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Short communication

Cholesterol Deficiency – new genetic defect transmitted to Polish Holstein-Friesian cattle

S. Kamiński, A. Ruść

University of Warmia and Mazury, Department of Animal Genetics, Oczapowskiego 5, 10-719 Olsztyn, Poland

Abstract

The aim of the study was to find out whether carriers of new genetic defect Cholesterol Deficiency (CD) occur in the population of Polish Holstein-Friesian bulls. Twenty seven bulls were included in the analysis. Bulls were selected as having in the pedigree known carrier of CD (Maughlin Storm CANM000005457798). All bulls were diagnosed by the test described by Menzi et al. (2016) by using allele-specific PCR. Among 27 bulls, 9 new CD carriers were found. Our results show that causal mutation for CD is already transmitted to Polish Holstein-Friesian cattle. The results are sufficient ground to take practical action in order to avoid further spreading of mutation causing CD.

Key words: bull, genetic defect, carrier, Cholesterol Deficiency

Introduction

Cholesterol Deficiency (CD) is a new autosomal monogenic recessive defect in Holstein cattle (OMIA 001965-9913). Calves being recessive homozygotes die within a period of days to months after birth as a consequence of the onset of idiopathic diarrhea (Kipp et al. 2015). A combined approach of a genome-wide association study (GWAS) and homozygosity mapping revealed a ~2.7Mb disease associated haplotype on BTA 11 (Kipp et al. 2015). The disease associated haplotype traces to the Holstein sire Maughlin Storm born in 1991 (VanRaden and Null 2015). Menzi et al. (2016) re-sequenced the entire genome of an affected calf and a healthy partially inbred male carrying one copy of the critical 2.24-Mb segment and detected a causal mutation – 1.3kb insertion of a transposable LTR element (ERV2-1) located in the coding sequence of the apolipoprotein B (APOB) gene. This insertion generated premature stop codon resulting in a truncation of the APOB protein to less than 140 amino acids. The 1.3kb insertion was confirmed by Schütz et al. (2016), who reported that the LTR element was inserted into exon 5 of the APOB gene (at BTA11:77,959kb) and is flanked by 6bp target site duplications typical to insertions mediated by retroviral integrases. APOB is an essential compound of chylomicrons and low-density lipoproteins. It seems then that the mutation represents a loss-of-function mutation similar to autosomal recessive inherited familial hypobetali-poproteinemia-1 (FHBL1) in humans (Young et al. 1988). Gross et al. (2016) reported that the causal mutation for CD affects lipid metabolism, steroid biosynthesis and cell membrane function in homozygous as well as heterozygous carriers and may result in unspecific symptoms like reduced fertility, growth, and health. The rapid exchange of genetic material by means of artificial insemination,

Correspondence to: S. Kamiński, e-mail: stachel@uwm.edu.pl

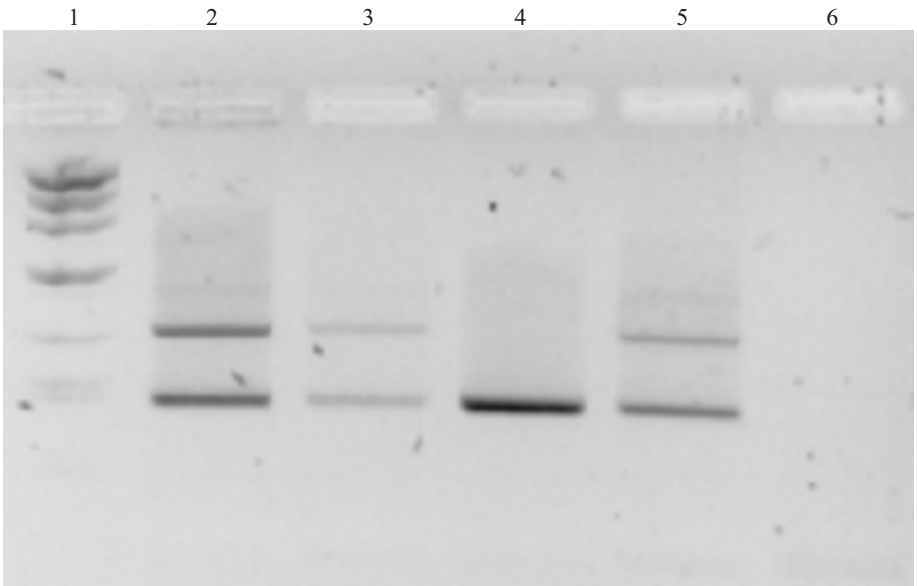

Fig. 1. Identification of CD carriers. From the left: line 1 molecular marker DRAMIX. Lines 2, 3 and 5 – CD carriers (both amplicons are present 249 bp and 436 bp); line 4 – normal animal (only lower band – 249 bp present); line 6 – negative control (PCR mix without DNA).

Table 1. Carriers of insertion within APOB gene causing Cholesterol Deficiency in sample of 27 Polish Holstein-Friesian bulls.

DNA id	Bull name	Bull id number	Known CD carrier in pedigree
5288	MAGENTA RED	US141304939	MR BURNS – sire of dam
1461	MOMENT	PL005100912369	STORM – sire of dam
1963	SALTOR ST	PL005106912998	STORM – sire of sire
2955	BARUBAR	PL005216372965	MR BURNS – sire
5292	PREDESTINE	US69177592	GOLDWYN – sire of dam
2859	JOSE	PL005137048444	GOLDWYN – sire
2778	INSERT ET	PL005142061698	GOLDWYN – sire
5799	nd	PL005252428817	MAGNETA RED – sire
5808	nd	PL005357031240	MAGNETA RED – sire

nd – no data.

import of semen, and transfer of embryos enables rapid transmission of mutation across populations. The aim of this report was to find out whether carriers of CD occur in the population of Polish Holstein-Friesian bulls.

Materials and Methods

Twenty seven Polish Holstein-Friesian bulls were included in the analysis. Bull were selected as having in the pedigree world known carrier of CD (Maughlin Storm CANM000005457798). Genomic DNA was isolated from the half volume of one commercial semen straw or ear tissue samples using the NucleoMag 200 Purification Kit or NucleoSpin Tissue Kit according to the manufacturer's instructions (Macherey-Nagel, Germany). All bulls were diagnosed by the test described by Menzi et al. (2016) by using 3 primers: forward common primer starting from wild sequence 5'GGTGACCATCCTCTCTCTGC3' and two primers discriminating wild sequence from mutant. Second wild reverse primer 5'AGTGGAACCCAG-CTCCATTA3' ensured amplification of 249 bp, but mutant forward primer 5'CACCTTCCGCTAT-TCGAGAG3' starting from inserted LTR element produced larger amplicon of 436 bp. To obtain a 436 bp or 249 bp fragment of bovine APOB gene, the following PCR mix was used: 20x PCR Buffer, 10x dNTP mix (2 mM each), 10 pmol each of 3 PCR primers (synthesized by Genomed, Poland), 10x PCR Enhancer, 25 mM $MgCl_2$, 0.5 µl Tfl polymerase (1U/µl), ca. 50 ng of genomic DNA and H_2O up to 25 µl (all chemicals used in PCR mix except primers

come from Epicenter, USA). The following thermal profile was used: pre-denaturation at 95°C for 3 min followed by 35 cycles of: 30 s 94°C, 30 s 60°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 1.5% agarose gel with ethidium bromide (EtBr), against DNA size marker DRAMIX (A&A Biotechnology, Poland). In the electrophoresis, 1x TBE was used as a buffer (0.45 M Tris, 0.44 M H_3BO_3, 0.5 M EDTA). The electrophoresis was run for 35 min under voltage of 100 V.

Results and Discussion

In Figure 1 typical picture of CD identification by PCR is shown. Carriers were identified as having two amplicons 436 and 249 bp in contrast to normal animals having only one band of 249 bp. Among 27 bulls which in pedigree files had known carriers of CD, 9 new carriers were found (Table 1). Kipp et al. (2015) estimated carrier frequency in German Holstein cattle as around 8.7% (among 3,400 screened). In another study, Schütz et al. (2016) found 12,5% carriers among Holstein bull born between 2012 and 2015 in Germany. Results presented in our paper show that causal mutation for CD is already transmitted to Polish Holstein-Friesian cattle and, in our opinion, it is sufficient ground to take practical actions in order to avoid further spreading of cholesterol deficiency defect. They should rely on the same rule applied to previous genetic defects (Czarnik et al. 2007, Ruść et al. 2013). Uncontrolled spreading of CD will decrease the fertility of cows since the higher number of carriers increase the chance of producing recessive homozygotes. Fertility is currently one of the most important trait and therefore any factors leading to its deterioration should be limited. Taking into account that the population of Holstein-Friesian cows in Poland is approximately 2,4 million, the policy limiting the number of carriers of any genetic defects will give substantial savings in the future.

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