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Identification of Complex Vertebral Malformation Carriers in Holstein and Guilan Native Cow Breeds in Iran Using SSCP Markers

Research Article

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ABSTRACT

Complex vertebral malformation (CVM) is an autosomal recessive hereditary disorder caused by a point mutation in position 559 in exon 4 of the SLC35A3 gene on chromosome 3 in Holstein dairy cattle. This mutation changes the function of uridine 5-diphosphate-N-acetylglucosamine transporter protein by the substitution of valine for phenylalanine at position 180 of this protein. The disease causes premature birth, aborted fetuses and stillborn calves. Latent recessive genes in heterozygous individuals can be identified with high accuracy and repeatability using PCR-SSCP technique. In the present study, blood samples from two different cow populations, including 100 Holstein cows and 100 Guilan native cattle were randomly collected. Specific primers were used to amplify the 177-bp fragment of exon 4 of the SLC35A3 gene. No heterozygous genotype was detected in the studied samples. The Lack of carriers could be a consequence of the selection against the defective gene and preventative programs for entering mutant genes into the populations or very low frequency of this gene in these populations. However, there is a risk for increased genetic defects prevalence and it is necessary to develop screening programs to identify the defective gene.

KEY WORDS cattle, complex vertebral malformation, PCR-SSCP, SLC35A3.

INTRODUCTION

The complex vertebral malformation syndrome (CVM) is a lethal autosomal recessively inherited disorder in Holstein breed. The CVM syndrome was identified at first in a Danish Holstein stock in 1999 (Agerholm *et al*. 2001), and during the following years it was also found in the United States, United Kingdom, Netherlands, and Japan (Nagahata *et al*. 2002). The vast international use of Carlin-M Ivanhoe Bell and the large number of animals descending from him, induced the spread of the CVM gene in Holstein cattle all over the world. By the turn of the 21st century, more than 30 percent of the best Holstein sires in both Denmark and Japan were CVM carriers (Rusc *et al*. 2007) (Table 1).

This syndrome has increased the frequency of abortion, premature birth and stillborn calves (Agerholm *et al*. 2004b; Nielsen *et al*. 2003). The major morphological changes in CVM-affected calves delivered after gestation day 260 consist of growth retardation, vertebral malformation, and bilateral symmetrical arthrogryphosis affecting the carpal and the metacarpophalangeal joints. Additional arthrogryposis of the posterior distal joints is present in some cases (Revell 2001; Agerholm *et al*. 2004a). The SLC35A3 gene codes an uridinediphosphate- *N*-acetylglucosamine transporter (Thomsen *et al*. 2006).

The molecular cause of CVM syndrome is a single- base transversion of guanine to thymine at nucleotide position 559 in exon 4 of SLC35A3 gene on chromosome 3, which

changes the amino acid sequence from a valine to a phenylalanine at position 180 of uridine 5'-diphosphate-N-acetyl-glucosamine transporter protein (Thomsen *et al.* 2006).

Table 1 Numbers and frequency of CVM carriers in Holstein bulls of some countries (from Rusc *et al.* 2007)

Country	Bulls tested	Carriers Bulls	
	No.	No.	%
USA	11868	2108	17.76
Germany	957	126	13.2
Sweden	228	52	23
Japan	40	13	32
Denmark	No data	No data	31

The frequency rate of recessive alleles due to lacking the application of molecular methods to identify carriers before 2002 has not been well recognized. In a study conducted on 605 Polish Holstein cows, 150 carriers of CVM disease were identified (Rusc *et al.* 2007). According to the harmful effects caused by the high frequency of this gene in cattle and its transmission to next generations, it is necessary to identify carriers of the disease in cow populations in Iran. The aim of this study was to assess the genetic structure of populations and calculate genotype frequency of G / G and G / T as well as identification of allele frequency of CVM in Holstein breed in Guilan province and Guilan native cattle.

MATERIALS AND METHODS

The blood samples of two populations including 100 Holstein cows from Sepidruod Livestock Company in Guilan province and 100 Guilan native cows and bulls from Fuman native breeding station in Guilan province were collected randomly and individually. DNA was extracted by salting out method (Javanrouh *et al.* 2006). Sequence information of SLC35A3 gene was available in the NCBI Gene Database (Thomsen *et al.* 2006) and position of single-base mutations responsible for CVM disease in the cattle with a record number of HU0302661 from Institute of Agricultural Sciences in Tjele, Denmark was prepared. Single strand conformation polymorphism (SSCP) marker was used to identify mutations in the 177 bp fragment correspond to the SLC35A3 gene. This method is defined as conformational difference of single-stranded nucleotide sequences of identical length as induced by differences in the sequences under certain experimental conditions. Alteration even in a single base can also cause changes in mobility of strand (Orita *et al.* 1989). In order to the SLC35A3 gene amplification, forward and reverse primers were used as indicated by Rusc *et al.* (2007). The sequences of the primers were as follow:

F: TCA GTG GCC CTC AGA TTC TC
R: CCA AGT TGA ATG TTT CTT ATC CA

Polymerase chain reaction (PCR) was performed in a final volume of 25 µL containing 50 ng genomic DNA, 5 µM each of the primers, 1X PCR buffer, 1.5 mM $MgCl_2$, 250 µM dNTPs and 1U Taq polymerase. The mixture was incubated at 95 ˚C for 5 min followed by 35 cycles of 94 ˚C for 30 sec, 58 ˚C for 30 sec, and 72 ˚C for 30 sec, with a final extension at 72 ˚C for 7 min.

Five microliters of PCR products and 4 µL of denaturing solution (50 mM NaOH, 1 mM EDTA) and 1 microliter of loading buffer (30% glycol, 0.25% bromophenol blue, 0.25% xylene cyanol) were mixed and denatured at 95 ˚C for 10 min, followed by a rapid chilling in ice for 10 min, then electrophoresed on 12% Polyacrylamide gel with a voltage of 320 V for 3 hours at 4 ˚C and detected by silver staining. By PCR amplification with the pair of primers, a specific 177-bp fragment of *SLC35A3* was obtained. With single-stranded conformational polymorphism (SSCP) analysis, 2 types of band patterns were expected corresponding to 2 kinds of genotype, named AA with 2 bands as healthy phenotypes and AB with 3 bands as carriers of recessive abnormalities.

RESULTS AND DISCUSSION

The extracted DNA from samples had high quality. Electrophoresis of amplified products from the SLC35A3 locus on 1.5% agarose gel demonstrated that the related 177 bp fragment has been well amplified without non-specific products.

This could be approved using size marker besides electrophoresed samples and was in agreement with the result obtained by Rusc *et al.* (2007). Binding patterns from conformation of single strand DNA (SSCP) on polyacrylamide gel showed no polymorphism in this gene. Only one genotype i.e. dominant homozygous was observed and no heterozygous genotype for the disease carriers was identified in the two populations studied (Figure 1). In the last few years, allele specific PCR (AS-PCR) and PCR-primer introduced restriction analysis (PCR-PIRA) techniques used to identify mutations in specific genes were costly and time consuming. In this study SSCP method was used due to high efficiency and detection of carriers with less cost and time.

Results obtained in the studied populations (100 Holstein cows and 100 Guilan native cows and bulls) showed no mutation in the SLC35A3 gene. The mutant defective genes of this disease were distributed throughout the world by sperm-producing companies due to lack of modern methods of detection and prevention.

Nagahata *et al*. (2002) studied 40 Japanese bulls using different methods such as analyzing autopsy and radiographic symptoms to identify phenotypic signs of calves affected by CVM. They identified 13 bulls with heterozygous genotype by using DNA polymerase chainreaction (PCR) analysis.

Meydan *et al*. (2010) identified 12 carriers of CVM among the 350 Holstein-Friesian cows in Turkey. In addition, a study was carried out in India on 52 cows of Caran Ferris population (from the Tharparkar native and Holstein Friesian crosses) in which 12 cows were identified as carriers of the disease (Mehdipour *et al*. 2010).

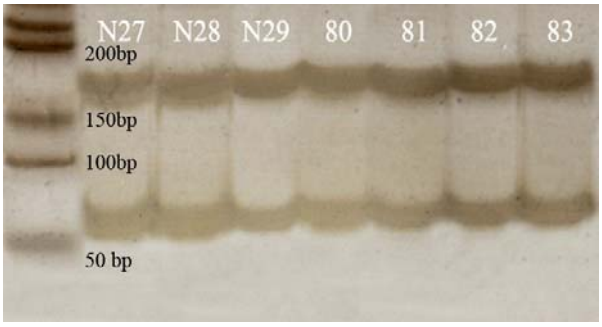

Figure 1 An example of banding patterns in SSCP method. Columns N27 to N29 are Guilan native cows and columns 80 to 83 are related to the Holstein breed

In the current research no carriers of the mutant alleles of CVM disease was detected in Holstein cattle from the Sepidruod farm in Guilan province. This result might be explained at least in apart absence of semen carrying this disease in this farm or unsuccessful insemination with sperm from carrier bulls.

However, because of the limited number of animals studied and the very low rate of the disease frequency as reported in other studies, it cannot be concluded that no recessive alleles are present in Holstein breed in Guilan and native populations.

As a result of genetic trend in native cattle and breeding programs to improve production performances, Holstein genes are being transferred to these cows. Regarding the fact that most genetic diseases mentioned are Holstein specific diseases, selection of imported bulls or semen must be examined to identify carriers of genetic diseases and prevent disease distribution.

CONCLUSION

The study demonstrates that carriers of CVM were not detected in the Holstein cows and Guilan native population. This is the first report on CVM in Holstein cows and Guilan native cattle reared in Guilan Province in north of Iran. The study demonstrates a need for further examination of more cattle in Iran, preferably by testing the breeding sires to avoid unrecognized spread of genetic disorders.

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