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*Short communication*

# Novel method for identification of the lethal mutation in bovine APAF1 gene and its preliminary prevalence in Polish Holstein-Friesian bulls

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## Abstract

The aim of the study was to develop a reliable and cost-effective method for detection of nonsense mutation in APAF1 gene causing lethal effect called HH1 (Holstein Haplotype1) and to evaluate its prevalence in a sample of Polish Holstein-Friesian bulls. One hundred seventy eight bulls born between 1996 and 2017 were included in the analysis. They were kept in four artificial insemination centers and have in the pedigree the known carrier of HH1. All bulls were diagnosed by novel PCR-SSCP technique. Specific amplicons of 261 bp APAF1 gene fragment were used to detect changes in single stranded conformation (SSCP) caused by nonsense mutation C/T responsible for HH1. Each new carrier was used to trace another potential carriers among their offspring available in Polish Holstein Bull Repository Database. Among 178 bulls, 85 HH1 carriers were found. Our results show that nonsense mutation in APAF1 gene is already transmitted and segregating in Polish Holstein-Friesian cattle and its frequency may increase if no action will be undertaken against actual carriers.

**Key words:** Holstein bull, lethal genetic defect, carrier, APAF1 gene

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## Introduction

Single Nucleotide Polymorphisms (SNP) spread evenly through entire genome of dairy cattle enables identifying homozygous stretches of DNA (haplotypes) which do not occur in the actual population because of its lethal effect (VanRaden et al. 2011). Among many lethal effects observed in Holstein-Friesian cattle (reviewed by Taylor et al. 2018), Adam et al. (2016) revealed the case, where bovine embryos being recessive homozygotes die in the first 35 days of gestation. They found a single nucleotide causal mutation located within APAF1 gene (Apoptotic Protease Activating Factor 1). In exon 11 replacement of Cytosine by Thymine (C/T) resulted in exchange of Glutamine by premature stop codon in amino acid position 579 (Q579X) (Adams et al. 2016). Because functional APAF1 peptide is required for embryo development, homozygosity for mutant allele results in natural spontaneous abortion manifested by lower conception rate of cows and lower non-return rate at 56 days. HH1 has caused an estimated 525,000 spontaneous abortions worldwide over the past 35 years, accounting for approximately $420 million in losses (Adams et al. 2016). The disease associated haplotype has been traced to the earliest born (1962) ancestor Holstein sire Pawnee Farm Arlinda Chief USAM000001427381 (Chief) known as the second most influential sire in the Holstein breed history (www.holstein.com). Since for over 40 years Polish dairy Black-and-White cattle has been improved by Holstein-Friesian bulls imported mostly from USA, it is assumed that also recessive lethal mutations were transmitted to subsequent generation of dairy cattle reared in Poland. Therefore, the aim of this study was to use fast and inexpensive method for screening potential carriers of Q579X polymorphism and to check its prevalence in a sample of Polish Holstein-Friesian bulls.

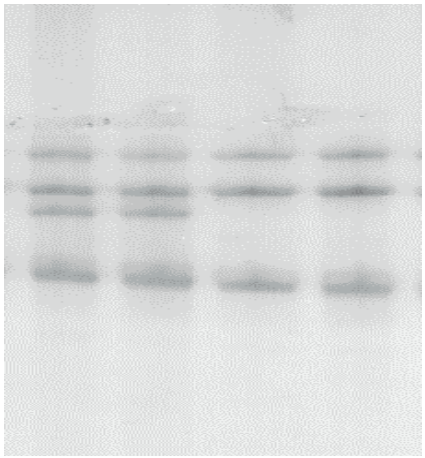
## Materials and Methods

One hundred seventy eight Polish Holstein-Friesian bulls born between 1996 and 2017 were included in the analysis. All bulls had in their pedigree history world-known carrier of HH1 - bull Chief. Genomic DNA was retrieved from Polish Repository of Holstein Bulls (PRHB). To amplify 261 bp fragment of APAF1 gene, the following primers were designed: forward 5' TTGGACGACAGCCATTTCCTA 3' and reverse 5' AATGCTTATTAAAAGTTCCA GCCC 3'. The following PCR mix was used: 1.5 µl 20x PCR Buffer, 1.5 µl 10x dNTP mix (2 mM of dATP, dCTP, dTTP, dGTP), 0.4 µl of each primer (10 pmol), 2.0 µl 10x PCR Enhancer, 1.5 µl 25 mM $MgCl_2$, 0.7 µl *Tfl* polymerase (1U/µl, Epiecntre, USA), 50 – 100 ng of DNA and $H_2O$ up to 25 µl. The thermal profile was used: pre-denaturation at 94° C for 3 min followed by 35 cycles of: 30 s 94°C, 30 s 61°C, 30 s 72°C finished by 10 min at 72°C (PTC-200 thermocycler, MJ Research USA). Depending on the efficiency of the PCR reaction, from 0.8 to 1.0 µl of PCR products were collected for the SSCP analysis and mixed with 3.0 µl of the denaturation solution (50 mM NaOH,1 mM EDTA) and 0.7 µl of loading buffer (30% glicol, 0.25% bromophenol blue, 0.25% xylene cyanol). The prepared samples were denatured for 13 min at 85° C in thermoblock, rapidly chilled on ice and then loaded into sample wells of the precast 15% polyacrylamide gels (Serva, Germany). Electrophoretic separations were carried out with the use of a system for horizontal electrophoresis Multiphor/ /MultiTemp III (Amersham, Sweeden) at 12°C according to the following programs: initial electrophoresis: 200 V, 20 mA, 10 min; electrophoresis: 375 V, 30 mA, 200 min. Gels were stained with silver (Promega, USA). The SSCP patterns were observed and analyzed in visible light emitted by a transilluminator (UVP, USA). PCR products forming different patterns in the SSCP reaction were sequenced (Genomed, Poland).

## Results and Discussion

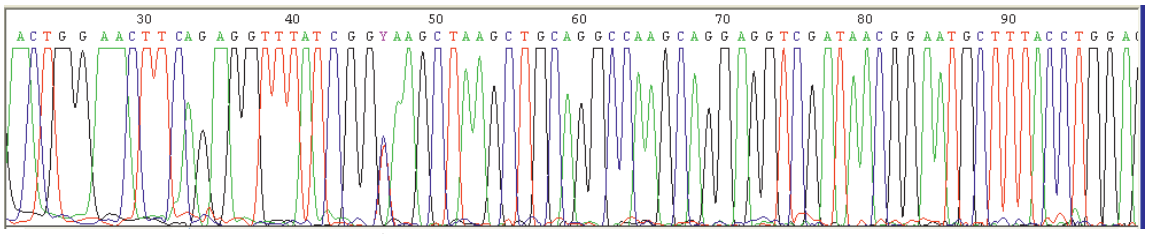
PCR-SSCP method turned to be very effective in clear identification of APAF1 mutation responsible for HH1. Two repeatable patterns of DNA bands were detected corresponding to the analyzed DNA samples originating from carriers of APAF1 mutation or wild homozygous animals. They were as follows: 3 bands in C/C homozygotes and a set of 4 bands in C/T heterozygotes (including 3 migrating with a velocity identical to that of homozygous animals) (Fig. 1a). Amplicons for six HH1 carriers and 2 free HH1 bulls were sequenced. There was complete conformity between genotypes identified by SSCP and sequencing (Fig. 1, b and c). The developed PCR-SSCP method is simple, fast (within 8 hour), reliable and inexpensive and therefore might be useful in screening programs. Moreover, the method is cost-effective alternative to method available in Illumina 10K SNP chip. In Poland, ca. 8000 individuals is screened annually by the SNP chip in the frame of genomic evaluation system (www.pfhb.pl). Therefore, the method can be addressed to remaining breeding herds in which testing for HH1 is planned. Each new carrier was used to trace another potential carriers among their offspring available in PRHB. Among 178 bulls, 85 HH1 carriers were found (47.75%). Although the data are roughly in accordance

**a.**

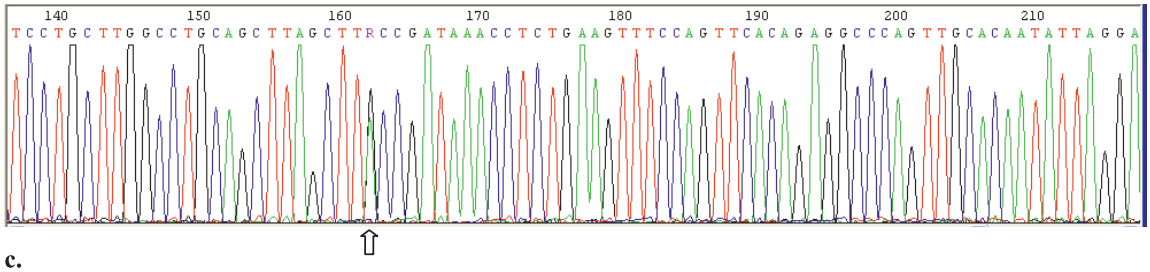


**b.**

forward

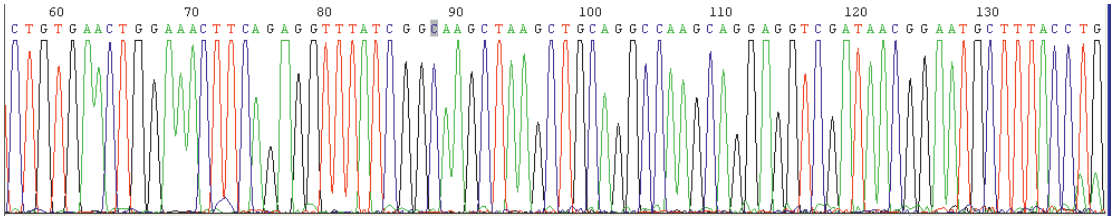


reverse



**c.**

forward



reverse

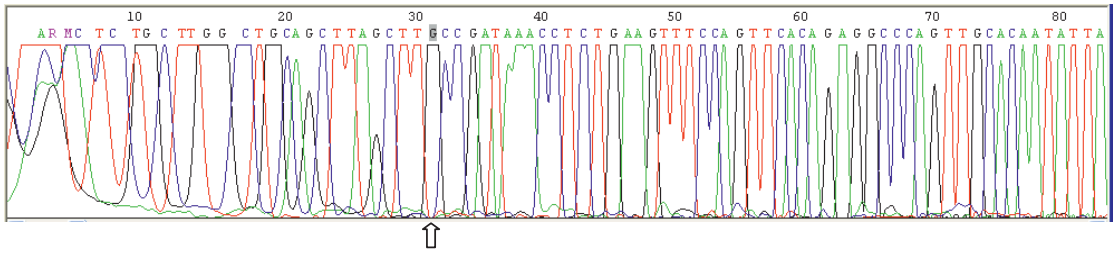


Fig. 1. Genotyping of APAF1 nonsense mutation responsible for HH1 by PCR-SSCP method.

a. SSCP patterns, from the left: line 1 and 2 – pattern characteristic for HH1 carrier (4 bands); line 3 and 4 – pattern characteristic for HH1 free individual (3 bands).

b. sequencing of PCR products showing HH1 carrier sample. In position indicated by arrow there is a substitution C>T (Y) on forward strand and A>G (R) on reverse strand.

c. sequencing of PCR products showing HH1 free sample. In position indicated by arrow there is C on forward strand and G on reverse strand.

with Mendelian inheritance, they do not reflect the actual frequency of carriers in entire population of bulls because they were sampled not randomly but were chosen from the offspring of HH1 mutation founder Chief in the follow-up analysis. The highest number of carriers were identified among available sons of sire MORTY PL000609521043 (16 positive out of 40 tested), sire FINLEY PL000609541249 (13 positive out of 34 tested) and sire MARION US130153294 (14 positive out of 26 tested). Since HH1 is one of the newest genetic defect detected 3 years ago only two reports on the frequency of carriers in Holstein cattle are available. Adams et al (2016) reported that the frequency of carriers dropped from 8% to 2%. In Japanese Holstein, Ghanem et al. (2018) found seven carriers out of 240 Holstein cows, giving the frequency of APAF1 mutant allele at 2.9%. Since sires MORTY, FINLEY and MARION were very popular among dairy cattle breeders in Poland, the initial results presented in the paper support the assumption that the prevalence of HH1 carriers in Poland can be similar to other countries in which semen from top American Holstein sires was intensively used. Results presented in the paper show that causal mutation for HH1 is already transmitted to Polish Holstein-Friesian cattle and is segregating in subsequent generations. Therefore it is postulated to undertake urgent action to avoid further spreading of HH1 lethal defect. All young sires and candidates for sire dams should be screened for APAF1 nonsense mutation in the same procedures which were already successfully applied for eradication of previous genetic defects, like Complex Vertebral Malformation (Ruść et al. 2013) or are currently implemented, like Brachyspina (Ruść and Kamiński 2015), Cholesterol Deficiency (Kamiński and Ruść 2016) and HH6 (Fritz et al. 2018, Kamiński 2019). The population of Holstein-Friesian cows in Poland is approximately 2,4 million (www.pfhb.pl), therefore the strategy limiting the number of HH1 carriers is desirable to reduce losses in fertility and profitability of dairy cattle production in the future.

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