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Original article

Muscle Weakness – new genetic defect transmitted to Polish Holstein-Friesian cattle

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Abstract

The aim of the study was to find out whether carriers of new genetic defect Muscle Weakness (MW) occur in the population of Polish Holstein-Friesian bulls. Fifty bulls were included in the analysis. Bulls were selected as having in the pedigree known carrier of MW. All bulls were diagnosed by DNA sequencing of *CACNA1S* gene containing single nucleotide substitution (rs3423414874) responsible for 97% of MW cases. Among 50 bulls, 19 MW carriers were found. Our results show that causal mutation for MW is already transmitted to Polish Holstein-Friesian cattle which is sufficient ground to take practical action in order to avoid further spreading of mutation causing MW.

Keywords: Holstein cattle, genetic defect, Muscle Weakness

Introduction

Muscle Weakness (MW) is a new autosomal monogenic recessive defect in Holstein cattle (OMIA 002819-9913). Calves being recessive homozygotes are unable to stand at birth and were found recumbent during the neonatal period with no detectable neurologic, infectious, or metabolic abnormalities. Most calves did not survive beyond 6 week of age (Dechow et al. 2022). The same team, using Genome-Wide Association Study for homozygosity screening mapped MW locus within 2.1 million region on the end of chromosome 16. Al-Khudhair et al. (2024) identified a variant in the *CACNA1S* gene (rs3423414874) as likely causal variant for MW. *CACNA1S* gene encodes a calcium channel, voltage-dependent, L type, α 1S subunit. CACNA1S is a part of calcium channels and is involved in transmitting the signal from the nervous system to the interior of the muscle necessary to cause muscle contraction (www.genecards.org). The causal mutation for MW was mapped at position BTA16 79,613,592 (according to *Bos taurus* genome assembly ARS-UCD1.3) as a missense mutation encoded on the negative strand predicted to alter a GGC codon to AGC and resulting in a glycine-to-serine amino acid substitution. This mutation registered under the number rs3423414874 has high concordance (97%) with the phenotype (Al-Khudhair et al. 2024). Globalization in exchange of genetic material by means of artificial insemination, 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 MW occur in the population of Polish Holstein-Friesian bulls.

Materials and Methods

Fifty Holstein-Friesian bulls were included in the analysis. Bulls were selected as having in the pedigree world known carrier of MW (SOUTHWIND Bell of Bar-Lee, US029HO05295, born in 1984). Genomic DNA was isolated from the half volume of one commercial semen straw using the NucleoMag 200 Purification Kit or NucleoSpin Tissue Kit according to the manufacturer's instructions (Macherey-Nagel, Germany).

Since the material for DNA isolation was commercial semen straws produced by artificial insemination centers according to appropriate veterinary regulations, no Ethic Commission Approval was required.

All DNA samples come from DNA Bull Repository consisting of bulls reared in Poland between 1991-2024 which were already tested for other genetic defects.

Bulls were diagnosed by sequencing of PCR product by using 2 primers: forward 5' TCCATCTGCCCA CAAGGTTC 3' and reverse 5'CACCCACGAGACT GTATCCG3' which ensured amplification of 397 bp. To obtain a 397 bp fragment of bovine *CACNA1S* gene, the following PCR mix was used: 10x PCR Buffer B with 15 mM MgCl2, 10x dNTP mix (2 mM each), 10 pmol each of 2 PCR primers (synthesized by Merck, Germany), 0.2 µl Taq DNA polymerase (5U/µl), ca. 50 ng of genomic DNA and H_2O up to 25 µl (all chemicals used in PCR mix except primers come from EURx Ltd., Poland). The following thermal profile was used: pre-denaturation at 95°C for 2 min followed by 35 cycles of: 30 s 95°C, 30 s 61°C, 30 s 74°C and finished by 7 min at 74°C. Reactions were performed in a PTC-200 thermocycler (MJ Research, USA). 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. After electrophoresis, specific PCR products were cut out from the agarose gel, purified using a Gel-Out kit (A&A Biotechnology, Gdańsk, Poland) and sequenced using an Applied Biosystems sequencer in Genomed Ltd (Poland). The sequences were analyzed using BioEdit v. 7.2.0 software.

Results and Discussion

In Figure 1 typical picture of MW carrier identification by sequencing is shown. In both strands overlapping peaks for A and G and C and T clearly show heterozygous status of this position in *CACNA1S* sequence. Unfortunately, the polymorphism cannot be detected by differential digestion of any commercially available restriction enzyme making sequencing the only method of genotyping. Among fifty bulls, 19 MW carriers were found. First, bull Seagull-Bay SUPERSIRE (US69981349) was confirmed as a MW carrier. The remaining MW carriers were descendants of known carriers: SOUTHWIND (3 bulls), SUPERSIRE (13 bulls) as well as Roylane Socra ROBUST (2 bulls) which is a father of SUPERSIRE. It has to be stressed however that according to Al-Khundhair et al (2024) mutation rs3423414874 in *CACNA1S* gene has incomplete penetrance, which means that 3% of carriers will not contribute to MW clinical symptoms. Mutation involved in MW was just discovered (Al-Khundhair et al. 2024), therefore there is no published data showing frequency of MW carriers in national Holstein cattle populations or in breeding companies selling semen for artificial insemination. Since bulls SOUTHWIND, SUPERSIRE and ROBUST were very popular around the world (including Poland),

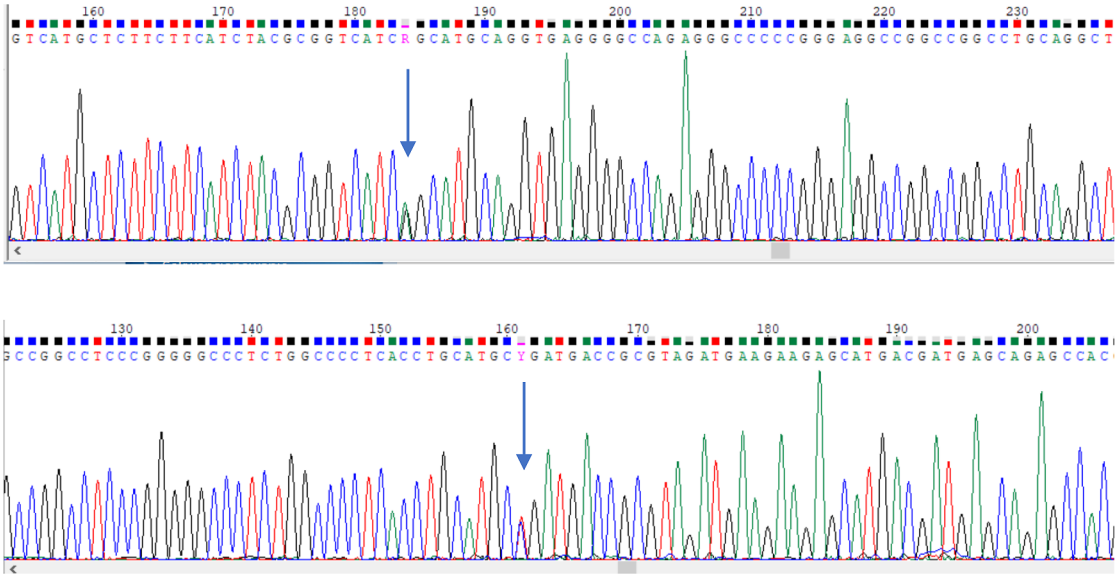

Fig. 1. Forward and reverse sequencing of PCR product (a fragment of *CACNA1S*) showing Muscle Weakness heterozygote (carrier). In position indicated by arrow there is a substitution A>G (R) on forward strand (upper) and C>T (Y) on reverse strand (below).

it can be expected that MW carrier frequency can achieve high level. Results presented in our paper show that likely causal mutation for MW is already transmitted to Polish Holstein-Friesian cattle and it is sufficient ground to take practical actions in order to avoid further spreading of this genetic defect. They should rely on the same rule applied to previous genetic defects (Czarnik et al. 2007, Kamiński 2023). Fast spreading of MW carriers will increase the chance of producing recessive homozygotes. Taking into account that the population of Holstein-Friesian cows in Poland is approximately 2,2 million (www.pfhb.pl), the policy limiting the number of MW carriers of any genetic defects will give substantial savings in the future.

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