Indian Journal of Animal Sciences **81** (9): 951–953, September 2011

Genetic polymorphism of SLC35A3 gene in Karan Fries bull calves

YATHISH H M[1], ASHWANI SHARMA[2], VIJAY KUMAR[3], ASIT JAIN[4], D CHAKRABORTY[5], AVTAR SINGH[6] and M S TANTIA[7]

National Dairy Research Institute, Karnal, Haryana 132 001 India

Received: 10 September 2010; Accepted: 30 April 2011

ABSTRACT

Solute carrier family 35 member 3 (SLC35A3) gene encodes the nucleotide sugar transporter that plays a pivotal role in the development of skeletal system. Point mutation from guanine (G) to thymine (T) at nucleotide position 559 of bovine SLC35A3 gene causes an inherited autosomal recessive disorder called complex-vertebral-malformation (CVM). In the present study 55 young Karan Fries bull calves born during the period of 2006–09, maintained at NDRI, Karnal were screened for the presence of CVM affected or carrier animals. PCR-RFLP band pattern using Pst I restriction enzyme showed the products of 287 bp in size, which confirmed the animals we screened are neither carrier nor affected. This work is important in suggesting our country to make the screening of HF crossbreds for various recessive genetic disorders as mandatory to contain their spread, eventually to make the animal husbandry an economical endeavour.

Key words: CVM, Karan Fries, PCR-RFLP, SLC35A3 gene

Vitiation in the functioning of SLC35A3 leads to the abnormalities of axial skeletal system. Substitution of guanine (G) by thymine (T) at position 559 in SLC35A3 gene is the molecular basis of CVM, which is a recessively inherited lethal disorder of Holstein Friesion' and its crossbreds. This transversion in turn causes the substitution of valine (V) by phenylalanine (PL) at position 180 (Thomsen *et al.* 2006). This defect is characterized by shortening of the cervical and thoracic parts of the vertebral column due to synostosis, symmetrical arthrogryposis in the front and occasionally in the hind legs, multiple hemivertebrae, scoliosis, protrusion of the tongue etc (Agerholm *et al.* 2001, Duncan *et al.* 2001, Revell 2001).

Extensive use of HF animals during the last 5 to 6 decades in crossbreeding programs to enhance the productivity of native animals in India has spread the CVM enormously. This calls the mandatory screening of HF and its crossbreds for genetic disease CVM. In India, crossbreeding is practiced on a large scale, the information relating to the genetic disorders which mainly concern HF cattle and its crosses become essential. At present, the information on occurrence of genetic disorders in Karan-Fries (KF) is very meager. Keeping this in view, our investigation was carried out to identify the single nucleotide polymorphism (SNPs) of SLC35A3in young KF bull calves, which causes mis. sense mutation i. e. CVM.

MATERIALS AND METHODS

All experimental procedures were approved by Institute's Animal Ethical Committee. Blood samples (10 ml) were collected from 55 KF young bull calves maintained at the institute (NDRI) using EDTA coated vacutainers. Genomic DNA was extracted from blood using OMEGA Midiprep Kit as manufacturer instruction. The quality and purity of isolated DNA was examined by 0.6% agarose gel electrophoresis and UV spectrophotometer, respectively.

Polymerase chain reaction-primer introduced restriction analysis (PCR-PIRA) is one of the most widely used techniques in SNP detection (Haliassos and Chomel 1989, Jacobson and Moskovits 1991). To create artificial restriction fragment length polymorphism (RFLP), 1 or 2 mismatch is usually introduced near the 3' end of the primer that is close to the mutation of interest. In the present study the mutation is near the end where forward primer is binding. In wild-type allele there is no any restriction site for any restriction

Present address:[1]Ph D Scholar (e mail: yathish.vety @gmail.com), Division of Animal Genetics, Indian Veterinary Research Institute, Izatnagar, Bareilly 243 122 India.

[2]Principal Scientist (e mail: ashwani.sharma@yahoo.co.in), [3,4] Ph D Scholar (e mail: jaisvet@gmail.com), [6]Principal Scientist (e mail: avtar54@gmail.com), Dairy Cattle Breeding Division.

[4]Assistant Professor (e mail: v.dr.dibyendu@gmail.com) Division of Animal Genetics and Breeding, Faculty and Veterinary and Animal Husbandry, SKUAST-J, R S Pura, Jammu 181 102

[5]Principal Scientist (e mail: tantiams@gmail.com), NBAGR, Karnal 132 001.

endonuclease around the point of mutation. So to make R E site of Pst I two nucleotides near 3' end of forward primer nucleotides corresponding to g and t bases of SLC35A3 gene fourth and fifth position toward 5' end are changed to nucleotides having T and C bases respectively. Pst I is a hexacutter and five nucleotides of R E site (CTGCA) are directly coming from 3' end of forward primer while sixth one (G) is synthesized. Now PCR product obtained, using genomic DNA having wild-type allele will show R E site for Pst I restriction endonuclease. So there will be a cut in PCR product from wild -type allele after restriction digestion. In case of CVM allele the 5 nucleotides of R E site come from forward primer but this time 6th one is T instead of G so Pst I restriction site destroyed. In such cases, there is no cut in PCR products after restriction digestion.

Polymerase chain reaction (PCR) was performed using primers consisting of 21 bases each (5'- CAC AAT TTG TAG GTC TCA CTG CA -3' and 5'- CGA TGA AAA AGG AAC CAA AAG GG -3') as propounded by Kanae *et al.* (2005). Targeted region of size 287bp in SLC35A3 gene was amplified. PCR was carried out in a total volume of 15 µl containing 7.4 µl milli Q water, 3 µl of 1X PCR buffer, 0.2 µl of 200 µM dNTPs, 1.0 µl of each primer (5 pmol/ µl), 0.2 µl of Taq polymerase (5U/µl), 1.2 l magnesium chloride (2.0 mM) and1.0 l of the template DNA was directly added into the cocktail in each lane of the PCR plate.

The PCR was carried out in bio-rad thermocycler. The PCR protocol involved an initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation (94°C for 45 sec), annealing (56°C for 45 sec) and extension (72°C for 60 sec) proceeded by 1 cycle of final extension (72°C for 10 min).

Amplification of the targeted region in SLC35A3 gene was validated through 0.6% agarose gel electrophoresis. 8.0 µl of the PCR product was subjected to restriction/ digestion with 4 units of Pst I restriction enzyme in incubator for 8 h at 37°C. The digested products were evaluated in 2% agarose gel electrophoresis and ethidium bromide staining, and were classified as CVM-free (homozygous for the normal allele), CVM-carrier (heterozygous) and CVM-affected (homozygous, V180P mutation) respectively.

PCR products were sequenced directly by dye terminator cycle sequence procedure on an ABI PRISM DNA sequencer. The gene frequency of the normal animals, carriers and affected for BLAD were calculated based on the Hardy-Weinberg principle.

RESULTS AND DISCUSSION

The size of PCR product was 287 bp and it was subjected to RFLP analysis using Pst I restriction enzyme. In normal calves, size of the PCR product yielded 2 fragments of size 264 bp and 23 bp (Fig.1), whereas, in carrier and affected calves (recessive homozygous), 3 fragments of size 287 bp, 264 bp, 23 bp and only 1 fragment of 287bp respectively.

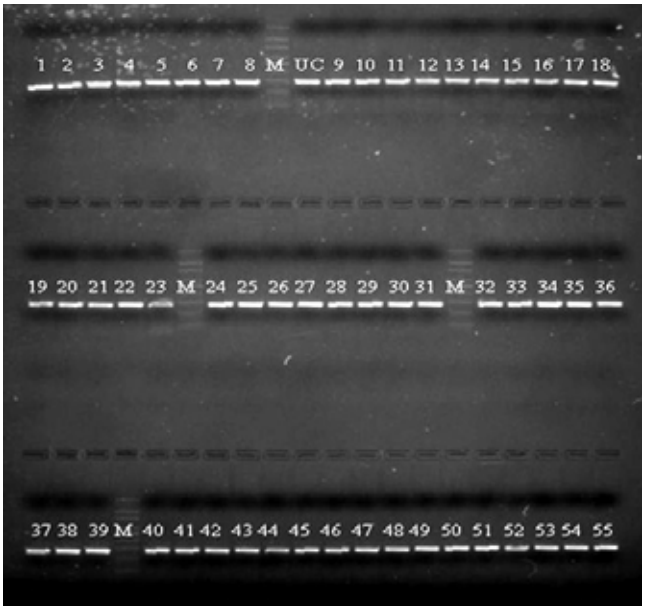

Fig. 1. *Pst I* digestion of PCR product on 2% agarose gel. Lane UC: Un-cut PCR product; lane M, 100 bp marker; lines 1-55, homozygous dominant genotypes.

PCR products of size 287 bp were used for DNA sequencing to insure the point mutation (G to T) in affected and carrier animals.

In our investigation, out of 55 bull calves screened, neither of the animals nor the carrier were found affected. So the estimated frequencies of CVM dominant and recessive alleles in the KF young bull calves population that we screened were 1 and 0 respectively. Development of artificial insemination enabled the advent of modern breeding practices in the universe. These practices involve importation of HF bulls or their semen, intense selection of bulls based on their daughters lactation performance and the widespread use of these few genetically superior bulls. During the last 3 decades, these practices have augmented the milk production potential of HF cows, its crossbreds and also the within breed genetic relationship. This increased relatedness is conducive for the expression of recessively inherited genetic diseases. This has made the screening a mandatory and routine practice for autosomal recessive disorders in farm-borne HF and its crossbreds prior to their introduction into the breeding programs. Even though the initial incidence of CVM is low, number of carriers could be substantially higher in coming days if the animals are not screened routinely. Spread of undesirable genetic disorder is hastened by the artificial insemination program. Therefore, vigilance is required to diminish the risk of dissemination of recessive defects resulting from increased selection pressure within the dairy industry, which is presently dominated by HF breed worldwide.

In India, Mahdi (2008) and Kumar (2009) reported the carrier bulls' frequency of 20.35 and 76.36% for CVM

respectively. The incidence of CVM carriers among top sires was 1776% in USA (Holstein Association USA 2006), 13.20% in Germany (Konersmann *et al.* 2003), 32.50% in Japan (Nagahata *et al.* 2002), 23.00% in Sweden (Berglund *et al.* 2004), 31.00% in Denmark (Thomsen *et al.* 2006), 25.00% in Poland (Kaminski and Rusc 2007). In contrast to the reported frequency of CVM, none of the animals found either affected or carrier in our investigation.

The research on recessively inherited genetic disorder will buttress an attempt to select animals using molecular markers. Routine and obligatory screening of HF animals contained the rapid spread of CVM in several countries. Restricted breeding and long-term investigations have enabled a great reduction of this threat to the population. This is corroborated by the result of our study. An integration of international and national associations of Holstein cattle breeders with the genetic defect detection programs is necessary to decrease the number of carriers and affected animals at a faster rate. Such actions will instill the confidence in international exchange of superior bull semen and in containing the local cattle population from being affected with the genetic defects. These actions will make the animal husbandry an economical endeavour as the losses incurred are reduced.

In conclusion, research on recessively inherited genetic disorder will definitely endorse the selection of animals using molecular markers. Rigor of hereditary disorders like CVM on the productivity and reproductive health status of herd could be mitigated by stringent adoption of the following actions: (i) an accurate maintenance of the pedigree of all cows for sire and maternal grandsire, (ii) data for the status of CVM in the sire and maternal grandsire of each cow in the herd should be maintained, (iii) selection index should be employed to identify the sires that are likely to be used for the next three months in the herd, (iv) use of semen from CVM carrier animals should be avoided on any cow whose sire or maternal grandsire is a carrier, and (v) modern genetic tools such as molecular markers would be used to identify undesirable genes causing CVM and to eliminate them in a rapid and efficient manner. The present findings and eradication of the CVM carriers are of great significance in India for animal improvement programs and in making the animal husbandry as well as dairy industry as an economical endeavour. This also calls for screening of other population of HF and its crosses in the country.

REFERENCES

Agerholm J S, Bendixen C, Andersen O and Arnbjerg J. 2001. Complex vertebral malformation in Holstein calves. *Journal of Veterinary Diagnostic Investigation* **13**: 283–89.

Berglund B, Persson A and Stalhammer H. 2004. Effects of complex vertebral malformation on fertility in Swedish Holstein cattle. *Acta Veterinaria Scandinavica* **45**: 161–65.

Duncan R B Jr, Carrig C B, Agerholm J S and Bendixen C. 2001. Complex vertebral malformation in a Holstein calf: Report of a case in the USA. *Journal of Veterinary Diagnostic Investigation* **13**: 333–36.

Haliassos A and Chomel J C. 1989. Detection of minority point mutations by modified PCR technique: A new approach for a sensitive diagnosis of tumor-progression markers. *Nucleic Acids Research* **17**: 8093–99.

Jacobson D R and Moskovits T. 1991. Rapid, nonradioactive screening for activating ras oncogene mutations using PCR-primer introduced restriction analysis (PCR-PIRA). *PCR Method and Application* **1**: 146–48.

Kaminski S and Rusc A. 2007. Prevalence of complex vertebral malformation among carriers Polish Holstein Friesian bulls. *Journal of Applied Genetics* **48**: 247–52.

Kanae Y, Endoh D, Nagahata H and Hayashi M A. 2005. Method for detecting complex vertebral malformation in Holstein calves using polymerase chain reaction-primer introduced restriction analysis. *Journal of Veterinary Diagnostic Investigation* **17**: 258–62.

Konersmann Y, Wemheuer W and Brenig B. 2003. Origin, distribution and relevance of the CVM defect within the Holstein-Friesian population. *Zuchtungskunde* **75**: 9–15.

Kumar V. 2009. 'Genetic screening of K F animals for bovine leukocyte adhesion deficiency syndrome (BLAD) and complex vertebral malformation (CVM).' M.V.Sc. Thesis, NDRI, Karnal.

Mahdi M. 2008. 'Genetic screening of Karan Fries bulls for bovine leukocyte adhesion deficiency and complex vertebral malformation.' M. Sc. Thesis, NDRI, Karnal.

Nagahata H, Oota H, Nitanai A, Oikawa S, Higuchi H, Nakade T, Kurosawa T, Morita M and Ogawa H. 2002. Complex vertebral malformation in a stillborn Holstein calf in Japan. *Journal of Veterinary Medicine* **64**: 1107–12.

Revell S. 2001. Complex vertebral malformation in a Holstein calf in the UK. *Veterinary Record* **149**: 659–60.

Thomsen B, Per Horn, Frank Panitz, Emoke Bendixen, Anette H, Petersen, Lars-Erik Holm, Vivi H Nielsen, Agerholm J S, Jens Arnbjerg and Christian Bendixen. 2006. A mis. sense mutation in the bovine SLC35A3 gene, encoding a UDP-N-acetyl glucosamine transporter, causes complex vertebral malformation. *Genome Research* **16**: 97–105.