Inheritance of A New Mutation Affecting Muscle Weakness Within a Common Haplotype in Holsteins

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Abstract

A haplotype associated with calf recumbency and mortality having a recessive effect but apparent incomplete penetrance was previously linked to the end of chromosome 16 (78.7 to 80.7Mbp). Genotype analysis of 5.6 million Holsteins indicated that the haplotype was common and traced back to 1952, with a key ancestor born in 1984 (HOUSA1964484, Southwind) identified from chip genotypes as homozygous for the suspect haplotype. Sequence data from Southwind, an affected calf, and the sire of the affected calf were scanned for candidate mutations. A mutation in the *CACNA1s* gene causing symptoms of recumbency (lately termed Holstein Early Onset Muscle Weakness; HMW) was homozygous in the affected calf and heterozygous in the calf's sire and Southwind. Improved methods for using pedigree to track new mutations within existing haplotypes were developed, and gene tests for the mutation were also included. For new mutations within existing common haplotypes, determining carrier status without gene tests is difficult, even with accurate pedigrees when the original haplotype has a high frequency.

Key words: HMW, CACNAS1, Recessive, Recumbency

Introduction

Computational genomic tools allow for monitoring known genetic diseases in dairy cattle, predict the rise of novel markers (causal/associated), and link them to newly identified disorders of a genetic nature (https://www.ars.usda.gov/ARSUserFiles/80420530/Publications/ARR/Haplotype%20tests_ARR-Genomic5.pdf). Recently, Holstein newborns exhibited higher death rates due to inability to stand and neuropathological symptoms described and termed as recumbency by Dechow et al. (2022). They identified the disorder using genotypes and pedigrees of affected calves that shared a haplotype on chromosome 16 (78.7 to 80.7Mbp on ARS-UCD1 map) and ancestral links traced back to a bull born in 2008.

The condition leading to calf recumbency is currently known as "Early Onset Muscle Weakness" (**MW**). Despite its clinical significance, official recognition of this disorder as a recessive trait is still in progress, and the nomenclature remains subject to finalization.

Carriers of recessive inheritance genes are usually identified and tracked using a haplotype-based tests and, once enough research data supports the identification of the actual causal variation, a gene test is developed as a diagnostic tool with high accuracy. Precisely monitoring the rise in frequencies of inherited haplotypes of recessive conditions is essential, and constant improvement of the haplotype-based test is critical. One such example is Holstein Haplotype for Cholesterol Deficiency (HCD), where a new mutation occurred in a commonly and highly frequent haplotype (VanRaden and Null, 2015).

The current study investigates the muscle weakness haplotype (**HMW**) using US data, reports a new mutation within it, and validates improved accuracy of the haplotype-based test by resolving status using pedigrees and gene test results.

Materials and Methods

The affected calves investigated by Dechow et al. (2022) were found to have a common ancestor born in 2008 (HOUSA64966739 Roylane Socra Robust-ET). By using a larger pool of chip-genotyped animals (over 5.5 million animals), the anticipated associated haplotype of the affected calves was found in further older ancestors (HOUSA1964484, Southwind Bell of Bar-Lee) born in 1984. Southwind carried the suspect haplotype and was also an ancestor of some recumbent calves that did not trace to Robust. Disregarding the association with the MW affected calves, the haplotype was common and was identified in further genotyped animals where the oldest (HOUSA1189870 Osborndale Ivanhoe) was born in 1952.

Sequence data for Southwind, an affected calf, and the sire of the affected calf were examined and to identify a potential causal mutation. *CACNA1s* mutation at position 79,613,592 was identified as the most likely variant with high association concordance. Sorting Intolerant From Tolerant (SIFT) software (https://sift.bii.a-star.edu.sg/) utilized to predict the deleterious consequence of this mutation.

A commercially developed gene test using the CACNA1s mutation was used to identify carrier status for 4,416 animals and reported by the Holstein Association USA (HAUSA). The method by VanRaden and Null, 2015 identifies recessively inherited haplotypes with a harmful effect was further improved and validated to identify the MW haplotype, and then sort genotyped animals that are carriers of the *CACNA1s* mutated MW haplotype. The revised algorithm also requires pedigree relatedness to the common ancestor of the affected calves. Then, it categorizes the haplotypes into the different genotype status groups (0 = noncarrier, 1 = carrier, 2 = homozygous defect, 3 = suspect carrier, 4 = suspect homozygous).

As of May 2023, Select Sires, ABS Global, Genex, and Semex companies utilized the MW gene test and publicly reported results for 2,609 tested animals. The reported results were examined, compared to the haplotype tests, and included in the haplotype determination algorithm to improve the accuracy of future calling of the MW carrier status.

Results & Discussion

Sequence data analysis confirmed the hypothesis of a new mutation. Southwind was homozygous for all sequence variants within the suspect region as expected but was heterozygous for the mutation at location 79,613,592 bp in the CACNA1s gene and was the earliest known carrier of the mutated haplotype (HMW). Thus, Southwind, or an ancestor between him and Ivanhoe was the source of a new mutation. The identified causal mutation was then utilized to develop a muscle weakness (MW) gene test.

Haplotype test results from the newly adopted method were further updated by matching haplotype data to the gene test results to rule out ancestors that inherited the common haplotype but not the mutation. Genotyped animals with unknown haplotype status may be resolved using gene tests for their ancestors (Table 1). This test examined 424,109 HO males (Table 2), and 5.9 million HO females (Table 3) genotyped as of July 2023. Before including 4,416 gene tests from HAUSA indirectly in the pedigree, 93.58% of males and 88.35% of females had certain haplotype codes (0, 1, or 2), and the other 4.96% of males and 11.11% of females had uncertain haplotype codes (3 or 4). That sex difference is because commercial females often have incomplete pedigrees and non-genotyped dams, whereas nearly all males have complete pedigrees and both parents are genotyped.

For animals with uncertain haplotype status, the inclusion of gene tests changed the haplotype status for 22% of the males but only 5% of the females. For example, of the 19,283 males with uncertain status, 3,574 resolved to noncarrier and 1,845 to carrier status. Very few (513) of the 5.6 million animals that previously

had certain status (< 0.1%) changed after including gene tests in the pedigree. The conducted research affirmed the accuracy of the newly adopted haplotype test method and a high concordance achieved in identifying actual noncarriers, carriers, and affected Holstein calves.

Popularity of Robust was responsible for the rapidly increasing trend of the mutation. The improved haplotype prediction method requiring a pedigree association to Robust and incorporating the CACNA1s mutation gene tests predicted only 220 HMW carriers by the end of 2009. The number of HMW carriers increased significantly over the next ten years to 66,432 carriers, of which 59,243 HMW carriers were animals genotyped in the last four years (2020 – present). Holstein Association USA and the dairy cattle industry are following our recommended preventative measurement by identifying muscle weakness disorder gene carriers and reporting affected animals using the gene test.

Another observation for recessively inherited diseases such as MW with incomplete penetrance and/or semi-lethal nature is that under-reported death losses by farmers are negatively affecting estimated effects calculated for mating carrier sires and maternal grandsires (data not shown in this study). Accuracy of reporting death losses from such inherited diseases could influence other similar phenotypes such as longevity traits (heifer livability, stillbirth. etc.) (Wiggans and Carrillo, 2022).

Conclusions

Haplotype-based testing methods for recessively inherited diseases such as MW are essential but are complex when new mutations occur. The accuracy of identifying carriers can be greatly improved by using pedigrees to track the haplotype source and incorporating gene test results when they are available. Farmers are encouraged to accurately report newborn death losses and/or cases of calves' early onset inherited disability as these accurately reported data could be correlated to infer confirmational estimates of the carriers of such recessive haplotypes.

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Table 1. Carrier status (count and %) before and after adding gene test results to the pedigree and haplotype information (males and females). Status 3 reports unsure heterozygote and 4 reports unsure homozygote.

Status before (rows) vs. after (columns)	0	1	2	3	4	Total (%)
0	5,497,696 (86.59)	513 (0.01)	1 (0)	91 (0)	0 (0)	5,498,301 (86.6)
1	31 (0)	133,105 (2.1)	9 (0)	13 (0)	0 (0)	133,158 (2.1)
2	0 (0)	0 (0)	796 (0.01)	0 (0)	0 (0)	796 (0.01)
3	27,271 (0.43)	7,866 (0.12)	0 (0)	634,717 (10)	0 (0)	669,854 (10.55)
4	1,048 (0.02)	1,058 (0.02)	221 (0)	0 (0)	44,470 (0.7)	46,797 (0.74)
Total (%)	5,526,046 (87.04)	142,542 (2.25)	1,027 (0.02)	63,4821 (10)	44,470 (0.7)	6,348,906 (100)

*Genotype status: 0 = noncarrier, 1 = carrier, 2 = homozygous defect, 3 = suspect carrier, 4 = suspect homozygous.

Table 2. Improved predictions are achieved when gene test results are incorporated into the population pedigree information (male data).

(old vs. new) gene code (%)	0	1	2	3	4	Total (%)
0	381,409 (89.93)	228 (0.05)	0 (0)	41 (0.01)	0 (0)	381,678 (90)
1	10 (0)	15,321 (3.61)	4 (0)	5 (0)	0 (0)	15,340 (3.62)
2	0 (0)	0 (0)	181 (0.04)	0 (0)	0 (0)	181 (0.04)
3	3,574 (0.84)	1,845 (0.44)	0 (0)	19,283 (4.55)	0 (0)	24,702 (5.82)
4	148 (0.03)	232 (0.05)	91 (0.02)	0 (0)	1,737 (0.41)	2,208 (0.52)
Total (%)	385,141 (90.81)	17,626 (4.16)	276 (0.07)	19,329 (4.56)	1,737 (0.41)	424,109 (100)

*Genotype status: 0 = noncarrier, 1 = carrier, 2 = homozygous defect, 3 = suspect carrier, 4 = suspect homozygous.

Table 3. Improved predictions are achieved when gene test results are incorporated into the population pedigree information (female data).

(old vs. new) gene code (%)	0	1	2	3	4	Total (%)
0	5,116,287 (86.35)	285 (0)	1 (0)	50 (0)	0 (0)	5,116,623 (86.36)
1	21 (0)	117,784 (1.99)	5 (0)	8 (0)	0 (0)	117,818 (1.99)
2	0 (0)	0 (0)	615 (0.01)	0 (0)	0 (0)	615 (0.01)
3	23,697 (0.04)	6,021 (0.1)	0 (0)	615,434 (10.39)	0 (0)	645,152 (10.89)
4	900 (0.02)	826 (0.01)	130 (0)	0 (0)	42,733 (0.72)	44,589 (0.75))
Total (%)	5,140,905 (86.77)	124,916 (2.11)	751 (0.01)	615,492 (10.39)	42,733 (0.72)	5,924,797 (100)

*Genotype status: 0 = noncarrier, 1 = carrier, 2 = homozygous defect, 3 = suspect carrier, 4 = suspect homozygous.