widely available but physical activity chances are few. The fat mass and obesity-related genes (FTO) that have the largest impact on body mass index (BMI) are the strongest of all known genes and are frequently linked to obesity susceptibility. I discovered a total of 09 mutations in the FTO gene of three goat breeds in this study: Barbari goats, Sindh Desi goats, and Tapri goats. Using DNA sequencing technology and PCR gel electrophoresis, find mutations. These mutations are explained and classified using the genetic code. 03 nonsense mutations and 04 deletion mutations were discovered in Barbari goats. Arginine, Lysine, and Alanine are encoded by the missense mutations discovered. In the Sindh Desi goat breed, a deletion was discovered, while in the Tapri goat breed, a missense mutation encoding the amino acid serine was discovered. Furthermore, our findings show that the Barbari goat breed is highly suitable for mixed breeds and has excellent meat properties, owing to its larger genetic variety.

Keywords: FTO gene, Goat, DNA sequencing, PCR, SNPs

Introduction

Obesity is one of the most prevalent public health issues affecting children and adolescents in our century, and its prevalence has risen dramatically in the last 30 years (WHO report 2012). Obesity is a complex illness whose aetiology is linked to both environmental and hereditary factors. Obesity among children in Pakistan is on the rise at an alarming rate. The main causes of childhood obesity, according to studies conducted in 2007, are genes associated to fat accumulation and obesity. Obesity has resulted in life-threatening implications for the majority of the world's population. Candidate genes for polygenic obesity include genes associated to fat mass and obesity. Herbert et al., 2008; Martinez A., et al., 2007).

The overall length of fat mass and obesity (FTO) genes in humans exceeds 400 kb and is found on chromosome 16q12.2. FTO is related to 3-methylthymine (3-meT) and 3-methyluracil (3-meU) in DNA and is a member of deoxygenase-dependent alpha ketoglutarate (ALKB) and nucleic acid demethylases. And adenosine (6-meA) in 6-methyl RNA is a non-heme protein (iron). Obesity is linked to a single nucleotide polymorphism in FTO intron 1. FTO is thought to be a gene that is

entirely linked to obesity, based on several experiments and investigations on various species (Dina et al., 2007; Frayling et al., 2007).

At the genomic level, it was discovered that the expression of the FTO gene can cause alterations in cell morphology in mice and humans' tissues and nerve cells (Gerken et al., 2007). The FTO gene, on the other hand, regulates and performs a wide range of actions, most of which involve brain cells, particularly those in the hypothalamus nucleus. Its primary function is to keep energy balance and active behaviour in check. Several investigations in rats, mice, and humans have also revealed that the FTO gene's m-RNA regulates the consumption of high-sugar diets. Aside from the startling example of FTO and higher ATP accumulation in brain cells, amino acid alterations also have an effect on FTO. The amount of ATP accumulated in adipose tissue is dramatically reduced, indicating a cell-centric strategy that prioritises energy enhancement. More fat in the body will have negative implications for people of all ages, races, genders, social and cultural backgrounds all over the world, including heart disease (CVD), atherosclerosis, blood pressure, type 2 diabetes, and liver disease. Cancer, steatosis, and embolism (Pitman et al., 2012; Balaban. G et al., 2004; Turconi. G et al., 2013).

Obesity can be classified into three types: 1-single gene (syndrome), 2-polygenic (syndromic), and 3-polygenic (non-syndromic, common, and multifactorial) (Choquet, H. et al. , 2011; Farooqi, IS. et al., 2006). Syndromic obesity can cause complex genetic symptoms due to decreased expression of FTO genes, but FTO genes mostly affect the abnormalities of obesity syndromes, accounting for 80 percent of global obesity cases. Single nucleotide polymorphism (SNP) testing is the most sensitive tool for discovering obesity-related alterations in humans. Farooqi, IS., et al., 2006; Herrera, BM., 2010; Hinney, A., et al., 2010).

FTO is thought to enhance hunger and cause weight gain. Weight loss may occur if an FTO gene outbreak or malfunction occurs. Mice without the FTO gene are relatively addicted, albeit their decreased body weight is a side effect of their growing deficiency, and they don't exist. The FTO gene, according to the study, is the source of mass obesity in humans. Obesity-related features are linked to genetic variations or mutations in FTO genes, according to extensive genome linkage studies (Scuteri. et al., 2007).

Changes in the FTO gene that are obese and related quirks have now been replicated in one-day human and pig genomic investigations (Fontanesi et al., 2009; Fan et al., 2009; Hunt et al., 2008; Chang et al., 2008; Frayling et al., 2009; Hunt Et al., 2008; Chang et al., 2008; Frayling et al., 2007). The traits of fullness, broadness, and longissimus muscles of at least five cattle breeds in China are referred to as the fat group or type and meat quality or meat-related genetic inclinations or characteristics (FTO) of the most current cow (Wei S. et al., 2011) The association of the FTO gene of the numerical fat streak of Hanwoo cattle in South Korea was increased due to an uncommon SNP in the management area (Chung ER et al., 2014). FTO gene polymorphisms have been associated to various types of obesity in cattle, pigs, and humans in all of the studies described above. Although there is no information on FTO gene polymorphisms in Pakistani goat breeds, the goal of this study is to find out. Three indigenous goat breeds from Khairpur District, Sindh Province, Pakistan, have FTO genes.

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Only in the last few years has technology been created that allows for the simultaneous analysis of hundreds of thousands (now more than one million) single nucleotide polymorphisms (SNPs). These genome-wide association studies (GWAS)-supporting technologies, combined with unprecedented international cooperation, have resulted in the collection of an increasing number of research cohorts that have become extremely powerful in detecting common genetic variants linked to diseases like obesity. As a result, the fast evolution of the environment and the introduction of new technology have magnified previously mild sensitivities in numerous ways.

Material and Methods:

Sampling

A total of 30 blood samples were taken from three different local goat breeds, including the Sindhi, Barbari, and Tapri goats from Taluka Khairpur, Taluka Sobhodero, and Taluka Gambat in Khairpur District, Sindh, Pakistan have been detected. The Canadian Animal Care Council's requirements are followed when caring for and handling these animals.

Extraction of DNA

Thermo Scientific Fisher Scientific's DNA kit n. (K0718) is used to extract DNA from white blood cells in a whole blood sample (www.thermofisher.com). PCR, gel electrophoresis, and traditional methods are used to identify and quantify the separated DNA samples. Following the conventional approach given by Sambrook and Russell, it was treated with 2% heparin and then stored at -20°C in (2002).

Primer Designing and Polymerase Chain Reaction (PCR) Amplification

Primer Premier 3 software was used to design the primers, which were then synthesized at CEMB Lahore. PCR amplification was carried out in a 20-L reaction mixture containing 50 mg template DNA, 10 pM per primer, 0.20 mM dNTP, 2.5 mM MgCl2, and Taq 0.5 U DNA polymerase, according to Zhang et al., (2007). (TaKaRa, Dalian, China). Initial denaturation at 95°C for 5 minutes, denaturation at 94°C for 30 seconds for 32 cycles, annealing at 58°C for 30 seconds, extension at 72°C for 30 seconds, and finally extension at 72°C for 10 minutes were used in the PCR.

Gel-Electrophoresis

In the electrophoresis chamber, the PCR products were checked on 1.5 percent agarose and stained with 200 mg/ml (ethidium bromide) gel. PCR was loaded with 1X TBE buffer at a voltage of 80 amperes, according to the method of de Yang et al., 2009).

Purification and Sequencing of DNA

The PCR products were purified and then sequenced by CEMB Lahore. The polymorphism comparison of the genetic material sequence determines the objects in the existing curriculum or line classification in NCBI (http://www.ncbi.nlm.nih.gov).

Data Analysis

Percentage and classification of SNPs was calculated with Microsoft excel sheet and online Bioinformatic analysis.

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Results

Quantification of Extracted DNA and PCR- Gel Electrophoresis

The amount of DNA in the extracted DNA samples was measured with a Nanodrop and found to be in the range of 30-103 ng/l, which was sufficient for PCR amplification. For high-quality DNA, the A260/A280 ratio should be between 1.7 and 2.0. The CAP-1 gene DNA quantification and PCR amplification gel images are shown in Figures 1 and 2.

Fig. 1. DNA quantification using nanodrop method.

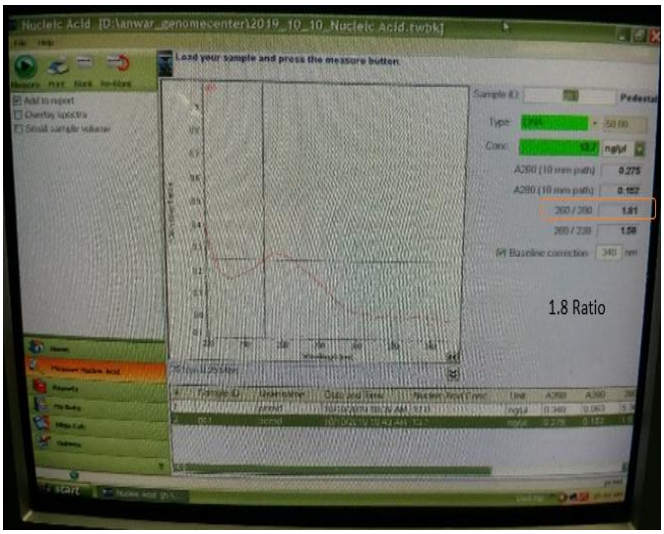

Fig. 2 Shows the PCR ampilied Product of Cap -1 gene on 1.5% Agarose gel

Note: Vertical bands: 1Kb DNA Marker and horizontal bands line: samples

Identification of Single Nucleotide Polymorphism

The current study's findings provide complete genomic data and explain the comparison of mutations and percentages in goat breed 03 in Khairpur, which includes Barbari goats, Sindh Desi goats, and Tapri goats. In this study, nine mutations in three goat breeds were discovered using DNA sequencing and gel electrophoresis technology. A thermal cycler accomplishes this by cooling and heating the test tube in a short amount of time.

The 210 bp portion of all goat breeds was amplified by PCR in this study, and the 210 bp fragment was sequenced to reveal the genetic code mutation. A total of 09 mutations in the FTO gene were discovered in this study using DNA sequencing, gel electrophoresis, and PCR amplification. There

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were also 04 total missense mutations and 05 deletion mutations found. For more information, see Tables 2 and 3.

Table 2 detailed position and type of mutation / Polymorphsim

Name of Breed	Position of mutation	Original Condon	Changed Condon	Original Amino Acid	Altered Amino Acid	Point Mutation
Barbari goat breed	109	GGA	CGA	Glycine (Non-Essential	Arginine (Essential)	Missense Mutation
	121 & 123	CCA	GCG	Proline (Non-Essential)	Alanine (Non-Essential)	Missense mutation
	131	AGA	AAA	Arginine (Essential)	Lysine (Essential)	Missense Mutation
	43	TAG	TA-	STOP	-	DELETION
	50	GAC	-AC	Asparagine (Non-essential)		DELETION
	64	AGG	-GG	Arginine (Essential)		DELETION
	76	AGG	-GG	Arginine (Essential)		DELETION
Sindh Desi Goat breed	328	TCT	T-T	Isolucine(Essential)		DELETION
Tapri Goat breed	277	TTT	TCT	Arginine (Essential)	Serine (Non-essential)	MISSENSE

Table 3 Percentage of mutation in all 03 goat breeds

Gene type	Breed type	Number of Identified SNP's	Percentage Formula	% Of Mutation
FTO Gene	Barbari	07	7/210x100	3.333%
	Sindh Desi	01	1/210x100	0.47%
	Tapri	01	1/210x100	0.47%

DISCUSSION

The FTO gene is absent from 16q12.2; the chromosome has 9 coding areas (exons) totaling 410.50 kb, the FTO gene comprises 505 amino acids, the subatomic mass is 58282 Daltons, and the monomer can be employed as the same. The source dimer is present. The FTO gene is found in many tissues at various levels of enhancement, particularly in the brain (Frayling et al., 2007). As the research improves, so does our understanding of the FTO gene's appearance, structure, and capabilities. The FTO gene is thought to code for 2-ketoglutarate subordinate nucleic acid demethylase, which catalyses the removal of methyl groups from 3-methyltylin in single-stranded DNA utilising Fe (II) and 2-oxoglutarate (2-OG), delivering carbon dioxide, formaldehyde, and succinic acid all at once (Gerken et al., 2007).

Polymorphism is defined as the presence of at least two different DNA sequences for a given DNA fragment, both of which are genetically distinct and essentially unique. These modifications might be as small as a single nucleotide base alteration to as large as a few hundred. The differences between the two types of polymorphisms, one caused by substitution of DNA bases and the other by addition

or substitution of base pairs, are mostly obvious. SNPs are the simplest type of polymorphism and can be found in the quality administrative region or throughout the genome. These alterations are known as double, triple, or quaternary alleles and are found at a ratio of one in 1,300 nucleotides in the human genome (Duncan and Miller, 1980).

The current study's findings provide complete genomic data and explain the comparison of mutations and percentages in goat breed 03 in Khairpur, which includes Barbari goats, Sindh Desi goats, and Tapri goats. In this study, nine mutations in three goat breeds were discovered using DNA sequencing and gel electrophoresis techniques. A thermal cycler does this by cooling and heating the test tube in a short amount of time.

The 210 bp region of all goat breeds was amplified by PCR in this study, and the 210 bp fragment was sequenced to reveal the genetic code mutation. A total of 09 mutations in the FTO gene were discovered in this study using DNA sequencing, gel electrophoresis, and PCR amplification. There were also 04 total missense mutations and 05 deletion mutations found.

Furthermore, the Barbari goat breed has a polymorphism of 3.333 percent, while the Sindh Desi and Tapri goat breeds each have a polymorphism of 0.47 percent. According to the current findings, the Barbari goat breed can be utilised for breed mixing, which improves meat quality.

5.1 CONCLUSION

The Barbari goat breed, the Sindh Desi goat breed, and the Tapri goat breed were all discovered to have mutations in the FTO gene 09 in this study. Using DNA sequencing and PCR gel electrophoresis, find mutations. These mutations are explained and classified using the genetic code. 03 nonsense mutations and 04 deletion mutations were discovered in Barbari goats. Missense mutations encoding arginine, alanine, and lysine have been identified, as have (01) deletions in Sindh goats and (01) missense mutations encoding the amino acid serine. Furthermore, our findings show that the Barbari goat breed is highly suitable for mixed breeds and has excellent meat properties, owing to its larger genetic variety.

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Conflict of Intrest

Authors declare no any conflict of interest.

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