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Determination of mucopolysaccharidosis IIID in some goat breeds

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Abstract: Mucopolysaccharidosis IIID (MPS IIID) or Sanfilippo D syndrome is an inherited lysosomal storage disease and caused by a deficiency of the N-acetylglucosamine-6-sulfatase activity, which involves in the catabolism of heparan sulfate. In addition to humans, canine, feline and murine, goats have been reported as possessing this lysosomal storage disease. The molecular defect in the MPS IIID goat has been previously identified. It has been observed that a molecular base for this defect is a nonsense mutation at nucleotide 322 (C→T) results in the change of the arginine codon to a stop codon. This mutation is not only to be related to stop enzyme function but also introduces a recognition site for *Alu*I that will enable carrier detection. The goal of this study is to find the presence of MPS IIID genetic disorder in some goat breeds. A total of 80 goat blood samples selected randomly from 4 different countries including Kyrgyzstan, Iraq, Northern Cyprus and Bosnia-Herzegovina were used as a sample. Polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) and DNA sequence data were utilized to identify this disorder in the goat populations. The result of this study demonstrates nonsense mutation which causes MPS IIID genetic disorder was not found in the goat breeds studied, while a silent mutation was found at nucleotide 354 (T→C) when compared with reference sequence.

Key words: Goat, genetic disorder, MPS IIID

1. Introduction

The mucopolysaccharidoses (MPS) are heritable lysosomal storage diseases (LSD) where the functional deficiency of any 1 of 11 lysosomal enzymes needed to degrade glycosaminoglycans (GAGs), formerly referred to as mucopolysaccharides [1–3]. Depending on the deficiency of the activity in any of these enzymes disrupts the degradation of GAGs and results in an accumulation of GAGs in lysosomes. As a result, lysosomal accumulation of GAGs leads to cell, tissue, and organ dysfunction [4–6]. One group of these diseases are known as a mucopolysaccharidosis type III (MPS III) or Sanfilippo syndrome. MPS III is an autosomal recessive disorder including four subtypes (A-D). Each is due to lack of one of four lysosomal enzymes that participate in the removal of sulfated N-acetylglucosamine residues during the degradation of heparan sulfate (HS) [2,7]. Models for four of the human MPS have been described and one of these models is caprine N-acetylglucosamine 6-sulfatase (GNS) deficiency represents the only animal analog of MPS IIID [8]. Caprine MPS IIID is an inherited lysosomal storage disease (LSD) caused by a lack in N-acetylglucosamine 6-sulfatase. In caprine MPS IIID, the GNS deficiency consequent to a single nonsense mutation [9]. Biochemical and immunohistochemical characterization of caprine MPS IIID has been reported and analyses showed increased accumulation of HS and free N-acetylglucosamine sulfate in central nervous system and somatic tissues [4,8]. Among affected goats, phenotypic variation in MPS IIID disease expression with mild and serious forms has been reported. However similar to human phenotype, the affected goat demonstrated delayed motor development, growth retardation, and accumulation of gangliosides in the central nervous system [10]. Caprine GNS's cDNA has been cloned and sequenced by Friderici et al. [9] and the cDNA defect in caprine MPS IIID has been detected by Cavanagh et al. [11]. A mutation test utilizing PCR has been introduced to carrier identification and prenatal testing for the disorder in goats [12]. The molecular base for this disorder was due to a nonsense C→T mutation in codon 102 from GNS cDNA of the affected animal. This mutation not only truncating the protein but also creates an *Alu*I restriction site, which make possible carrier or affected animal detection by RFLP. Caprine MPS IIID is only identified in Nubian goats and their crosses. The goal of this study is to find the presence of MPS IIID genetic disorder in some goat breeds from different countries. It is thought that the information obtained as a result of this

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research will contribute to both goat genetic information and human health studies as well.

2. Materials and methods

2.1. Collection of sample and DNA extraction

In this study, blood samples originated from goats were obtained from four different sampling countries: Kyrgyzstan, Iraq, Northern Cyprus and Bosnia--Herzegovina. A total of 80 samples are selected randomly from different goat breeds grown in universities and private companies in various cities of these countries. Information about the samples analyzed in the study was shown in Table. Goat blood samples were collected by puncture of jugular vein into sterile tubes containing EDTA. Total genomic DNA was extracted from blood samples by standard salting-out extraction method [13]. The quality and quantity of extracted DNA was checked on 1% agarose gel electrophoresis and spectrophotometer at A_{260}/A_{280} nm, respectively.

2.2. Polymerase chain reaction and enzyme digestion

The primers used for amplification of the GNS gene including mutation site MPS IIID were reported by Leipprandt et al. [12] with the following forward (F) and reverse (R) nucleotide sequences; F: 5′-CTT ATG TGC CAA GTG CTC TC-3′ and MPS IIID- R: 5′-CCT CCA GAG TGT TGT TAA CC-3′. PCR amplifications were conducted in 25 µL with 1 µL of genomic DNA (100 ng/µL), 200 µM each dNTP, 0.10 µM of each primer and 1.25 U Taq DNA polymerase (Fermentas EP0401). Reactions were performed in a thermal cycler (BioRad MyCycler) with the following conditions: 94 °C for 7 min followed by 35 thermal cycles of 30 s at 94 °C, 30 s at 55 °C, 30 s at 72 °C and final extension at 72 °C for 10 min. After the reactions, PCR products were digested with *Alu*I (Fermentas® ER0011) at 37 °C for at least 2 h and were subjected to electrophoresis on 2% agarose gel stained with ethidium bromide at 100 V for approximately 1.5 h. Visualization of bands was carried out under ultraviolet transillumination (Kodak Gel Logic 200) and the size of the amplified fragments was compared with the 50 bp DNA ladder (Fermentas GeneRuler SM0371).

2.3. DNA sequencing

The PCR products were sequenced using a BigDye Terminator v3.1 cycle sequencing kit and an ABI PRISM 3130 automatic sequencer (Applied Biosystems, Foster City, USA). Amplification primers were used for sequencing, and each fragment was sequenced in both directions. Raw sequencing data were visualized in FinchTv Version 1.4.0 as chromatograms. Sequences were aligned by using the ClustalW method, a component of the program MEGA 6.0 [14] and then saved as a MEGA alignment file. DNA sequencing results of 80 samples were aligned to *C. hircus* GNS gene reference sequence (U17694.1).

Table. Sampling abbreviation and sample size (n) of goat breeds.

Breed	Abbreviation	n
Kyrgyzstan Angora	AGR	10
Kyrgyzstan Native	KRGY	15
Kyrgyzstan Cashmere	KSMR	10
Iraq Shami	SM-I	10
Iraq Hair	KIL-I	10
Cyprus Native	KBRY	15
Bosnia Native	BSNY	10
Total		**80**

3. Results and discussion

A 96-bp fragment containing the mutation site of GNS gene that causing MPS IIID genetic disorder was produced. These PCR products were subsequently digested with the restriction enzyme *Alu*I and separated on a 2% agarose gel electrophoresis. Since the mutation creates an *Alu*I recognition site, the affected animals' amplicons are cut to 66 bp. Normal animals contains no site for *Alu*I that will have only the 96-bp band, and carrier animals will have 96-bp, 66-bp, and 30-bp band. After digestion with *Alu*I, all samples showed a negative result for the nonsense mutation at nucleotide 322 (C→T) of GNS gene (Figure 1).

DNA sequencing results also indicated no mutation at this site. As a comparison with the reference sequence, it was determined that all the samples got same substitutions at nucleotide 354 a T changed to C (c.354T>C). This substitution is a silent mutation, which determined in the cDNA of all animals, changing a T to C in codon 112 of the 559 amino acid GNS coding sequence (Figure 2).

After the describing, a naturally occurring caprine MPS IIID genetic disorder in Nubian goats, the caprine GNS's cDNA was cloned and sequenced, and the mutation causing the defect was determined. Finally, the PCR-based test was described and subsequently the observed genotypic frequency for this defect was reported to be 25.2% carrier and 1.3% affected Nubian goats [15]. Unfortunately, there are very few studies to determine this genetic defect in goat breeds. A total of 39 purebred Anglo Nubian goats and 82 crossbred Anglo Nubian × Thai Native goats were tested by RFLP and then 5 DNA samples were sequenced by Wasiksiri et al. [16]. RFLP results in this study showed no mutation at nucleotide 322; however, sequenced data indicated polymorphism of base C at nucleotide 354. The researcher attributed the absence of the mutation causing the disorder to insufficient sample size and elimination of the affected goats. Gedik and Kavuncu [17] investigated the presence of MPS IIID genetic defect in Turkish native goat breeds (total 120 sample from 13 different breed) by

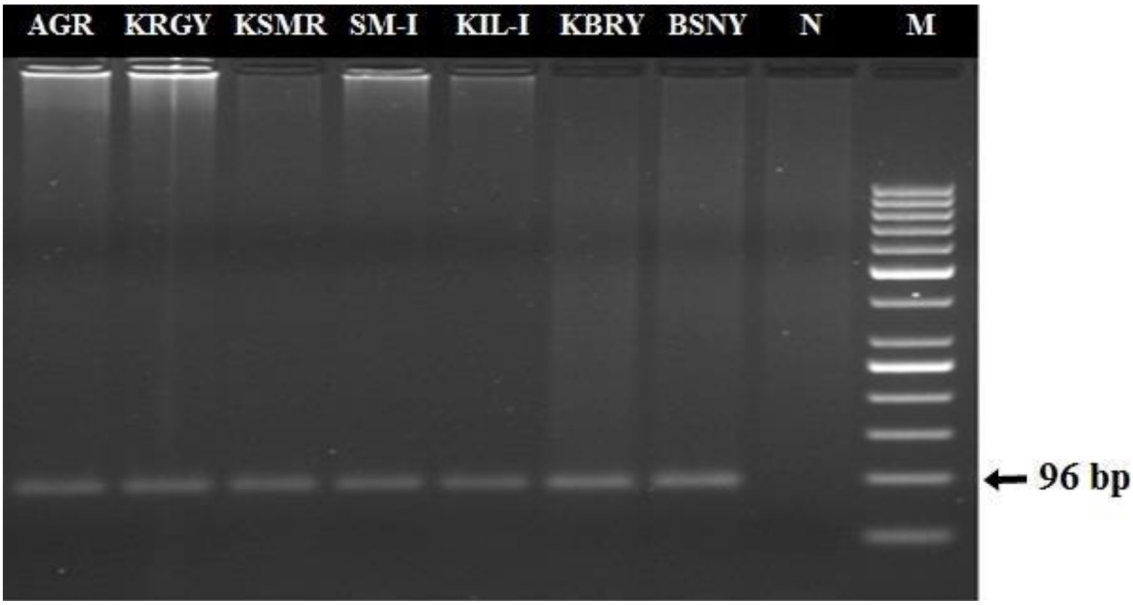

Figure 1. PCR-RFLP results of seven samples. N; negative control with no DNA, M; 50 bp DNA ladder. PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism, DNA: Deoxyribonucleic acid, bp: base pairs, AGR: Kyrgyzstan Angora, KRGY: Kyrgyzstan Native, KSMR: Kyrgyzstan Cashmere, SM-I: Iraq Shami, KIL-I: Iraq Hair, KBRY: Cyprus Native, BSNY: Bosnia Native.

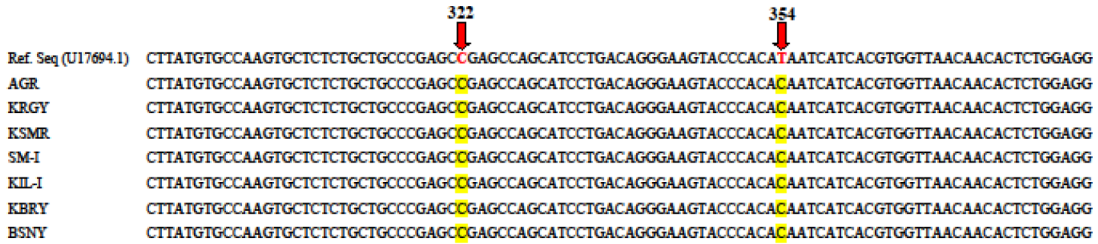

Figure 2. Alignment of goat GNS gene with reference sequence (Ref. Seq U17694.1). GNS: N-acetylglucosamine 6-sulfatase gene, AGR: Kyrgyzstan Angora, KRGY: Kyrgyzstan Native, KSMR: Kyrgyzstan Cashmere, SM-I: Iraq Shami, KIL-I: Iraq Hair, KBRY: Cyprus Native, BSNY: Bosnia Native, A: Adenine, C: Cytosine, G: Guanine, T: Thymine.

PCR-RFLP and DNA sequencing. The results showed that Turkish native goat breeds had no mutation that causes disorder. As a result of sequencing, all sample were found to be polymorphic at nucleotide 354. In this study, similar results were found as in the previous two studies. While the c.322 C>T mutation was not found, the c.354T>C silent mutation was found. The first domesticated farm animals were goats and they have been serving human in many aspects for about 11,000 years. They have socioeconomically high value and an important role in feeding humans in many parts of the world especially in the rural areas and developing countries. Because of their high adaptability to extreme conditions and different diets, they are always thought as very useful animals for their good productivity. While goats are very important and valuable animal, they are the least studied species amongst the ruminants. According to the OMIA (Online Mendelian Inheritance in Animals), the database of genes containing inherited disorders and traits, the number of recorded disorder or traits which key mutation known are only 14 in goats. These records for cattle (284) are nearly twenty times and for sheep (54) about four times higher than goats. MPS IIID is an inherited LSD first identified in human and then found in Nubian goats. In human medicine, Nubian goats have been used as a model to study human diseases. Although various tissues are affected, lysosomal accumulation of GAGs in the central nervous system is generally responsible for main clinical symptoms observed in human and goats. The main symptom in affected goats is failure to grow. Goats with this disorder are smaller than normal at birth and grow slowly. Some of them grow as normal for the first three months and

after that stopped growing. This growth retardation can be confused with numerous nutritional, infectious, and parasitic problems. Some affected goats lack muscle mass, appear slab-sided, sometimes with blocky heads and may have a compromised immune system. Sometimes they become deaf or blind. Although affected animals can grow up to breed, they often experience reproduction difficulties. While progressive neurological signs appear in adult goats, research should be conducted to determine whether there is any association with other diseases affecting the central nervous system, such as scrapie. The data that can be obtained as a result of research to be carried out for this purpose would be useful both for animal breeding and human health. Unfortunately, genetic disorders are minor concern in animal production, but increase in the frequency and amount of carrier animals of these alleles/defects might cause important economic losses. Testing the herd may help to prevent economic losses due to genetic disorders. On the other hand, the study to be done in this area will also provide useful information on genetic diversity and bring fresh insights goat domestication and their dispersal.

4. Conclusion

Goat that has long been considered the poor man's cow is probably the most well-adapted farm animal and distributed globally. Currently, there are over 1 billion goats around the world. Up to last ten years, goat gene variants determining phenotypes mostly have been identified by candidate gene approaches. With the start of goat genome project, more goat causative variants are expected to detect. New molecular technologies, genomic tools and databases help to gene identification and genomic analysis to find harness genetic variations. With sequencing of goat genome and systems approaches to genetic evolution animal welfare and production will develop in future. Caprine MPS IIID studies have mainly been performed to examine human diseases and goats are used as a model. Studies to identify this disease in goat breeds are very few and inadequate. This disease has been identified only in the Nubian goats until now and only has been studied in Turkish native goat breeds. This research is the first study to detect the GNS gene mutation in some goat breeds originated from different countries. Although there is no GNS mutation was found, silent mutation was found at nucleotide 354. As a recommendation, for detection of GNS mutation there is a need to carry out further studies using more sample size and other goat breeds. Moreover, further studies should be done to investigate whether the silent mutation we have found could be related to MPS IIID genetic disorder or other traits/diseases. It is hoped that this research will lead studies attempting to reveal genetic causes of MPS IIID and other genetic disorders in goats.

Conflict of interest

The authors declare that they have no conflict of interest.

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