A hypomorphic mutation in the *ATP2A1* gene increases muscle mass yet compromises meat quality of Belgian Blue cattle

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Abstract

We herein describe a novel phenotype related to meat quality in Belgian blue cattle breed, named 'tough and dry meat syndrome'. A genome-wide association study pinpointed a single significantly associated locus on chromosome 25, encompassing the *ATP2A1* gene coding for the SERCA1 protein. A previous reverse genetic screen identified a breed-private missense mutation *(R143W)* in this gene. It segregates at high frequency (0.15) without deviation from expected genotypic proportions and was proven to be causative for this syndrome. The prevalence of the condition is estimated at 12.5% and its severity correlates with *W143* allelic dosage. The mutation is partially dominant with a penetrance of 43% and 100% in *R/W* and *W/W* animals respectively. Retrospective examination of *W143* frequency revealed a clear linear upward trend, likely due to the fact that the *W143* variant has a positive effect on muscle mass. Direct selection against the mutation is underway.

Introduction

Belgian blue cattle (BBC) breed is known for its exceptional double-muscling phenotype due to the fixation of a loss-of-function mutation in the *myostatin* gene (Grobet *et al.*, 1997). It is directly associated with a lower collagen content in muscle resulting in a superior meat tenderness much appreciated by consumers. But, since several years, meat cutters reported the occasional observation of meat cuts that were described as 'tough and dry'. Most of the affected muscles were located in high-value cuts in the hindquarter: silverside (m. *glutaeobiceps*), topside (m. *semimembranosus*) and top rump (m. *quadriceps femoris*). In the worst cases the eye-muscle (m. *longissimus dorsi*), the underblade (m. *subscapularis*) and the shoulder tender (m. *teres major*) were also impacted. Here we report the identification of a mutation in the *ATP2A1* gene associated with a major negative effect on meat quality.

Materials & Methods

Carcass phenotyping. Meat quality phenotypes were classified into six discrete categories (C) of increasing severity (0, 1, 1.5, 2, 2.5 and 3). Carcasses without any macroscopic meat defect are classified as C0. Upon cutting of the carcasses, all affected animals (C > 0) exhibited exudate at the surface of the *m. semimembranosus*, and a 'tough and dry' feeling of the *m. adductor femoris* and inner parts of the *m. glutaeobiceps* and *m. semimembranosus* (C1 and 1.5), as well as of the *m. quadriceps femoris, m. semitendinosus, m. longissimus dorsi, m. subscapularis* and *m. teres major* for the most severe cases (C2 to 3).

Genome-wide association studies (GWAS)

For the association studies, DNA samples were genotyped on custom LD12K (1st cohort) or MD50K (2nd cohort) EuroGenomics SNP arrays (Illumina, Inc) as described in Charlier *et al.*, 2016. After QC, 11,088 and 47,473 autosomal SNPs were kept in the 1st and 2nd cohorts, respectively. GWAS between SNP or haplotypes and meat quality (0 = normal; 1 = 'tough

and dry') were performed using GLASCOW (Zhang *et al*., 2012). The genomic relationship matrix was estimated either based on SNP similarity or on haplotype similarity as described in Zhang *et al.* (2012). For this, haplotypes were reconstructed with Beagle 4.1 (Browning and Browning, 2007) and then grouped at each marker position into 10 clusters of ancestral haplotypes (hidden states; HS) based on their similarity with the model described in Druet and Georges (2010) using HiddenPHASE.

Results

Using genotypes from 38 affected meat pieces (cases) and from 1,058 unrelated breed-age-matched animals as controls, we obtained a single genome-wide significant signal on chr25 with the two models (single SNP and 10 HS)(Fig 1A&B). The most significant variant was a missense variant *(R143W)* in the *ATP2A1* gene encoding the SERCA1 protein ($p = 3.6x10^{-21}$). The maximum 10 HS signal ($p = 6.2x10^{-23}$) overlapped this variant, and was due to the enrichment in cases of a single haplotype that was in near perfect linkage disequilibrium ($r^2 \geq 0.91$) with the *ATP2A1 R143W* variant (Fig 1C). The SERCA1 protein is a Ca^{2+} ATPase that is embedded in the membrane of the sarcoplasmic reticulum of muscle fibers, pumping Ca^{2+} ions from the sarcoplasm back into the reticulum thereby controlling muscle relaxation (eg. Wuytack *et al.*, 2002). Loss-of-function mutations in *ATP2A1* cause autosomal recessive Brody myopathy in humans (eg. Brody, 1969; Odermatt *et al.*, 1996), congenital muscular dystonia I in BBC (Charlier *et al.*, 2008), and perinatal lethality in *ATP2A1* knock-out mice (Pan *et al.*, 2003). Since, missense *ATP2A1* variants have been associated with recessive pseudo-myotonia of variable severity in at least four other beef cattle breeds (OMIA 001464-9913). The *R143W* mutation is located in *ATP2A1* coding exon 5 and affects a highly conserved residue in the first cytoplasmic loop of SERCA1 (Fig1 D-F). *R143W* is only reported in BBC. It had a frequency of 15% in the GWAS control population, yet of 55% in the 38 affected samples ($p = 9.5x10^{-23}$). Based on these initial findings, we performed a systematic survey for 'tough and dry' meat cuts in Belgian slaughter-plants. We first examined 496 carcasses of adult Belgian Blue cows, 62 (12.5%) were considered to be affected to various extend. A striking feature was the systematic increased loss of fluid from the carcass (referred to as 'drip') during the cooling process. Upon cutting of the non-C0 carcasses, they exhibited exudate at the surface of the respective affected muscles (see M&M). Samples of the m. *glutaeobiceps* were collected from 26 affected (5 C1; 9 C1.5; 9 C2; 3 C2.5) and 21 non-affected C0 animals and analysed for standard meat quality traits. Briefly, non-C0 samples had altered colour (darker) and colour stability, texture (higher hardness, gumminess, chewiness, and shear force), and moisture content (reduced EZ-drip, thawing and cooking loss; increased dry matter content) compared to C0 controls. We genotyped 116 case (non-C0) samples as well as 127 unaffected (C0) controls. A case-versus-control GWAS confirmed the chr25 association, maximizing at the *ATP2A1 R143W* position with individual SNP ($p = 1.7x10^{-36}$) and the HS10 ($p = 4.8x10^{-54}$) models. The frequency of the *W143* allele was 8.2% in the 'hyper' C0 controls, while it was 55.1% in non-C0 cases. We further tested (within cases) whether there was an association between *R143W* genotype and severity of the condition by regressing dry meat score (C1 to 3) on dosage of the *W* allele (1 or 2). The average score of *R/W* cases was 1.46 while it was 1.88 for *W/W* cases ($p = 0.0005$). We retrospectively examined the change in frequency of the *W143* variant in the general BBC population over time (>65,000 animals from 2014 to 2020).

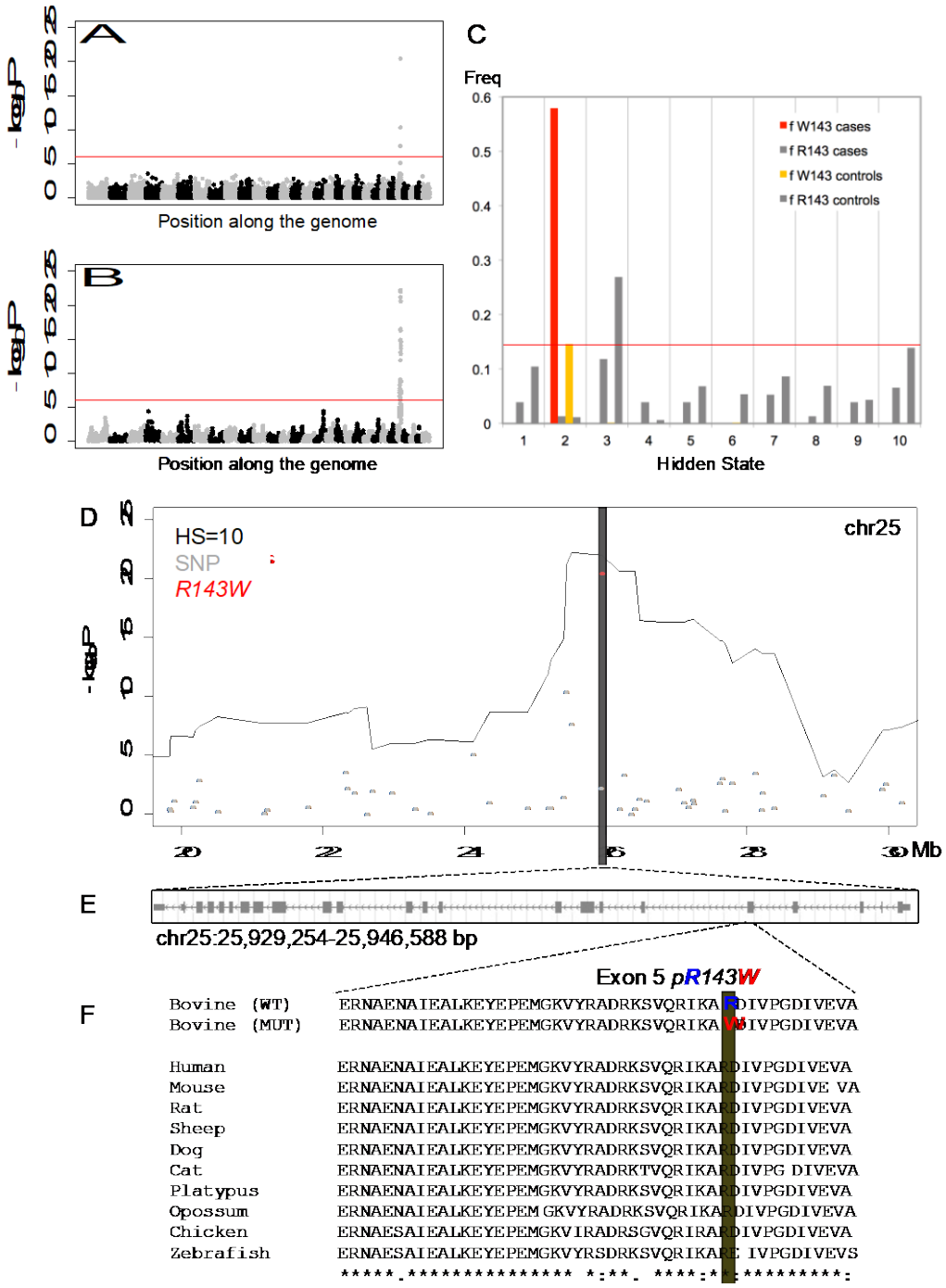

Figure 1. Mapping and fine-mapping of the 'tough and dry meat' phenotype. Manhattan plots show the respective significance of a single SNP model **(A)** and a ten hidden-states (HS) model **(B)**; the horizontal red line indicates the significance threshold; alternating colors (black and grey) mark the limits between each of the 29 autosomes. **(C)** Frequency of the 10 HS in the 38 cases and the 1058 controls at peak position; within each HS, frequency is given for haplotypes carrying the *W143* mutation or the wild-type allele in cases and in controls; the horizontal red line indicates the *W143* freq. in the population. **(D)** Association signals for a single SNP model (grey dots) and a 10HS model (black line). **(E)** Gene model for the *ATP2A1* gene (adapted from UCSC browser). **(F)** *ATP2A1* exon 5 protein alignment across vertebrates highlighting the *R143W* aa change; * marks invariant aa.

It showed a clear linear upward trend from 14.5% in 2014 to 17.5% in 2020. To understand what may cause this trend, we studied the effect of the *W143* variant on 22 phenotypes that are systematically recorded in BBC as described in Gualdrón Duarte *et al*. (2020). Significant (Bonferroni-corrected) positive effects were observed for five traits pertaining to muscular development of the front and hindquarters (data not shown).

Discussion

We herein provide very strong evidence that a likely hypomorphic *ATP2A1* missense variant (*R143W*) is the cause of a newly discovered 'tough and dry meat' phenotype in BBC.

Assuming that the prevalence of the condition is 12.5% in the BBC population and given the genotypic proportions observed in cases and controls, we estimate that the penetrance of the anomaly is 43% in heterozygotes and 100% in homozygotes, which at present account for 30% and 3%, respectively. We can estimate the depreciation of the non-C0 carcasses at roughly 20% by assuming that the affected cuts can only be valorized as minced meat. Further work is still needed to determine which factors affect the penetrance of the *W143* mutation. We have since confirmed that the defect is present as well in young bulls with a similar prevalence (data not shown). The frequency of the *W143* mutation appears to have been steadily increasing in the BBC population. We showed that this is likely due to the fact that the *W143* allele has a positive effect on muscle mass. It thus represents another example of the increase in frequency of a variant with a major deleterious effect because of its perceived positive impact on muscle mass - the phenotype that remains the primary selection goal in BBC. Knowing the causative mutation, will allow for effective counter selection against the *W143*. However, as heterozygotes may have a high probability to be affected (>40%), avoiding matings between carrier animals would not be sufficient to control the condition. Animals carrying the *W143* mutation should be identified by direct genotyping and progressively eliminated from the breeding stock.

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