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Short communication

Genotyping Charolais and Angus bulls for Progressive Ataxia by PCR-RFLP method

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Abstract

The paper presents a method for genotyping Charolais and Angus beef bulls for the causative mutation within KIF1C gene responsible for Progressive Ataxia (PA). The method involves amplifying a 406 bp fragment of the KIF1C gene using PCR and detecting the polymorphic site with the Mvn I restriction enzyme. Due to its simplicity, speed, and low cost, this method is accessible to an average laboratory equipped with a PCR thermocycler. Using this method, 62 bulls (46 Charolais and 16 Angus) currently used in artificial insemination in Poland were tested, among which 7 PA carriers were identified, exclusively among Charolais bulls. Detecting PA carriers does not mean they need to be eliminated, as according to the literature, they exhibit better growth and muscling traits.

Keywords: cattle, Charolais, Angus, PCR-RFLP technique, genetic defect, carrier

Introduction

Progressive Ataxia (PA) is a hereditary neurodegenerative disorder affecting cattle of the Charolais and Angus breed registered in Online Mendelian Inheritance of Animals dataset under id number 000527-9913 (Nicholas et al. 2024). First described in the 1970s (Palmer et al. 1972), the disease has since been identified in multiple countries with Charolais populations. Molecular genetic studies have identified a PA causal mutation on chromosome 19 within exon 4 of KIF1C gene at position g.26407668C>T (according to NC_037346.1, reference sequence ARS-UCD1.3). Substitution C by T causes amino acid exchange R on Q at 203 amino acid position (Duchesne et al. 2018). KIF1C gene encodes a kinesin-like motor protein responsible for intracellular transport along microtubules and plays a critical role in neuronal function, particularly in axonal transport and oligodendrocyte activity. Loss of KIF1C function disrupts cellular transport systems, leading to impaired maintenance of myelin and neuronal degeneration. The mutant *KIF1C* allele was estimated at 13% in French Charolais cattle (Duchesne et al. 2018). For over three decades PCR-based methods were successfully used in genotyping dairy cattle for inherited diseases (Jorgensen et al.

1993). Among many PCR variations, the PCR-RFLP method, due to its simplicity, reliability and low cost, has gained great popularity until current time as demonstrated in recent work used to identify carriers of the latest genetic defect in Holstein bulls (Kamiński 2025). The aim of the study was to develop a PCR-RFLP test to detect causal mutation for PA and to assess its occurrence in Charolais and Angus bulls currently used in Artificial Insemination (AI) in Poland.

Materials and Methods

All available Charolais and Angus bulls offered by 3 artificial insemination centers operating in the territory of Poland in 2025 were included in the study (46 Charolais and 16 Angus). 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). Polymorphism C>T within the KIF1C gene was identified using a Polymerase Chain Reaction (PCR) followed by digestion of a specific restriction enzyme. A pair of primers (forward: 5'GGGCCCTACCTTCTCTGAGT3' and reverse: 5'TGCTCCTCTGACTCAGGTGA3') were designed using Primer3 software (Untergasser et al. 2012) and used to amplify a 406 bp fragment of the KIF1C gene. The PCR thermal profile consisted of 35 cycles of 95°C for 25 s, 60°C for 25 s and 74°C for 30 s and a final extension at 74°C for 10 min. PCR was performed in a reaction mix containing 70-80 ng of genomic DNA, 0.4 µL (25 pM) of each primer, 2.0 µL PCR Buffer (10x, Biotools, B&M Labs), 1.2 µL dNTPs mix (2.0 mM each), 1 unit of Eurx Taq DNA Polymerase and deionized water added to reach a volume of 25 µL. Eight µL of specific PCR products were digested with 0.6 u of Mvn I restriction enzyme (recognition site: CGCG, Thermo Scientific) during 45 min incubation at 37°C. Restriction fragments were electrophoresed in standard 2.5 % agarose gel stained with ethidium bromide (1mg/ml). Amplicons obtained from DNA of one KIF1C CC and 7 CT bulls 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 (Genomed Ltd, Poland). The forward and reverse strands were analyzed using BioEdit v. 7.2.0 software.

Results and Discussion

An example of genotyping KIF1C polymorphic site C>T is shown in Fig. 1. Within the entire amplicon (406 bp), for Cytosine in the polymorphic site (C allele, wild), Mvn I enzyme (recognizing sequence CGCG) was able to find this site and cut a 406 bp amplicon into two smaller fragments: 213 and 193 bp. For the T allele (mutant), the recognition sequence for Mvn I was disrupted and therefore amplicon was retained uncut (406 bp). For heterozygous (CT) individuals, three fragments occurred: 406, 213 and 193 bp. Sanger sequencing of bulls genotyped as PA free (CC) and PA carrier (CT) confirmed the reliability of genotyping with the use of Mvn I enzyme (Fig. 2). The reproducibility of PA genotyping by PCR-RFLP method was 100% (meaning that all tested samples gave the expected PCR product, suitable for restriction enzyme digestion). For 7 bulls, PA carrier status was confirmed by 406 bp amplicon sequencing. The obtained fluorogram images for both strands were fully consistent with the PCR-RFLP results (data not shown), confirming the high reliability of the developed method. Comparing to sequencing, the current method is reliable and fast since the total genotyping time takes only 6-7 hours. The method is also inexpensive since the reagents used are low in price and easily available. Another advantage of the PCR-RFLP method is that genotyping can be set for one or many individuals without significant additional cost/per sample. These features make the method described available to an average veterinary diagnostic laboratory with a PCR thermocycler either for screening a population of bulls or a single bull which is suspected to be a potential PA carrier. Since the mutated allele is not cut by the restriction enzyme, there is a risk that an inactive restriction enzyme could produce false positive results. Therefore, it is recommended to include a control sample that is homozygous (healthy) in each genotyping batch, where cutting 406 bp amplicon into two fragments (213 and 193 bp) will indicate that the enzyme was active and that the lack of cutting is due to the mutation, not to a technical artifact. Moreover, if PA carrier is detected, it is recommended to confirm the result through sequencing.

Among 62 bulls, 7 bulls were PA carriers, all were Charolais breed, 1 and 6, of Czeck and French origin, respectively. It gives 11.29% PA carrier frequency and a mutant allele frequency – 5.65%. In Poland, a relatively small number of Charolais and Angus bulls is used for insemination, so the estimated PA carrier frequency may vary depending on the number of bulls. Broader screening for PA was done by Bischofberger et al. (2024) who analyzed 1315 Charolais cattle and found that the *KIF1C* mutation occurs in a frequency of 11.75% in the German Charolais population.

Unlike most lethal defects, PA should not be viewed entirely negatively. Duchesne et al. (2018) note that carriers of the causative mutation for PA exhibit certain beneficial traits, primarily higher body weight and better overall muscle assessment, which are crucial pro-

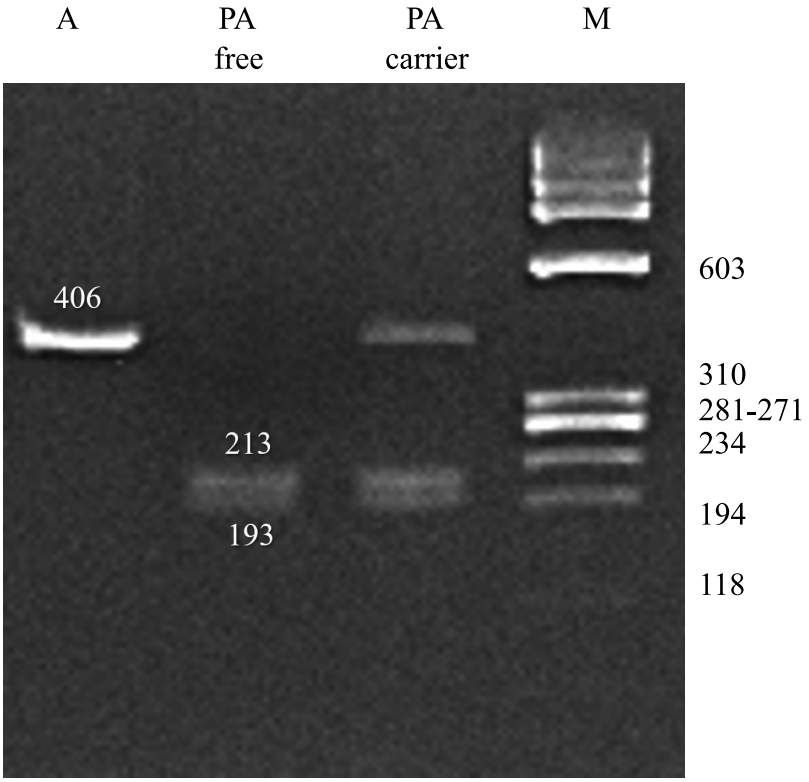

Fig. 1. Example of electrophoresis of 406 bp amplicons of KIF1C gene containing a polymorphic site C>T causing Progressive Ataxia (PA) detected by the use of the Mvn I restriction enzyme. A – amplicon 406 bp uncut, PA free – bull with CC genotype (406 bp amplicon cut to 213 and 193 bp), PA carrier – bull with genotype CT (406, 213 and 193 bp); M – DNA size marker PhiX174/Hae III (only fragments useful for genotyping are shown).

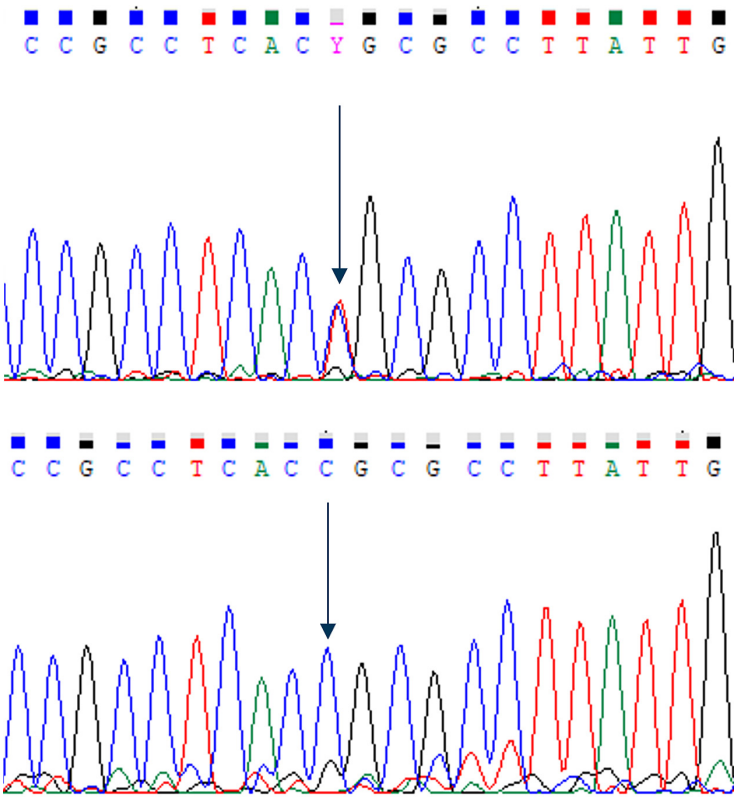

Fig. 2. Fragment of a 406 bp amplicon sequence of KIF1C gene obtained from bulls genotyped for PA by the PCR-RFLP method. Polymorphic site is indicated by an arrow. Bull free of PA has CC genotype (down) and bull being PA carrier (upper) has CT genotype. Y indicates heterozygote (C or T). Genotypes were confirmed on reverse strand (data not shown).

duction traits in this breed. Consequently, they propose so-called balancing selection, which means avoiding matings that lead to the appearance of recessive homozygotes, while preferring heterozygotes (carriers). These observations were confirmed by Siddikui et al. (2022) and Bischofberger et al. (2024), who showed that in both PA dominant homozygous animals and PA carriers there was a higher estimated breeding value for weight gain and muscle development, although the authors do not determine whether these positive effects can be attributed to genetic variants of the KIF1C gene or to a haplotype of other loci linked to the KIF1C gene.

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Authors Declarations

Ethics approval

Material for DNA isolation was commercial semen straws produced by AI centers according to appropriate veterinary regulations, no Ethic Commission Approval was required.

Use of generative artificial intelligence

No tools for generative AI was used in the preparation of manuscript

Conflict of interest

The author declares no conflict of interest

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