



Detecting the Causal Mutation for Mucopolysaccharidosis IIID in a Nubian Goat Herd in Mexico

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ABSTRACT

Background: Mucopolysaccharidosis type IIID is caused by a deficiency of N-acetylglucosamine-6-sulfatase enzyme, which increases heparan sulfate accumulation in lysosomes and generate cytoplasmic vacuolation in central nervous system and somatic tissues. Goats kids with this disorder exhibit delayed motor development, growth retardation and sudden death. This is a transition mutation of cytosine to thymine at nucleotide 322 of the genetic sequence. The objective of this study was to determine the allelic frequency of the glucosamine N-acetyl-6-sulfatase gene (GNS) that causes mucopolysaccharidosis type IIID (MPS IIID) in a population of Nubian goats in Colima, Mexico.

Methods: Genomic DNA was isolated from blood samples of 158 Mexican Nubian goats. The 96 bp fragment of GNS gene including the nonsense mutation was PCR amplified. The PCR amplicon was subjected to RFLP using *AluI* restriction enzyme to identify polymorphism at nucleotide position 322 (C>T). Genotypes were scored. Using Hardy-Weinberg equilibrium (HWE) testing we determined the population to be in genetic equilibrium.

Result: Of the entire Nubian goat population genotyped, eight of which were MPS IIID carriers (1 male and 7 females). Observed allelic frequencies were 0.975 for the wild-type allele C and 0.025 for the mutant allele T. MPS IIID was first found in goats at the end of the last century, with considerable higher frequencies, but recent advances in diagnosis and marker-assisted selection against genetic diseases appear to have lowered MPS IIID mutation frequencies in North American Nubian goats. We identified for the first time the variant causing MPS IIID in Nubian goats in Mexico.

Key words: Caprine, Lysosomal storage disease, PCR-RFLP.

INTRODUCTION

Mucopolysaccharidosis type IIID (MPS IIID) is a lysosomal storage disease caused by a defect in the lysosomal enzyme N-acetylglucosamine 6-sulfatase (G6S/GNS) involved in glycosaminoglycan (GAG) degradation. Deficiency in this enzyme leads to GAG accumulation in the lysosomes of most tissues. It is a progressive multisystem disease (Thompson *et al.*, 1992; Cortizo, 2021). Accumulation of non-catabolized heparan sulfate glycosaminoglycan triggers a marked increase in cytoplasmic vacuolization within the central nervous system and somatic tissues (Jones *et al.*, 1998; Downs-Kelly *et al.*, 2000). Presence of this disease in goats varies. Primary symptoms include delayed motor development, delayed growth and possible death from sudden congestive cardiac insufficiency shortly after sexual maturity (Friderici *et al.*, 1995). In Nubian goats with this disease the mutation occurs within the GNS genetic sequence; it is a point mutation cytosine to thymine transition in nucleotide 322 of the genetic sequence, corresponding to codon 102 of the G6S enzyme of 559 amino acids, switching the decoding from an arginine to a stop codon (R322X) and resulting in a nonsense mutation producing a truncated non-functional protein (Cavanagh *et al.*, 1995; Friderici *et al.*, 1995).

Nubian goats are often found in commercial herds and have spread across the world for production of meat

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and milk (Agossou and Koluman, 2017), it could be because they have the highest fat and protein content in milk (Šlyžienė *et al.*, 2020), it also produces more lactose and dry non-fat milk solids per liter of milk than other breeds of dairy goats (Zazharska *et al.*, 2018). As a dual milk/meat type, it has considerable genetic diversity in various

genomic regions related to these traits (Cheng-Li *et al.*, 2023). Mexico is no exception. Nubian goats are valued for their rustic nature that works well in small-scale rural production in dry areas of Western Mexico. Despite the breed's extensive presence, there are very few studies on the incidence of caprine mucopolysaccharidosis IIID (Hoard *et al.*, 1998; Clavijo *et al.*, 2010; Wasiksiri *et al.*, 2013; Gedik y Kavuncu, 2017; Gedik y Kavuncu, 2021). In Mexico, information on hereditary goat diseases is scarce and it would be of great value to document MPS IIID allelic frequency in Nubian goat populations to understand the disease's actual incidence. There are no studies on disease frequency in either Mexico or Latin America. It would also be valuable to be able to test for MPS IIID to identify reproducing carriers, helping reduce mortality in young goats that might have been erroneously attributed to infectious or opportunistic diseases, due to lack of knowledge or lack of convenient identification of MPS IIID within a herd. The objective of this study was to determine allelic frequency of the GNS gene that causes mucopolysaccharidosis IIID (MPS IIID) in a Nubian goat herd in Mexico.

MATERIALS AND METHODS

Bioethics statement

This project was conducted in accordance with ethical standards for work with experimental animals and is in compliance with internal Bioethics Regulations (CC/NN11-12/00/2012).

Animals and sample collection

We collected blood samples of a herd (coordinates 19.11814 -130.79194) of 158 Nubian goats from the municipality of Colima, state of Colima, Mexico. After cleaning and disinfection of the sampling area, we punctured the jugular vein and collected the blood samples in vacuum tubes with EDTA as anticoagulant, then labeled the tubes with each animal's registration number and transported the samples to the laboratory under refrigeration (4-6°C).

Genotyping

The experimentation was carried out between the months of February and November 2023 at the Institute of Animal Biotechnology of the University of Guadalajara. We extracted DNA from whole blood using the Quick-DNA™ 96 Kit (Cat. D3010, Zymo Research, Irvine, California, USA). We amplified the GNS gene with the following primer sequence: Direct F: 5'-CTTATG TGC CAA GTG CTC TC-3' and Inverse R: 5'-CCT CCA GAG TGT TGT TAA CC-3', according to the methodology of Leipprandt *et al.* (1995) and commercially synthesized by Invitrogen, Carlsbad, California, USA. We carried out polymerase chain reaction (PCR) amplification in volumes of 20 µL in a 2720 Endpoint Thermal Cycler of 96 wells (Applied Biosystems, Foster City, California, USA). The reaction mixtures contained 50 ng genomic DNA, 1.5 mM MgCl₂, 0.1 µM of each primer, 0.4 mM dNTP and 2.4 U

of DreamTaq DNA polymerase (Thermo Fisher Scientific Inc., Glen Burnie, Maryland, USA). Our endpoint PCR procedure included an initial denaturation step of 95°C for 5 min, followed by 35 cycles of three steps: 95°C for 30 seconds, 53°C for 30 seconds and 72°C for 30 seconds and a final step of 72°C for 10 minutes.

We used *AluI* restriction endonuclease (Cat. ER0011, Fermentas, Burlington, Ontario, Canada, 2.5 U at 37°C for 16 hours) to digest the amplicons for RFLP analysis. We analyzed the digested PCR products using 4% agarose gel electrophoresis and dyed them with ethidium bromide tincture. We visualized DNA bands with ultraviolet light and sized the amplified fragments with a 50 bp DNA ladder (Cat. SM0371, Fermentas Gene Ruler, Burlington, Ontario, Canada). PCR generated a 96 bp amplicon containing the nonsense mutation in nucleotide 322 (C→T) of the GNS gene, responsible for the genetic disease MPS IIID. Since the mutation creates a recognition site for *AluI*, the enzymatically digested amplicons of carrier goats are cut at 66 bp and 30 bp. Animals homozygous for wild-type allele CC that have no recognition site for *AluI* would only have a band at 96 bp and carrier animals heterozygous for CT would have bands at 96 bp, 66 bp and 30 bp. We generated a genotype database using microsoft excel to calculate allelic frequencies and tested hardy-weinberg equilibrium (HWE) using Cervus 3.0.7 software package (Kalinowski *et al.*, 2007).

RESULTS AND DISCUSSION

PCR-RFLP genotyping

We genotyped a total of 158 goat samples, eight of which (1 male and seven females) were identified as heterozygous carriers and we confirmed their polymorphisms by the number and size of amplicons digested by the *AluI* restriction enzyme and visualized by gel electrophoresis (Fig 1).

Allelic frequencies and hardy-weinberg equilibrium

Observed allelic frequencies were 0.9747 for wild-type allele C and 0.0253 for the mutant allele T that causes MPS IIID. Observed frequencies for the Nubian goat herd were CC (150), CT (8) and TT (0). Expected frequencies based on Hardy-Weinberg equilibrium (HWE) were CC (150.1013), CT (7.7975) and TT (0.1013). Chi-square test results ($\chi^2 = 0.1066$) indicate that the differences between observed and expected genotype frequencies were not significant ($P < 0.05$), thus the population is considered in Hardy-weinberg equilibrium. The fact that allelic frequencies remained constant across generations may indicate that GNS mutation frequencies in this herd has been constant for some time, without fluctuation from phenomena such as selection or gene flow.

There are few studies on the frequency of the GNS genetic mutation responsible for MPS IIID in Nubian goats, possibly because the primary objective of these prior studies was not the identification, selection or genetic improvement

in goats, but rather the use of goats as an animal model for the human disease known as Sanfilippo syndrome, to evaluate possible treatment therapies (Downs-Kelly *et al.*, 2000; Haskins *et al.*, 2002). Given also the low level of scientific production in goats, in contrast with other domestic species of higher value or economic interest, it's difficult to find an up-to-date snapshot of this disease and its frequency in the Nubian breed (Gedik and Kavuncu, 2017; Gedik and Kavuncu, 2021). Likewise, the few relevant veterinary publications noted the absence of the mutation causing MPS IIID in the goat populations being studied and this could be due to several factors and circumstances, both in the animals themselves and in the study methodologies. In studies that genotyped Nubian goats it's possible that reproducers were free of the mutation and did not belong to the affected lineages, or were predominantly selected for milk production, or the mutation never entered the population, or even that the mutation existed but the affected homozygous animals never reached reproductive age or were intentionally removed by selective breeding (Wasiksiri *et al.*, 2013). As for study methodologies, by selecting as study samples breeds other than Nubian, the possibility of finding the MPS IIID mutation drops considerably unless the breed has some Nubian ancestry, since up to now the mutation has never been identified in other breeds. For example, Gedik and Kavuncu (2017) sampled 120 goats of 13 native Turkish breeds and they either found no presence of MPS IIID mutations. In a subsequent study they selected 80 goats of 7 breeds from 4 different countries, similar results were found to the previous study, the MPS IIID mutation was not found (Gedik and Kavuncu, 2021). We chose Nubian goats and

we found eight carriers of the MPS IIID mutation out of 158 genotyped goats. We estimated the allelic frequency for mutation variant T as 0.025, a lower frequency compared to prior literature as described by Clavijo *et al.* (2010) where 691 animals were genotyped, 165 identified as heterozygous (0.24) and 13 as homozygous for the mutation (0.02) and also earlier studies such as Hoard *et al.* (1998) where 25.2% or 139 of 552 Nubian goats analyzed were heterozygous and 1.3% or 7 of them were homozygous for the MPS IIID mutation. In the present study eight goats were identified as heterozygotes, one is male and seven are female, while Hoard *et al.* (1998) found seven animals homozygous for the MPS IIID mutation, all females. The difference in the number of carriers or mutant homozygotes by sex could be due to productive reasons; normally the number of males is lower than the number of females in the herd if it has predominantly dairy production. In that study, the number of males sampled was one for every ten females, so the possibility of identifying a male with the mutation is lower. Furthermore, it is suggested that females identified with the mutation appear to be less affected than the first goats reported with caprine MPS IIID.

Thanks to ongoing advances in genomics and molecular diagnostics and of selection assisted by molecular markers, the frequency of hereditary diseases caused by harmful variants has tended to go down with time (Andersson, 2001). However, scientific and technological progress is not the homogenous across the world and in less technologically advanced countries there is a real risk of persistence of recessive mutations, which is why it's important for breeders and veterinarians to integrate DNA

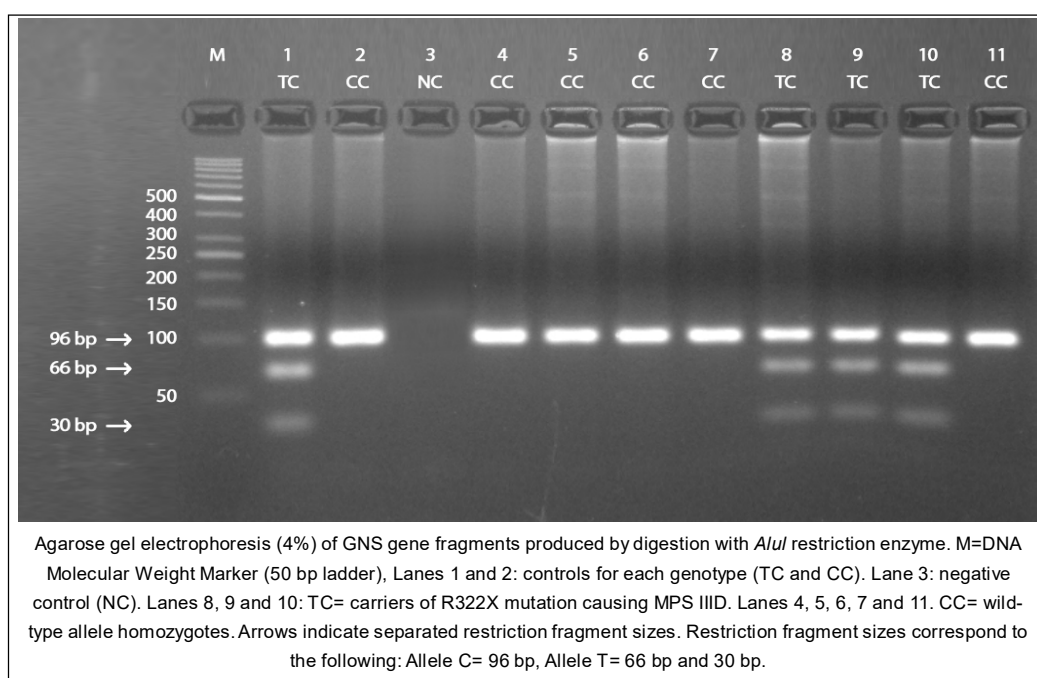


Fig 1: Allelic discrimination of GNS gene.

test results into their selection strategies, to successfully reduce the frequency of mutations that cause hereditary diseases like MPS IIID.

CONCLUSION

We identified the variant that causes the genetic disease mucopolysaccharidosis type IIID in eight goats of the Nubian breed in Mexico, confirming for the first time the presence of the mutation in the country.

Conflict of interest

The authors, declare that there are no conflicts of interest to announce.

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