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Short communication

Missense mutation in SDE2 gene – new lethal defect transmitted into Polish Holstein-Friesian cattle

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Abstract

The aim of the study was to find out whether carriers of new lethal mutation in SDE2 gene occur in the population of Polish Holstein-Friesian bulls. Eighty seven bulls were included in the analysis. Bulls were selected as having in the pedigree known carrier of SDE2 mutation (bull Mountain USAM000002070579). All bulls were diagnosed by PCR amplification of 524 bp fragment of SDE2 gene followed by digestion of Bcc I restriction enzyme. Heterozygotes (carriers) were confirmed by sequencing. Each new carrier was used to trace another potential carriers among its offspring available in Polish Holstein Bull Repository Database. Among 87 bulls, 50 new SDE2 carriers were found. The study has shown that mutation in SDE2 gene causing early embryo mortality is already transmitted to Polish Holstein-Friesian cattle. The results are sufficient to initiate the screening program to reveal new carriers and to avoid further spreading of SDE2 lethal mutation.

Key words: Holstein bull, lethal genetic defect, carrier, SDE2 gene

Introduction

Holstein Haplotype 6 (HH6) is a new autosomal recessive defect in Holstein cattle (OMIA 002149-9913). Bovine embryos being recessive homozygotes die in the first 35 days of gestation (Fritz et al. 2018). A combined approach of a genome-wide association study (GWAS) and homozygosity mapping revealed a ~1.1Mb disease associated with HH6 on BTA 16 (Fritz et al. 2018). The disease associated with the haplotype traces to the Holstein sire MOUNTAIN USAM000002070579 born in 1987. The same team resequenced the entire genome interval of affected calves and a healthy males carrying one copy of the critical segment, and detected a causal mutation – substitution of Adenine by Guanine within gene SDE2 coding Telomere Maintenance Homolog2 protein. This mutation is assigned as g.29773628A>G and registered in NCBI SNP database under the accession number rs434666183. A>G substitution diminishes initiation codon ATG to ACG and in effect translation starts at the subsequent closest ATG codon which truncates SDE2 protein by 83 amino acids including very conservative cleavage site. This leads to dysfunction

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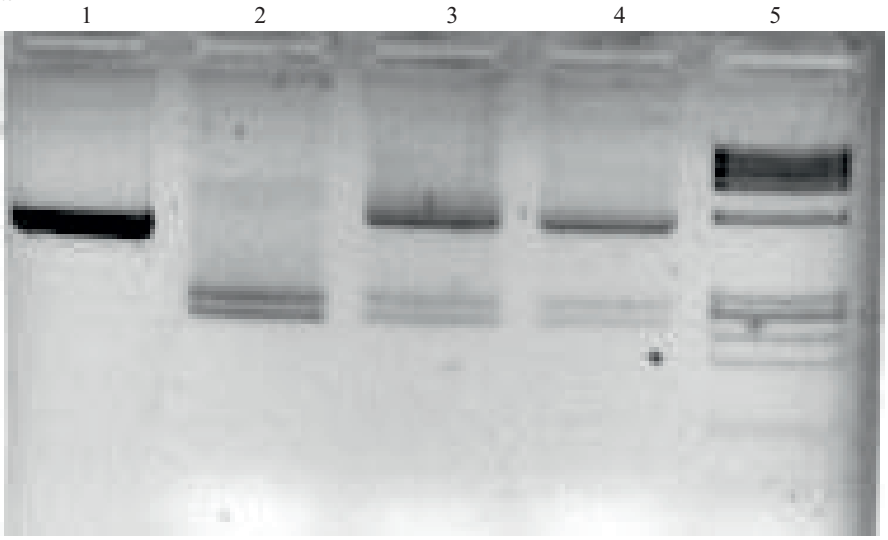
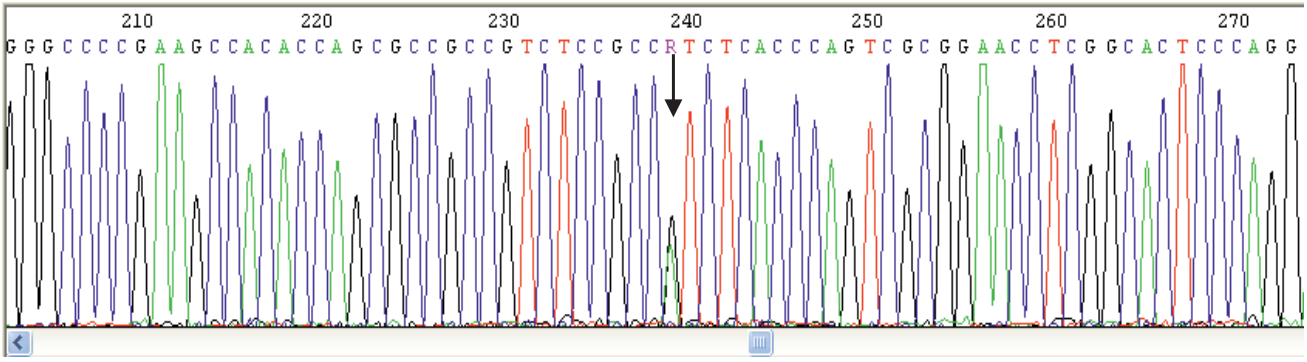
a



b

forward sequence



reverse sequence

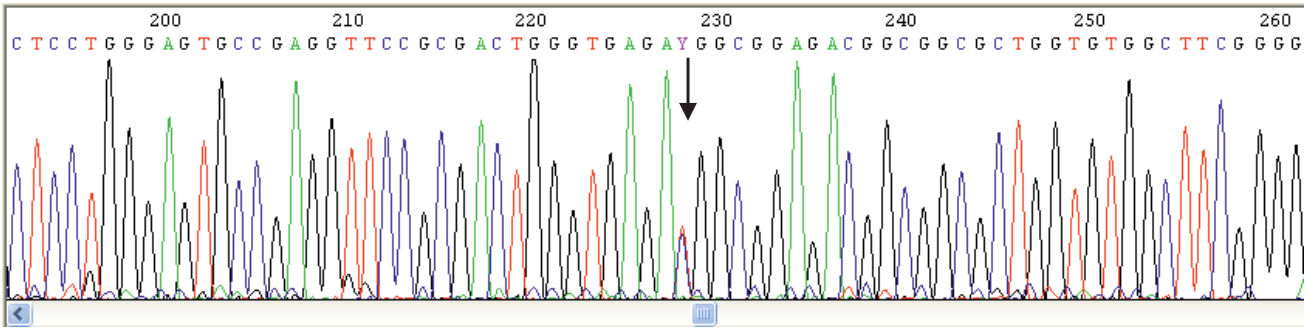


Fig. 1. a. example of SDE2 mutation genotyping by restriction enzyme Bcc I. Line 1 – PCR product undigested (524 bp); Line 2 – bull with AA genotype (amplicon cut into two fragments 278 and 246 bp); Line 3 and 4 – bull carriers (uncut fragment 524 bp being G allele and 278 and 246 bp (A allele). Line 5 – DNA mass marker PhiX174 Hae III.
b. forward and reverse sequencing of PCR products showing SDE2 heterozygote (carrier). In position indicated by arrow there is a substitution A>G (R) on forward strand and C>T (Y) on reverse strand.

of the SDE2 protein causing slower embryo growth and their abortion which is manifested by lower conception rate of cows and lower non-return rate at 56 days. Intensive exchange of genetic material through artificial insemination, import of top bull semen, and international transfer of embryos make easier spreading of any mutation across local populations. Since Polish Holstein-Friesian cattle has been strongly influenced by USA Holstein bulls for about 50 years, it is hypothesized that causal mutation for HH6 was transmitted and is now segregating in the population of Polish Holstein-Friesian bulls.

Materials and Methods

Eighty seven Polish Holstein-Friesian bulls were included in the analysis. Bulls were selected as having in the pedigree world known carrier of HH6 (bull Mountain USAM000002070579). Genomic DNA was retrieved from Polish Repository of Holstein Bulls stored in the University of Warmia and Mazury (Olsztyn, Poland). To amplify 524 bp fragment of SDE2 gene, primers designed by Fritz et al. (2018) were used: forward 5'GACGGAAG CCCTCACTATCA3' and reverse primer 5'CTTCTCTTAGCAACGCCTCG3'. The following PCR mix was used: 20x PCR Buffer, 10x dNTP mix (2 mM each), 50 pmol each of 3 PCR primers (synthesized by Genomed, Poland), 25 mM $MgCl_2$, 1.5 unit of Taq polymerase (Eurix, Poland), ca. 50 ng of genomic DNA and H_2O up to 20 µl (all chemicals used in PCR mix except primers come from Epicenter, USA). PCR was run under the following thermal conditions: pre-denaturation at 95°C for 3 min followed by 35 cycles of: 30s/94°C, 30 s/61°C, 30 s/72°C and finished by 5 min at 72°C. Reactions were performed in a Mastercycler 5330 thermocycler (Eppendorf, Germany). Specificity and efficiency of PCR reaction products were analyzed in 1 % agarose gel with ethidium bromide (EtBr). Ten µl of PCR products were digested by Bcc I restriction enzyme (BioLabs, USA) to check polymorphic site. To confirm genotypes amplicons were purified by the use of GelOut kit (AA Biotechnology, Poland) and sequenced (Genomed, Warszawa, Poland). Sequences of amplicons were analyzed by Chromas software.

Results and Discussion

An example of genotyping for SDE2 missense mutation is shown in Fig 1a. Amplicons obtained from bulls with Adenine in the polymorphic site (A allele) were recognized by Bcc I (CCATC) and therefore were cut into two smaller fragments (278 and 246 bp). For G allele Bcc I site was lost, so 524 bp fragment remained uncut. Therefore, for carriers 3 fragments were observed: 524 bp (G allele) and 278 and 246 bp (A allele). This method is less time- and cost-consuming than sequencing used by Fritz et al. (2018). It can be especially useful for labs where sequencing is not a routine techniques and outside service is necessary. Genotyping by the use of Bcc I enzyme was validated by re-genotyping of carriers by sequencing (Fig. 1b). Each new carrier was used to trace another potential carriers among their offspring available in the Polish Holstein Bull Repository Database. Among 87 bulls, 50 new SDE2 carriers were found (57.47%). The data do not reflect the actual frequency of carriers because the bulls were not randomly chosen from the population but were selected from the offspring of SDE2 mutation founder MOUNTAIN in the follow-up analysis. The highest number of carriers were identified among available sons of sire MARION US130153294 (14 positive out of 27 tested) and Duch sire ADDISON NL839380546 (11 positive out of 15 tested). Since HH6 is the newest genetic defect detected this year there is no reports on the frequency of carriers in Holstein cattle except the work of Fritz et al. (2018). They screened 29.000 animals and found 1.3% of HH6 carriers in French Holsteins. Analysis of bull MOUNTAIN male offspring in SYMLEK database (Polish official software system for registering of dairy cattle pedigree, insemination and milk production) revealed 155 his sons including 55 born in Poland (oral communication). Therefore we can suspect that the SDE2 missense mutation was widely spread in the Polish Friesian-Holstein cattle. Our initial results support this assumption since bulls MARION and ADDISON were top bulls and were very popular among dairy cattle breeders in Poland. The present results show that causal mutation for HH6 is already transmitted to Polish Holstein-Friesian cattle and is segregating in subsequent generations. Therefore we postulate undertaking urgent action to avoid further spreading of HH6 lethal defect. All young sires and candidates for sire dams should be screened for SDE2 missense mutation using the same procedures which were already successfully applied to previous genetic defects, like Bovine Leukocyte Adhesion Deficiency (Czarnik et al. 2007), Complex Vertebral Malformation (Ruść et al. 2013) or are currently implementing, like Brachyspina (Ruść and Kamiński 2015) and Cholesterol Deficiency (Kamiński and Ruść 2016). Uncontrolled spreading of SDE2 missense mutation will certainly decrease the fertility of cows because the higher number of carriers increases the chance of producing recessive homozygotes. Since the population of Holstein-Friesian cows in Poland is approximately 2,4 million, the policy leading to limit the number of carriers of any genetic defects is reasonable and should reduce losses in fertility and increase profitability of dairy cattle production in the future.

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