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Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Identification of BLAD and citrullinemia carriers in Chinese Holstein cattle

Jianbin Li[1,2]*, Hongmei Wang[2], Yi Zhang[1], Minghai Hou[2], Jifeng Zhong[2]*, Yuan Zhang[1]

[1] College of Animal Science and Technology, China Agricultural University, 2 Yuanmingyuan West Road, Beijing, 100094 China

[2] Dairy Cattle Research Centre, Shandong Academy of Agricultural Science, 159-1 Gongye North Road, Jinan, 250100 China

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Bovine leukocyte adhesion deficiency (BLAD) and citrullinemia are two autosomal recessive genetic diseases in Holstein cattle and both result in death of homozygous animals. Through the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technology and DNA sequencing, one citrullinemia and three BLAD carriers were found in a population of 615 Chinese Holstein cattle, including 436 cows and 179 bulls. Citrullinemia and BLAD carrier frequency was 0.16% and 0.49%, respectively, in tested Chinese Holstein cattle. In this study, the first citrullinemia carrier occurring in Chinese Holstein cattle is described, although the world frequency of this disorder is very low.

KEY WOERS: BLAD / citrullinemia / Chinese Holstein cattle / PCR-RFLP

The widespread use of elite sires by means of artificial insemination in livestock breeding leads to the frequent emergence of recessive genetic defects, which cause significant economic and animal welfare concerns [Charlier *et al*. 2008]. Bovine leukocyte adhesion deficiency (BLAD) and Citrullinemia are two Holstein-specific autosomal recessive genetic disorders. BLAD results in death of homozygous animal

*Corresponding author: msdljb@163.com

that is disable to defend itself from pathogens. Afflicted animals show a series of severe symptoms, including ulcers of oral membranes, gingivitis, chronic pneumonia, impaired wound healing, anorexia, chronic diarrhea, and chronic dermatitis. The main reason is that their leukocytes lack β_2-integrins needed for mobilized leukocytes to pass through capillary walls to fight infection. The molecular biological mechanism of BLAD was characterized in 1991. The condition is caused by a missense mutation at position 383(A-G) in the *CD18* gene, resulting in a substitution of aspartic acid to glycine at amino acid 128 (D128G) – Shuster *et al*. [1992]. Citrullinemia is a rare metabolic disorder in Holstein dairy cattle. Affected animals lack argininosuccinate synthetase crucial to urea cycle. The mutation responsible for citrullinemia has been characterized as a single-base substitution (C-T) in exon 5 of argininosuccinate synthetase (*ASS*), which converts the CGA codon that codes for arginine-86 to TGA, a translational termination codon. This conversion results in a truncated peptide product (85 amino acids instead of 412) deprived of functional activity. Afflicted animals show impaired urea cycle leading to ammonia accumulation in blood and tissues, present the unsteady gait, aimless wandering, apparent blindness, head pressing, convulsions, and death within a week [Dennis *et al*. 1989].

BLAD and citrullinemia have been reported to occur in many countries [Robinson *et al*. 1993, Grupe *et al*. 1996, Hajime 2004], however, not in China. In China, Holstein cattle were bred through introductive crossing of native cows with elite Holstein bulls, using semen straws or embryos imported from other countries including US, Canada, and recently Australia, where the two genetic defects in question had been reported. In light of this it was decided to investigate whether there are BLAD and citrullinemia in dairy cattle of China.

Material and methods

Collected were 436 blood samples from Chinese Holstein cows in Shandong province of China and 179 frozen semen samples of young Holstein bulls from two AI stations involved in progeny testing programme. Genomic DNA was isolated according to a method described earlier by Chen *et al*. 2006]. In order to detect the mutation responsible for BLAD and citrullinemia, a polymerase chain reaction and restriction analysis (PCR-RFLP) was performed according to Shuster *et al*. [1992] and Dennis *et al*. [1989], respectively. DNA sequencing of heterozygous animals was carried out to confirm the PCR-RFLP results.

Polymerase chain reaction (PCR) for *CD18* was set up in a volume of 25 µl containing Taq DNA polymerase buffer (2.5 µl), 100 ng DNA template (1 µl), 100 µM of each primer (0.5 µl, respectively), 50 mM $MgCl_2$ (0.9 µl), 200 mM dNTPs (0.5 µl), 1U Taq polymerase (2.5 µl) and redistilled water (16.6 µl). The mixture was incubated in 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 sec, with a final extension at 72°C for 8 min. The PCR components and conditions

for the amplification of the exon 5 for *ASS* gene were the same as given above except for the primers and annealing temperature, which was 53°C.

The PCR products were analysed separated in 2.5% agarose gel stained with ethidium bromide and visualized under UV-transilluminator. *CD18* and *ASS* were digested overnight with *TaqI* and *AvaII* (*Taq*I and *Ava*II) restriction enzymes, and then electrophoresed in 2% agarose gel for the *CD18* and in 11% polyacrylamide gel (SDS-PAGE) for *ASS* gene.0 PCR products for heterozygous individuals were sequenced for further identification.

Results and discussion

Three BLAD carriers, including two cows and one bull, and one citrullinemia carrier were detected with described methods in 615 Chinese Holstein cattle (Fig. 1); respective frequency was 0.49% and 0.16%. Pedigree information indicates that the BLAD carriers detected were related to common ancestor "Osborndale Ivanhoe". The Citrullinemia carrier could not be traced back to common ancestor "Linmack Kriss King" because the genealogy was recorded for only two generations.

At present, although the number of carriers of both conditions has decreased remarkably, they still exist in the world population of Holstein cattle. In USA, BLAD carriers frequency was 14.1%, occurrence rate of BLAD was estimated to be 0.2% at birth in 1992 [Shuster *et al*. 1992]. In Denmark, BLAD carriers and afflicted animals' frequency was 21.5% and 0.5% respectively [Jorgensen *et al*. 1993]. In Japan, BLAD carrier rate was 5.4%, with no occurrence [Nagahata *et al*. 1993]. In Poland, 4.8% of Holstein bulls tested were BLAD carriers [Natonek 2000], and 0.8% in 2006 [Czarnik *et al*. 2007]. In Turkey, two BLAD carriers were found in 120 Holstein cattle which corresponded to mutant BLAD allele frequency of 0.84% [Akyüz *et al.* 2006], and no carriers were found in 170 Holstein cows [Oner et al. 2010]. In India, the percentage of BLAD carriers in Holstein-Friesian (HF) and HF crossbred population was estimated to be 3.23% [Patel *et al.* 2007]. Frequency of BLAD carriers varies currently from 0.8 to 3.45% of cattle considered by cited authors [Schütz *et al*. 2008]. In Pakistan, frequency of the mutant allele of BLAD in Friesian-Friesian and Friesian-Sahiwal population was estimated to reach 0.01[Nasreen *et al.* 2009]. The incidence of bovine citrullinemia occurred for the first time in Australia, where 13% of sires in one AI centre were found to be heterozygous for the mutation in 1989 [Healy 1996]. In USA and Germany, the incidence frequency of citrullinemia was found very low [Robinson *et al*. 1993, Grupe *et al*. 1996]. In other countries, such as India and Turkey, no carriers of citrullinemia were reported [Patel *et al*. 2006, Oner *et al*. 2010]. Generally, the citrullinemia mutation frequency is very low, which is in accordance with results of this study.

The BLAD and citrullinemia carriers have been found in Chinese Holstein cattle. China has about 13 million Holstein cattle, similar to the other countries, AI breeding programme being conducted for the genetic improvement of productivity

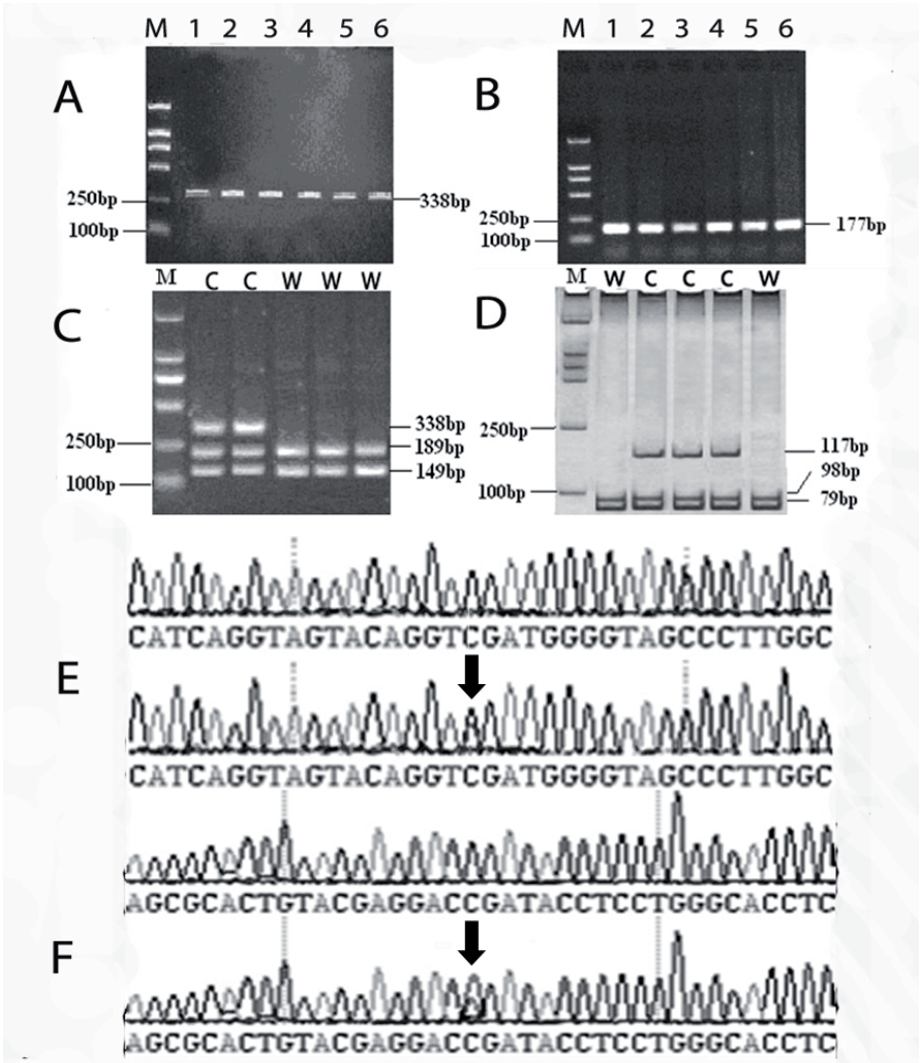

Fig. 1. PCR products, PCR-RFLP and partial sequences of *CD18* and *ASS* gene in Chinese Holstein cattle. **A:** Polymerase chain reaction (PCR) products of *CD18*. M – Marker, Line 1-6 – PCR products of *CD18* (338 bp). **B:** Polymerase chain reaction (PCR) products of *ASS*. M – Marker, Line 1-6 – PCR products of *ASS* (177bp). **C:** PCR-RFLP result of *CD18* gene. **C** – carriers (three fragments, 338 bp, 189 bp and 149 bp respectively), W – wilds (two fragments, 189 bp and 149 bp). **D:** PCR-RFLP result of *ASS* gene, C – carriers (three fragments, 177 bp, 98 bp and 79 bp, respectively), W – wilds (two fragments, 98 bp and 79 bp, respectively). **E:** DNA sequencing by anti-sense primer. A T/C mutation was observed in the *CD18* gene of carriers detected by PCR-RFLP. **F:** DNA sequencing by sense primer. A C/T mutation was observed in exon 5 for the *ASS* gene of carriers detected by PCR-RFLP.

by using elite bulls. The programme inevitably leads to drastic reduction in effective population size, and consequently increases the risk of dissemination of inherited defects. It is important to establish the screening methods of various genetic defects, allowing producers and breeders to test their suspicious cows and all breeding bulls and combine the pedigree information to avoid the at-risk matings.

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