



Detection of N-Acetylglucosamine-6-Sulfatase (GNS) Gene Mutation Causing MPS IIID Genetic Disorder in Turkey Native Goats

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ABSTRACT

Mucopolysaccharidosis IIID (MPSIIID) is an inherited lysosomal storage disease resulting from lack of the activity of the N-acetylglucosamine-6-sulfatase, which is one of the enzymes involved in the step-wise degradation of heparan sulfate. MPS IIID in goats has been described and characterized. Goats with this disorder demonstrate delayed motor development and growth retardation. Even if they reach sexual maturity, they will suddenly die from congestive heart failure. A molecular base for this disorder is a nonsense mutation at nucleotide 322 (C→T) results in the change of the arginine codon to a stop codon, which leads to stop enzyme function. However, this also creates a recognition site for *AluI* restriction enzyme for a genetic analysis. The aim of this study is to detect the presence of MPS IIID genetic disorder in Turkish native goat breeds. A total of 120 blood samples from 13 different native goat breeds were used as a material. Polymerase chain reaction (PCR) - Restriction fragment length polymorphism (RFLP) and DNA sequence data were utilized to identify the goat populations. The result of this study indicates nonsense mutation that causes MPS IIID genetic disorder is found in none of Turkish Native Goat breeds. However, a silent mutation was found at nucleotide 354 (T→C) when compared with reference sequence.

1. Introduction

The mucopolysaccharidoses (MPS) are lysosomal storage diseases characterized by inherited deficiencies of lysosomal enzymes catalyzing the stepwise degradation of glycosaminoglycans (GAGs) (Liour *et al* 2001, Neufeld and Muenzer 2001). Depending on the enzyme deficiency, the catabolism of GAGs may be blocked and results in an accumulation of GAGs or partially degraded GAGs in lysosomes of cells of various tissues, and an increase in their excretion in urine. Intralysosomal accumulation of GAGs eventually leads to cell, tissue, and organ dysfunction (Coutinho *et al* 2012). One group of these disease is known as a Mucopolysaccharidosis type III (MPS III) or Sanfilippo syndrome. MPS III is an autosomal recessive disorder including four subtypes (A-D) characterized by the inability to one of the four enzymes involved in lysosomal degradation of heparan sulfate (HS), a GAGs (Mok *et al* 2003). Animal models for this syndrome

including feline, canine, murine and caprine have been described (Thompson *et al* 1992).

Caprine MPS IIID is caused by a deficiency in N-acetylglucosamine 6-sulfatase (GNS) activity in lysosomes due to a single base mutation in the 5' coding sequence of this enzyme. The consequent lack of GNS activity in goats leads to the primary accumulation of uncatabolized HS in lysosomes and marked cytoplasmic vacuolation in the central nervous system and somatic tissues (Downs-Kelly *et al* 2000, Jones *et al* 2004). There is phenotypic variation in MPS IIID disease expression with mild and severe forms affected goats among which delayed motor development, growth retardation and early deaths are main symptoms (Smith and Sherman 2009).

Analysis of caprine GNS's cDNA cloning and sequencing was introduced for the first time by (Friderici *et al* 1995) based on the result determination of cDNA defect in caprine MPS IIID has been made in the subsequent research (Cavanagh *et al* 1995). Finally, the PCR-based test has been described to identify the disorder in goats (Leipprandt *et al* 1995). The molecular

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base for this disorder is a nonsense mutation, changing a C to T in codon 102 of the 559-amino acid GNS gene. This mutation also creates a recognition site for *AluI* restriction enzyme which make possible PCR-RFLP.

Up to now this genetic disorder is only identified in Nubian goats and their crosses. The aim of this study is to detect the presence of MPS IIID genetic disorder in Turkish native goat breeds.

2. Materials and Methods

Sample collection and DNA isolation

Blood samples were collected from different goat breeds reared in some universities and private farms in various cities in Turkey. Information about the samples used in the study is presented in Table 1.

Table 1
Sampling location and sample size (n) of Turkish native goat breeds

Breed	Abbreviation	Sampling location	n
Honamlı	HNM	Antalya	10
Kilis	KLS	Kilis, Hatay, Urfa	12
Norduz	NRD	Van	10
Gökçeada	GKC	Çanakkale	10
Malta	MLT	Edirne, Konya	10
Saanen	SNN	İzmir, Konya	10
Halep	HLP	Konya, Antep	10
Akkeçi	AKK	Ankara	5
Kıl	KIL	Ankara	6
Ankara	ANK	Konya	10
Gürcü	GRC	Ardahan	7
İspir	ISP	Rize	10
AbazaXKaçkar	AXK	Artvin	10
Total			120

Blood samples were collected by puncture of jugular vein into sterile tubes containing EDTA. The genomic DNA was extracted from 500 µl of whole blood samples using the standard salting-out method (Miller *et al.* 1988). The quality of DNA was checked on % 1 agarose gel electrophoresis and quantity by spectrophotometer at A_{260}/A_{280} nm.

Polymerase Chain Reaction and Enzyme Digestion

The primer used for amplification of the GNS gene including mutation site MPS IIID- F: 5'-CTT ATG TGC CAA GTG CTC TC-3' and MPS IIID- R: 5'-CCT CCA GAG TGT TGT TAA CC-3' are described by (Leipprandt *et al.* 1995). The PCR reaction was carried out 1 µl of genomic DNA, 200 µM each dNTP, 0.10 µM of forward and reverse primers and 1.25 U Taq DNA polymerase to make final volume 25 µl. PCR

conditions were 94 °C for 7 min followed by 35 thermal cycles of 30 sec at 94 °C, 30 sec at 55 °C, 30 sec at 72 °C and final extension at 72 °C for 10 min. PCR products were checked for right band size using 2% agarose gels. The amplicons produced were digested with *AluI* at 37 °C for at least 2 hours, and fragments were separated in a 2% agarose gel stained with ethidium bromide by electrophoresis.

DNA sequencing

The sequence PCR was done with the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, USA), using amplification primers. PCR fragments were sequenced using an ABI PRISM 3130 automatic sequencer (Applied Biosystems, Foster City, USA) in both directions. Raw sequencing data were visualized in "FinchTv Version 1.4.0" as chromatograms. Sequences were aligned by the ClustalW method, a component of the program MEGA 6.0 (Tamura *et al.* 2013) and saved as a MEGA alignment file. DNA sequencing results of 120 samples were aligned to *C. hircus* GNS gene reference sequence (U17694.1).

3. Results and Discussion

96 bp fragments of the GNS gene that contains the mutation site causing MPS IIID genetic disorder have been amplified by PCR. After digestion with *AluI*, all samples showed a negative result for the 322 (C-T) mutation of GNS on both heterozygous and homozygous recessive genotypes (Figure 1). DNA sequencing results also showed no mutation at this site.

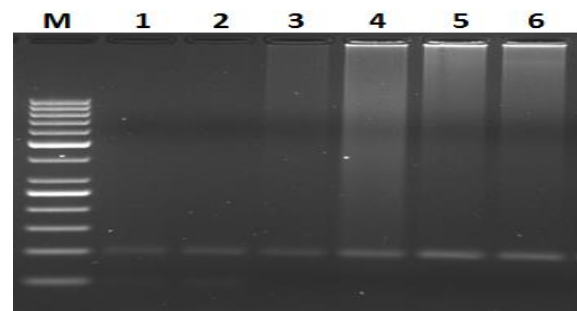


Figure 1
PCR-RFLP results of 6 samples. M; 50 bp DNA ladder.

As a comparison with the reference sequence, it was determined that all the samples were polymorphic at position nucleotide 354. This polymorphism has been determined to be a point mutation in the CAT (C-T) code responsible for the synthesis of the histidine amino acid, and that the new CAC codon resulting from this mutation is a silent mutation as it encodes histidine amino acid (Figure 2).

		322	354
U17694	CTTATGTGCCAAGTGTCTCTGCTGCCCGAGCGAGCCAGCATCTGACAGGGAAGTACCCACA		
HNM	CTTATGTGCCAAGTGTCTCTGCTGCCCGAGCGAGCCAGCATCTGACAGGGAAGTACCCACA		
KLS	CTTATGTGCCAAGTGTCTCTGCTGCCCGAGCGAGCCAGCATCTGACAGGGAAGTACCCACA		
NRD	CTTATGTGCCAAGTGTCTCTGCTGCCCGAGCGAGCCAGCATCTGACAGGGAAGTACCCACA		
GKC	CTTATGTGCCAAGTGTCTCTGCTGCCCGAGCGAGCCAGCATCTGACAGGGAAGTACCCACA		
MLT	CTTATGTGCCAAGTGTCTCTGCTGCCCGAGCGAGCCAGCATCTGACAGGGAAGTACCCACA		
SNN	CTTATGTGCCAAGTGTCTCTGCTGCCCGAGCGAGCCAGCATCTGACAGGGAAGTACCCACA		
HLP	CTTATGTGCCAAGTGTCTCTGCTGCCCGAGCGAGCCAGCATCTGACAGGGAAGTACCCACA		
AKK	CTTATGTGCCAAGTGTCTCTGCTGCCCGAGCGAGCCAGCATCTGACAGGGAAGTACCCACA		
KIL	CTTATGTGCCAAGTGTCTCTGCTGCCCGAGCGAGCCAGCATCTGACAGGGAAGTACCCACA		
ANK	CTTATGTGCCAAGTGTCTCTGCTGCCCGAGCGAGCCAGCATCTGACAGGGAAGTACCCACA		
GRC	CTTATGTGCCAAGTGTCTCTGCTGCCCGAGCGAGCCAGCATCTGACAGGGAAGTACCCACA		
ISP	CTTATGTGCCAAGTGTCTCTGCTGCCCGAGCGAGCCAGCATCTGACAGGGAAGTACCCACA		
AXK	CTTATGTGCCAAGTGTCTCTGCTGCCCGAGCGAGCCAGCATCTGACAGGGAAGTACCCACA		

Figure 2

DNA sequencing alignment of Turkish native goats breed with reference sequence.

MPS IIID genetic disorder in goat was identified in 1992 by Thompson and his colleagues (Thompson *et al.* 1992). The genotyping frequency for this disorder has been reported to be 25.2 % carrier and 1.3 % affected Nubian goats in USA (Hoard *et al.* 1998). Since then there are only few studies on the topic in the world. (Wasiksiri *et al.* 2013) did not find the GNS gene mutation in Thailand's pure Anglo-Nubian goats and their crosses. There might be different reasons not to find the mutation that causes MPS IIID genetic disorder other than its real absence. (Wasiksiri *et al.* 2013) outlined some reasons for failure to detect the mutation in Nubian goat and their crosses of Thailand, such as insufficient sample size and elimination of the animals affected before getting the blood sample. Similar possibilities might be true for Turkish native and other goat breeds. They also did DNA sequence analysis on only 5 randomly selected samples. DNA sequence analysis is consistent with our results, the same polymorphism was also found in this study. Moreover, there is still no ample information about newly identified silent mutation and MPS IIID genetic disorder. This invites detailed studies on Turkish native and other goat breeds.

Goats, important domestic animals in many parts of the world, have served human for ages. These hardy ruminants can exist in harsh environments in which other livestock species would perish. Goats grow and reproduce under extreme conditions from rugged mountain areas where winters are bitter cold to desert regions where it is hot and dry, water and forage are limited.

Although goat is very important and valuable animal for human in many aspects, it is the least studied species among 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 is only 10 in goat whereas 135 in cattle and 48 in sheep. There should be more studies on identification of the gene variant responsible for defects /disorders, breed and production traits in goats.

Although its presence in various tissues has adverse effect, lysosomal accumulation of GAGs in the central nervous system is mostly responsible for its main symptoms. Because of the appearance of progressive neurological signs in adult goats in MPS IIID disease, researches should be conducted to determine whether there is any association with other diseases affecting the central nervous system, such as Scrapie. The information that can be obtained as a result of researches to be carried out for this purpose will be important both for animal breeding and human health. Furthermore affected animals showing poor growth and decreased muscle mass should be investigated in relation to other traits/diseases. On the other hand the work 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

Studies on caprine MPS IIID mostly have been made for investigation of human diseases and goats are used as a model. Number of studies to determine this disease in goat breeds are very few and insufficient. This disease has been identified only in the Nubian goats until now and has not been studied in other goat breeds.

This study is the first report to detect the GNS gene mutation in Turkish native goat breeds. 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 that are going to be conducted to reveal genetic causes of MP-IIID and other genetic disorders in goats.

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