



Genome-wide association study of economically important traits in Charolais and Limousin beef cows



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ABSTRACT

Genomic selection has proven effective for advancing genetic gain for key profit traits in dairy cattle production systems. However, its impact to-date on genetic improvement programs for beef cattle has been less effective. Despite this, the technology is thought to be particularly useful for low heritability traits such as those associated with reproductive efficiency. The objective of this study was to identify genetic variants associated with key determinants of reproductive and overall productive efficiency in beef cows. The analysis employed a large dataset derived from the national genetic evaluation program in Ireland for two of the most predominant beef breeds, viz. Charolais (n = 5 244 cows) and Limousin (n = 7 304 cows). Single nucleotide polymorphisms (SNPs) were identified as being statistically significantly associated (adj. P < 0.05) with both reproductive and productive traits for both breed types. However, there was little across breed commonality, with only two SNPs (rs110240246 and rs110344317; adj. P < 0.05) located within the genomic regions of the *LCORL* and *MSTN* genes respectively, identified in both Charolais and Limousin populations, associated with traits including carcass weight, cull-cow weight and live-weight. Significant SNPs within the *MSTN* gene were also associated with both reproduction and production related traits within each breed. Finally, traits including calving difficulty, calf mortality and calving interval were associated with SNPs within genomic regions comprising genes involved in cellular growth and lipid metabolism. Genetic variants identified as associated with both important reproductive efficiency and production related traits from this study warrant further analyses for their potential incorporation into breeding programmes to support the sustainability of beef cattle production.

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Implications

In beef cow production systems, reproductive performance is central to production efficiency and can impact the profitability and sustainability of beef cow production. Genomic selection breeding programs have proven effective for advancing the genetic gain of productivity related traits in cattle; however progress for reproductive related traits has been much slower, primarily due to the low heritability. This study reports single nucleotide polymorphisms (SNPs) significantly associated with reproduction and production related traits in Limousin and Charolais beef cows. Following appropriate validation, SNPs identified may be utilised in genomic selection breeding programs to advance genetic gain in beef cows.

Introduction

Beef cows serve an important role, worldwide, in converting low quality forage to high quality protein for human consumption (Diskin

and Kenny, 2016). This is coupled with the fact that beef cow herds are typically maintained on land that is not suitable to support intensive dairy or crop production. Moreover within beef cow production systems, the calf is the sole output; consequently, reproductive performance is key to production efficiency and is also a major determinant of profitability and overall sustainability (White et al., 2015; Ahmadi et al., 2017). In conventional cow-calf systems, improvements in reproductive performance and longevity have been shown to have a larger impact than improvements in end production traits (Diskin and Kenny, 2016). Achieving a high level of reproductive efficiency is underpinned by producers being cognisant of and achieving many key targets throughout the production cycle, for example age at first calving, calving interval, conception/pregnancy and calving rate (Diskin and Kenny, 2016).

Genomic selection has proven effective for advancing genetic gain of cattle for a number of productivity related traits (Mrode et al., 2018). However, genetic improvement programs for traits associated with reproductive efficiency, particularly for beef cattle, have been much slower to develop (Berry et al., 2014). This is primarily a consequence of the low heritability estimates (0.02–0.04, Berry et al., 2014) and general difficulty in recording of reproductive performance related traits in

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beef cows (Martínez-Velázquez et al., 2003; Donoghue et al., 2004; Berry et al., 2014). In addition, reproductive related traits are typically expressed late in the life of the animal and thus traditional breeding techniques require more time to evaluate the reproductive potential of individual animals (De Melo et al., 2017). However, even with these limitations the level of genetic variation present in beef cattle is sufficiently large enough to allow for breeding programs to improve reproductive efficiency (Berry and Evans, 2014). Thus the propagation of superior genetics for reproductive efficiency in beef cows is possible and ultimately the identification of robust genetic variants which could be harnessed as part of a genomically assisted selection program will assist in the accurate identification of reproductively efficient animals. Additionally this will also support greater economic and environmental sustainability of beef cow enterprises.

The objectives of this study were to (1) identify genetic variants associated with key determinants of reproductive efficiency, including fertility and longevity and, (2) identify genetic variants associated with key determinants of production efficiency, including live-weight and carcass characteristics, which may impact reproductive related traits. This was undertaken using phenotypic and genotypic data for Charolais and Limousin cows in the Irish national genetic evaluation database. Previous studies have evaluated the genetic architecture of production related traits in both Charolais and Limousin cows (Kause et al., 2015; Purfield et al., 2019a), however there is less information available for SNPs involved in the reproductive potential of these breeds (Purfield et al., 2019b). Genetic variants identified as associated with both reproduction and production traits from this study can be incorporated into the national beef cattle breeding programme in Ireland and elsewhere, ultimately improving the sustainability of beef cow enterprises, worldwide.

Materials and methods

Genotypic data and quality control

Records from 12 548 purebred Charolais and Limousin cows, genotyped using the custom built International Dairy and Beef Single Nucleotide Polymorphism (SNP) chip (IDB; version 2; Mullen et al., 2013) were available for this study. The following quality controls were applied; any SNP, within breed, with less than 90% call-rate, a minor allele frequency less than 1%, resided on the sex chromosomes, or had an unknown position using the UMD3.1 reference genome were removed from the dataset using PLINK (Purcell et al., 2007). Only animals with a call-rate of >90% were retained for further analysis. Missing SNPs were then imputed using FImpute (Sargolzaei et al., 2014). Following quality control, 15 144 SNPs remained for 7 301 Limousin cows and 15 137 SNPs for 5 238 Charolais cows.

Phenotypes

The national genetic evaluations for beef cattle in Ireland are based on a multi-breed population and are undertaken using the MIX99 software. Estimated breeding values (EBVs) for 6 production traits including, carcass weight, carcass conformation, carcass fat, cull-cow weight, live-weight, and feed intake, as well as 7 functional reproductive related traits including docility, age at first calving, calving interval, gestation length, cow survival, calf mortality, and calving difficulty (dairy heifer calving difficulty; dairy cow calving difficulty; beef heifer calving difficulty and beef cow calving difficulty) along with their respective reliabilities were available for all cows from the national genetic evaluation obtained from the Irish Cattle Breeding Federation (ICBF; McCabe et al., 2019). Pedigree information of these animals was also available from ICBF; the pedigree was traced back 5 generations where available and consisted of 59 529 animals. The effective record contribution of each animal was estimated using the Harris and Johnson (2010) method. Deregression of the EBVs was undertaken

using the secant method with a full animal model in the Mix99 software suit.

Genome-wide association studies

Single-SNP genome-wide association analyses were carried out for each breed separately using a linear mixed model approach in GCTA (Genome-wide Complex Trait Analysis, Yang et al., 2011). The genomic relationship matrix was constructed following method 1 outlined by VanRaden (2008), which was included in the model to account for population relatedness. Following quality control procedures all remaining SNPs were scored as 0, 1, or 2 representing the number of copies of the allele each animal carries and were included as fixed effects in the model one at a time. The following model was used:

$$y = \mu + bx + u + e$$

where y is the deregressed EBV, μ is the mean, b is the fixed effect of SNP x , and $u \sim N(0, G\sigma_u^2)$ is the vector additive genetic effects, where G is the genomic relationship matrix and σ_u^2 is the additive genetic variance, and $e \sim N(0, I\sigma_e^2)$ is the vector of random residual error, where I is the identity matrix and σ_e^2 is the residual variance. Manhattan plots were created for each trait tested within each breed separately using the QQman package in R (Turner, 2014).

Meta-analysis

A meta-analysis was conducted for each of the traits across the two breeds using the weighted Z-score method in METAL (Willer et al., 2010); this method generates a Z-score for each SNP based on the p-values and the direction of the SNP effect. Z-scores for each SNP are combined across populations into a weighted Z-score:

$$Z = \frac{\sum z_i w_i}{\sqrt{\sum w_i}}$$

where w_i is the square-root of the sample size of breed i , z_i is the Z-score of breed i .

Gene annotation and functional analyses of significant SNPs

The chromosomal location of SNPs identified as significantly associated with the traits tested was used to determine the gene nearest to each SNP through utilisation of Bedtools software (version 2.27.1). Additionally the biological consequence of each significant SNP was determined using the Variant Effect Predictor tool within Ensemble, using the SNP ID as the search parameter.

Results

Genome-wide association analyses

A total of 77 SNPs were significantly associated with economically important traits tested in the current genome-wide association study (GWAS; adj. $P < 0.05$) across both breed types. Specifically, results comprised of 47 associations for Limousin and 30 associations for Charolais. Across each population significant associations were evident for calving interval, calf mortality, calving difficulty, carcass conformation, carcass fat, carcass weight, live-weight, feed intake and cull-cow weight. No significant associations were evident for docility, age at first calving, gestation length or cow survival across either population. In each of the Charolais and Limousin populations, 12 and 14 significant SNP associations, respectively, were identified with reproduction related traits. The majority of the significant associations identified within this study were between the Limousin population and the production traits (33 significant associations). Similarly a greater number of significant SNPs was

Table 1
Single nucleotide polymorphisms significantly associated with reproductive related traits in Limousin cows.

Trait	SNP ¹ ID	P-Value ²	Gene (nearby)	SNP location ³	
Calving interval	rs109286373	*	<i>EIF1B</i>	–7101	
	rs110065568	***	<i>MSTN</i>	Missense variant	
	IDBv2020000591	***	<i>MSTN</i>	Missense variant	
	rs385065278	***	<i>MSTN</i>	3' UTR variant	
Calving-difficulty (beef cow)	rs110240246	**	<i>LCORL</i>	–265508	
Calving-difficulty (beef heifer)	rs109986401	*	<i>ADRA1B</i>	153333	
	rs109434016	**	<i>PDGFA</i>	–50488	
	rs110879087	***	<i>FABP6</i>	27660	
	rs109443848	*	<i>ENSBTAG0000047127</i>	1353	
	rs132913613	**	<i>PIK3R2</i>	753	
	rs110834249	*	<i>TANC2</i>	Intron variant	
	rs209000640	*	<i>MAP2K6</i>	471237	
	Calf mortality	rs110756766	***	<i>NR5A2</i>	113980
		rs43706486	***	<i>MAPK14</i>	3' UTR variant

¹ SNP= Single nucleotide polymorphisms² P-values: *adjusted-P<0.05, **adjusted-P<0.01, ***adjusted-P<0.001³ SNP location relative to nearest gene

also identified for the production traits (18 significant associations) in the Charolais population when compared to the reproduction traits in the same population. Statistically significant associations are presented in Tables 1–4 for reproductive traits in Limousin cows, reproductive traits in Charolais cows, production traits in Limousin cows and productive traits in Charolais cows, respectively. Of the 77 SNPs identified in this study, 4 SNP associations were found to be commonly significant (adj. $P < 0.05$) with the same trait across each breed type. These included the rs110344317 SNP which was commonly associated with carcass weight in both breeds analysed, as well as rs110240246, which was commonly associated with carcass weight, cull-cow weight and live-weight across Limousin and Charolais populations. Additionally 6 SNPs identified across both breeds were found to be located within the *MSTN* genomic region, including those associated with calving interval, carcass weight, carcass conformation, carcass fat, feed intake and cull-cow weight in Limousin, as well as calving interval, calf mortality, carcass weight, live-weight and cull-cow weight in Charolais. Across all traits, the majority of significant SNPs within the Limousin and Charolais populations were associated with carcass weight (Fig. 1) and dairy-cow calving difficulty (Fig. 2), respectively.

META-analysis

Meta-analysis revealed 17 SNPs as commonly associated (adj. $P < 0.05$) with both reproduction and production related traits across each breed type population. Meta-analysis results are presented in Table 5

and similar to the individual GWAS analyses conducted, the majority of these significantly commonly associated SNPs were located within the *MSTN* genomic region.

Discussion

Reproductive performance is key to production efficiency and consequently is also a key determinant of profitability and overall economic and environmental sustainability of beef cow enterprises (White et al., 2015; Ahmadi et al., 2017). Genomic selection has proven effective for advancing genetic gain for economically important traits in livestock, with the level of genetic variation in beef cattle sufficiently large to allow for successful breeding programs for reproductive efficiency (Berry and Evans, 2014). Thus the objective of this study was to identify genetic variants associated with key determinants of reproductive efficiency in beef cows, including fertility and longevity. Additionally economically important production type traits were also evaluated. Results from this study identified 37 SNPs significantly associated with reproductive and production efficiency related traits across both Charolais and Limousin cattle. However only 2 of these SNPs were identified as common across the two breeds types analysed, these included rs110240246 and rs110344317 located within the genomic regions of the *LCORL* and *MSTN* genes, respectively. Similarly, the meta-analyses conducted across GWAS results for each trait also showed a clear similarity for the association of traits tested with both *MSTN* and *LCORL* SNPs. Together these results indicate the importance of these genomic

Table 2
Single nucleotide polymorphisms significantly associated with reproductive related traits in Charolais cows.

Trait	SNP ¹ ID	P-Value ²	Gene (nearby)	SNP location ³
Calving interval	rs110344317	***	<i>MSTN</i>	Stop gained
Calf mortality	rs110344317	***	<i>MSTN</i>	Stop gained
	rs109463390	*	<i>USP3</i>	–27516
Calving-difficulty (beef heifer)	rs384418600	***	<i>GRH</i>	Intron variant
	rs210381089	***	<i>GRH</i>	Intron variant
	rs109463390	**	<i>USP3</i>	–27516
	rs384418600	***	<i>GRH</i>	Intron variant
	rs210381089	***	<i>GRH</i>	Intron variant
Calving-difficulty (dairy cow)	rs384133137	**	<i>MAPK13</i>	3' UTR variant
	rs384418600	***	<i>GRH</i>	Intron variant
	rs210381089	***	<i>GRH</i>	Intron variant
Calving-difficulty (dairy heifer)	rs384133137	**	<i>MAPK13</i>	3' UTR variant

¹ SNP= Single nucleotide polymorphisms² P-values: *adjusted-P<0.05, **adjusted-P<0.01, ***adjusted-P<0.001³ SNP location relative to nearest gene

Table 3
Single nucleotide polymorphisms significantly associated with production related traits in Limousin cows.

Trait	SNP ¹ ID	P-Value ²	Gene (nearby)	SNP location ³
Live-weight	rs110240246	**	<i>LCORL</i>	−265508
	rs41650764	***	<i>ENSBTAG00000047821</i>	−108609
	rs718553050	***	<i>PMEL</i>	Missense variant
Carcass weight	rs42607469	**	<i>PTPRD</i>	1703065
	rs109567582	**	<i>LCORL</i>	−824021
	rs110240246	***	<i>LCORL</i>	−265508
	rs109090869	**	<i>LCORL</i>	−760779
	rs41650764	**	<i>retired</i>	−108609
	rs41624597	**	<i>PPP3CA</i>	201323
	rs110065568	**	<i>MSTN</i>	Missense variant
	IDBv20200000591	***	<i>MSTN</i>	Missense variant
	rs385065278	***	<i>MSTN</i>	3' UTR variant
	rs136293037	**	<i>ABCG2</i>	2549
	rs110344317	***	<i>MSTN</i>	Stop gained
	rs110233897	*	<i>SLC40A1</i>	41546
	rs110233897	***	<i>SLC40A1</i>	41546
	rs41638273	***	<i>SLC40A1</i>	15786
Carcass conformation	rs29012920	*	<i>PROC</i>	Intron variant
	rs110065568	***	<i>MSTN</i>	Missense variant
	IDBv20200000591	***	<i>MSTN</i>	Missense variant
	rs210928543	**	<i>MSTN</i>	3' UTR variant
	rs385065278	***	<i>MSTN</i>	3' UTR variant
	rs211389737	*	<i>MSTN</i>	3' UTR variant
	rs110344317	***	<i>MSTN</i>	Stop gained
	rs110065568	***	<i>MSTN</i>	Missense variant
	IDBv20200000591	***	<i>MSTN</i>	Missense variant
	rs385065278	***	<i>MSTN</i>	3' UTR variant
	rs110065568	***	<i>MSTN</i>	Missense variant
	rs110240246	*	<i>LCORL</i>	−265508
	rs41650764	**	<i>retired</i>	−108609
	Carcass fat	IDBv20200000591	***	<i>MSTN</i>
rs385065278		***	<i>MSTN</i>	3' UTR variant
rs110065568		***	<i>MSTN</i>	Missense variant
rs110240246		*	<i>LCORL</i>	−265508
Feed intake	rs110240246	**	<i>retired</i>	−108609
	IDBv20200000591	***	<i>MSTN</i>	Missense variant
	rs385065278	**	<i>MSTN</i>	3' UTR variant
	rs718553050	***	<i>PMEL</i>	Missense variant
Cull-cow weight	rs110240246	*	<i>LCORL</i>	−265508
	rs41650764	**	<i>retired</i>	−108609
	IDBv20200000591	***	<i>MSTN</i>	Missense variant
	rs385065278	**	<i>MSTN</i>	3' UTR variant

¹ SNP= Single nucleotide polymorphisms² P-values: *adjusted-P<0.05, **adjusted-P<0.01, ***adjusted-P<0.001³ SNP location relative to nearest gene

regions to both reproductive and production type traits in the two breeds of beef cows used in this study. Furthermore our results have also identified SNPs specific to each Limousin and Charolais breed type in relation to both reproductive and production traits evaluated.

As mentioned above the individual breed GWAS conducted in this study identified the rs110240246 SNP as significantly associated with reproductive and production efficiency related traits across both Charolais and Limousin cattle. This SNP, which is located in an intergenic region upstream of the *LCORL* gene, was associated with carcass weight, cull-cow weight and live-weight across both breeds. However, the direction of the SNP effect was opposing, with negative associations evident for Charolais and positive associations identified for the Limousin population, suggesting differential genomic regulation for these traits across the two breed types used in this study. An additional positive association between this SNP and beef-cow calving difficulty was also apparent within the Limousin cow population. The *LCORL* gene codes for a ligand dependent nuclear receptor co-repressor, functioning as a transcriptional repressor and the rs110240246 SNP has previously been implicated within other published studies. For example, Akanno et al. (2018) identified the rs110240246 SNP as associated with economically important traits including bodyweight, weaning weight, pre-weaning daily gain, average daily gain on feedlot, yearling weight, hot carcass weight, back-fat thickness, rib-eye area and lean meat yield in multi-breed and crossbred beef cattle. Similarly, Lindholm-Perry et al. (2011), Lu et al. (2013) and Lee et al. (2019) all reported associations of this SNP with production related traits including feed intake, gain, meat and carcass traits (Lindholm-Perry et al., 2011), bodyweight, metabolic bodyweight, hot carcass weight and rib-eye area (Lu et al., 2013) and weaning weight in Limousin cattle (Lee et al., 2019). Through further gene expression analyses Lindholm-Perry et al. (2013) suggested that

Table 4

Single nucleotide polymorphisms significantly associated with production related traits in Charolais cows.

Trait	SNP ¹ ID	P-Value ²	Gene (nearby)	SNP location ³
Carcass conformation	rs137811338	*	<i>DUSP10</i>	−147801
	rs109247499	*	<i>FST</i>	Missense variant
	rs382784953	*	<i>FST</i>	Intron variant
Carcass fat	rs137811338	*	<i>DUSP10</i>	−147801
	rs109247499	*	<i>FST</i>	Missense variant
	rs382784953	*	<i>FST</i>	Intron variant
Carcass weight	rs110240246	***	<i>LCORL</i>	−265508
	rs110344317	***	<i>MSTN</i>	Stop gained
	rs110240246	***	<i>LCORL</i>	−265508
Live-weight	rs43705628	**	<i>ENSBTAG00000037580</i>	−296478
	rs110344317	***	<i>MSTN</i>	Stop gained
	rs109636878	**	<i>LRP4</i>	Missense variant
	rs110240246	**	<i>LCORL</i>	−265508
Feed intake	rs137811338	*	<i>DUSP10</i>	−147801
	rs109247499	*	<i>FST</i>	Missense variant
	rs382784953	*	<i>FST</i>	Intron variant
Cull-cow weight	rs41257524	*	<i>ENSBTAG00000027937</i>	2426
	rs110240246	***	<i>LCORL</i>	−265508
	rs110344317	***	<i>MSTN</i>	Stop gained

¹ SNP= Single nucleotide polymorphisms² P-values: *adjusted-P<0.05, **adjusted-P<0.01, ***adjusted-P<0.001³ SNP location relative to nearest gene

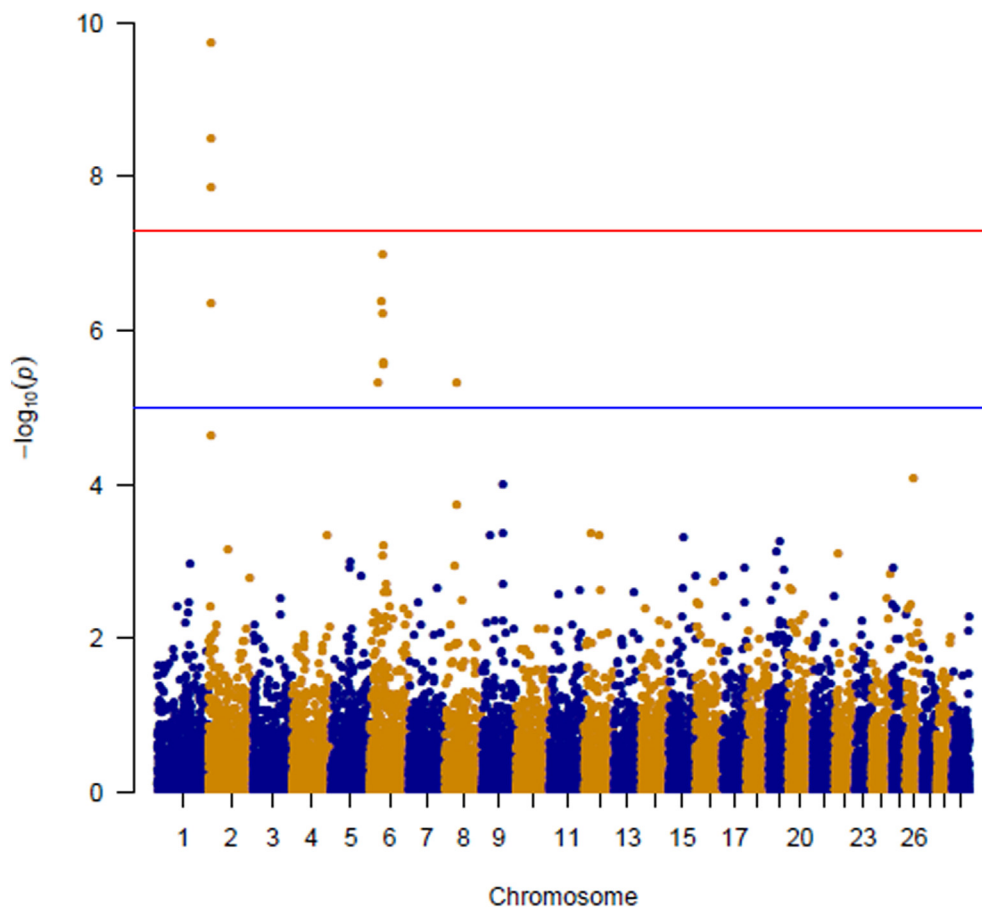


Fig. 1. Manhattan plot for carcass weight in Limousin cow population. The blue line indicates the suggestive P-value threshold ($-\log_{10}(1e-05)$). The red line indicates the Bonferroni genome-wide significance P-value threshold ($-\log_{10}(5e-08)$).

the *LCORL* gene contributes to the variation observed in feed intake and average daily gain in beef cattle. Additionally, our results also suggest an involvement of this SNP (rs110240246) in reproductive efficiency manifested through the association with beef-cow calving difficulty in the Limousin cow population. This same SNP was reported as associated with calving ease in Piedmontese cattle (Bongiorni et al., 2012). Together these results suggest a role for the rs110240246 SNP in reproductive efficiency in beef cows. In addition, two further SNPs (rs109567582 and rs109090869) located upstream of *LCORL* were identified as significantly positively associated with carcass weight within the Limousin population. Both of these SNPs were previously reported as associated with yearling weight in Korean cattle (Kim et al., 2013). The rs109090869 SNP has also been found to be associated with carcass weight (Li, 2012) and both bodyweight and yearling weight in Limousin beef cattle (Lee et al., 2019). Thus, our results reinforce the influence of these SNPs and the importance of the *LCORL* genomic region on production and reproduction related traits. However, despite results clearly showing an involvement of *LCORL* towards both reproductive and productive related traits the precise biological functions of this effect remain to be elucidated fully (Lindholm-Perry et al., 2011). Notwithstanding this, studies have suggested that the aforementioned effects may be mediated through the interaction of *LCORL* with ubiquitin C (Kim et al., 2011), which is involved in various cellular processes including cell cycle regulation and signal transduction (Kimura and Tanaka, 2010).

Although few SNPs were common between the breed types analysed in this study, results showed commonality within specific genomic regions were apparent between Charolais and Limousin cows for reproductive and production type traits. This was particularly evident within the *MSTN* gene on chromosome 2, where SNPs associated

with both reproductive and production efficiency traits were apparent. Additionally SNPs within this genomic region were also identified as commonly associated with reproductive and production traits across both breed types through meta-analyses, representing the majority of results from this analysis. The *MSTN* gene was first observed as a negative regulator of skeletal muscle mass in mice (McPherron et al., 1997). In cattle, this gene is responsible for muscular hypertrophy (Grobet et al., 1997; McPherron and Lee, 1997) and is widely known as the causal variant for multiple muscularity and carcass traits (Casas et al., 2000; Allais et al., 2010). For example, within the Charolais population the rs110344317 SNP, also known as the Q204X mutation, was associated with production related traits including live-weight, carcass-weight and cull-cow weight, as well as with reproductive related traits including, calf mortality and calving interval. Moreover, the same SNP was also associated with carcass weight and carcass conformation in the Limousin population. This specific mutation of the *MSTN* gene has previously been reported to be associated with production traits including carcass weight, fat and conformation in Charolais sires and carcass fat in Limousin sires (Purfield et al., 2019a), and with muscular development of hind quarter, inner thigh and the width of the thigh in both Limousin and Charolais cattle (Doyle et al., 2020). Indeed, Doyle et al. (2020) reported commonality between Charolais and Limousin breeds within the *MSTN* gene, suggesting that the genetic architecture of the muscularity traits tested in their study may be similar in those breeds due to the similar origins of the two breed types. Moreover, the relationship between *MSTN* SNPs and both reproduction and production type traits has previously been attributed to the role of this gene in muscle mass accretion which may impact the development of other tissues or organs within the body including the reproductive tract (Fiems, 2012).

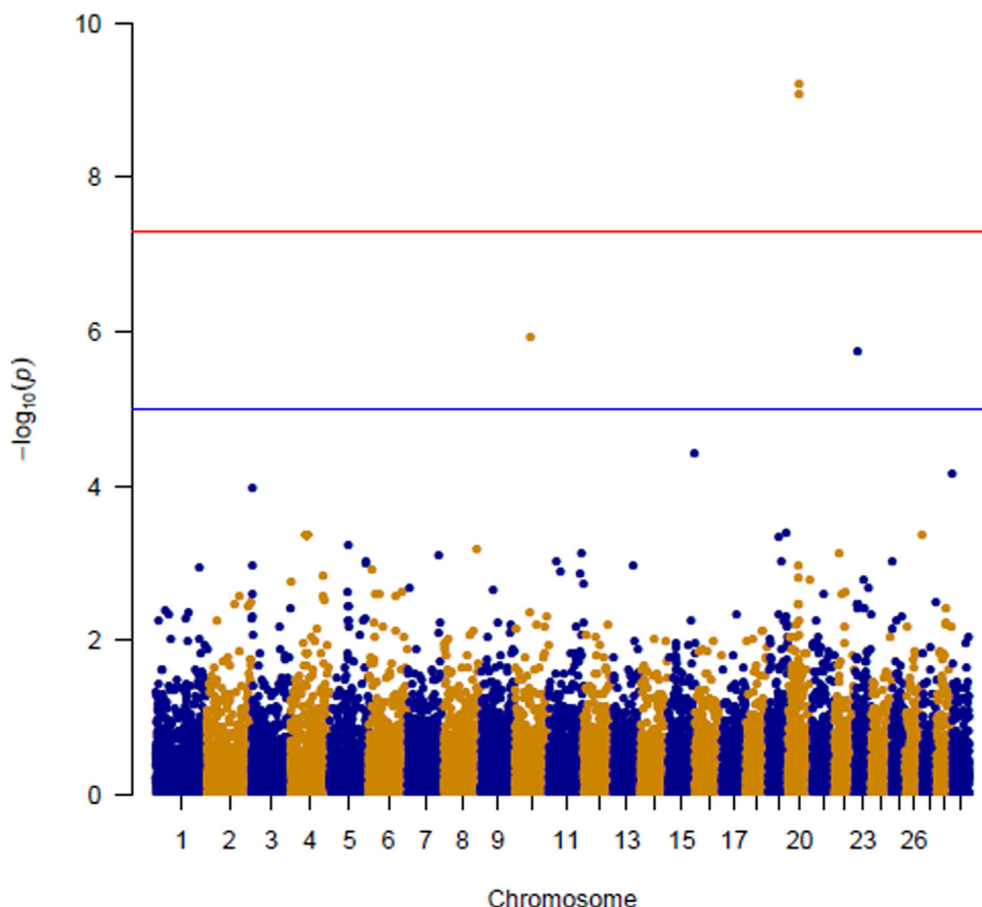


Fig. 2. Manhattan plot for dairy cow calving difficulty in Charolais cow population. The blue line indicates the suggestive P-value threshold ($P < 1 \times 10^{-5}$). The red line indicates the Bonferroni genome-wide significance P-value threshold ($P < 5 \times 10^{-8}$).

Additional SNPs within the *MSTN* genomic region were also identified in the Limousin breed as significantly associated with many of the economically important traits examined. These included rs110065568, which was associated with carcass conformation, carcass fat, carcass weight, calving interval and feed intake. The presence of this particular SNP has been found to result in increased muscularity and reduced

external and intramuscular fat (ICBF, 2016). Purfield et al. (2019a) recently reported an association of this SNP with carcass fat in Charolais sires. Moreover, Djari et al. (2013) reported the same non-synonymous SNP within the corresponding transcript in bovine skeletal muscle tissue. Further evidence for the involvement of this SNP with reproductive related traits was provided by Cushman et al. (2015) who observed an association of the rs110065568 SNP with puberty in beef heifers, suggesting that the presence of the SNP results in delayed puberty attainment in heifers. Another statistically significant SNP (IDBv 20200000591) from the current study within the Limousin population may result in double muscling, larger birth weights, increased dystocia and meat tenderness (ICBF, 2016). This SNP was specifically associated with carcass conformation, fat, weight, calving interval and cull-cow in the Limousin cow population used in this study. Additional SNPs including rs211389737 and rs210928543 were also associated with carcass conformation in Limousin cows in the current study. Overall our results further establish the influence of the *MSTN* genomic region in regulating both body growth and reproductive related traits in beef cattle.

A clear difference between the two breed types utilised in the current study was apparent through the difference in genes harbouring SNPs coding for production related traits including feed intake, carcass conformation and carcass fat. Within the Limousin cows, SNPs associated with these traits included those within or near the following genes: *PROC* (Protein C), *SLC40A1* (Solute carrier family 40 member 1) and *MSTN* for carcass conformation, and *MSTN* for both carcass fat and feed intake. However, the same three SNPs within the *DUSP10* (Dual specificity phosphatase 10) and *FST* (Follistatin) genes were associated with these traits within the Charolais population. These three SNPs included rs137811338 which lies upstream of the *DUSP10* gene

Table 5

Individual breed genome-wide association study results for all generic variants in Limousin and Charolais cows that reached genome-wide significance following meta-analysis.

Trait	SNP ¹ ID	P-value ²	Nearest gene
Calving-difficulty (beef heifer)	rs110879087	*	<i>FABP6</i>
Calf mortality	rs110344317	***	<i>MSTN</i>
Carcass conformation	rs110065568	***	<i>MSTN</i>
	rs110344317	***	<i>MSTN</i>
Carcass weight	rs109090869	**	<i>LCORL</i>
	rs109621511	*	<i>PLCXD3</i>
	rs110344317	***	<i>MSTN</i>
	rs110392766	*	<i>PKD2</i>
	rs41638407	*	<i>ARL15</i>
Calving interval	rs110344317	***	<i>MSTN</i>
Cull-cow weight	rs110344317	***	<i>MSTN</i>
Live-weight	rs109090869	*	<i>LCORL</i>
	rs109636878	*	<i>LRP4</i>
	rs110344317	***	<i>MSTN</i>
	rs110392766	**	<i>PKD2</i>
	rs41582594	**	<i>RNASEH2B</i>
	rs41625651	**	<i>SNX16</i>

¹ SNP= Single nucleotide polymorphisms

² P-values: *adjusted- $P < 0.05$, **adjusted- $P < 0.01$, ***adjusted- $P < 0.001$

and rs109247499 and rs382784953 SNPs, which are located within the coding region of the *FST* gene. The *DUSP10* gene codes for a protein involved in the regulation of MAPK (Mitogen-activated protein kinase) signalling pathway, ultimately functioning in the negative regulation of growth and proliferative processes (Mishra et al., 2017). Thus the association between a SNP located within this genomic region with traits aligned to growth may not be unexpected. However, the identification of SNPs within the *FST* gene and their association with such production related traits is somewhat unexpected given the direct function of the *FST* gene in reproductive processes as an inhibitor of FSH secretion and signalling (Gore et al., 2005). This is further established through previous reports of SNPs within the *FST* gene associated with reproductive related traits in cattle. Specifically, this included significant associations with somatic cell score (Cochran et al., 2013) and as well as with both heifer and cow conception rates (Ortega et al., 2016) with the rs109247499 SNP. However, although SNPs within the *FST* gene may represent novel SNPs for production related traits within Charolais cows, the identification of SNPs within the *SLC40A1* gene and their association with animal production related traits builds upon existing published knowledge (Saatchi et al., 2014; Higgins et al., 2018; Lee et al., 2019). For example, within the current study we identified two SNPs (rs110233897 and rs41638273), both of which lie downstream of the *SLC40A1* gene, as associated with carcass conformation in the Limousin population. Additionally, the rs110233897 SNP was also associated with carcass weight in the same population. The *SLC40A1* gene codes for ferroportin, which is involved in the process of transporting iron from the duodenal epithelial cells into the bloodstream as well as in the transfer of iron between maternal and fetal circulation (Donovan et al., 2005). Saatchi et al. (2014) also reported potential roles for these two SNPs in economically important traits in Limousin cattle, specifically the rs41638273 SNP was associated with ease of calving, birth weight, marbling and maternal weaning weight and the rs110233897 SNP associated with rib-eye muscle area, weaning weight and yield grade. Similarly, Lee et al. (2019) also reported associations of the rs11923897 SNP with bodyweight, marbling, rib-eye area and yield grade. Furthermore in another recent study from our group, the rs41638273 SNP was found to be implicated in feed efficiency in beef cattle (Higgins et al., 2018). These results suggest that SNPs within this genomic region may be involved in a number of economically important traits and thus warrant further investigation.

As outlined above, few SNPs were identified as commonly associated with the traits examined across both breed types through the individual GWAS. However notwithstanding this, results from this study do show commonality for the function of genes in genomic regions affected for similar traits across both Charolais and Limousin populations. This is particularly evident through the SNPs reported as associated with calving difficulty across both breeds, where SNPs within genes involved in the regulation of cellular division and growth processes were apparent for both breed types. Specifically these included *ADRA1B* (Adrenoceptor alpha 1B; rs109986401), a G-protein coupled receptor; *PDGFA* (rs109434016), platelet-derived growth factor subunit; *PIK3R2* (rs132913613), a phosphoinositide-3-kinase regulatory subunit and; *MAP2K6* (mitogen-activated protein kinase 6; rs209000640) all associated with beef heifer calving difficulty in Limousin cows and *GHR* (Growth hormone receptor; rs384418600, rs210381089), involved in the somatotrophic signalling axis, associated with both beef heifer and dairy cow calving difficulty; *MAPK13* (Mitogen-activated protein kinase 13; rs384133137), involved in MAPK signalling associated with dairy cow calving difficulty; and *USP3* (Ubiquitin specific peptidase 3; rs109463390) a ubiquitinase involved in S-phase of the cell cycle, associated with beef heifer calving difficulty in Charolais cows. The rs109986401 SNP downstream of the *ADRA1B* gene has previously been implicated with reproductive function in cattle. For example, this SNP was reported by Tenghe et al., 2016 as associated with the commencement of luteal activity in dairy cattle. Additionally, SNPs within genes of the

somatotropic axis signalling pathway have previously been shown to be associated with reproductive traits including services per conception and daughter pregnancy rate for *GHR* (Schneider et al., 2013) and resumption of ovarian cyclicity for *GHR*'s downstream effector, *IGF1* (Insulin-like growth factor 1; Nicolini et al., 2013). A similar outcome was also apparent for other traits tested including calf mortality and calving interval, where within the Limousin population SNPs associated with these traits were also within genomic regions including genes involved in growth processes. These include the rs43706486 SNP, associated with calf mortality, which lies within the *MAPK14* (Mitogen activated protein kinase 14) gene, which is involved in the MAPK signalling cascade for growth and proliferation. Additionally, the rs109286373 SNP, which is located upstream of the *EIF1B* (Eukaryotic translation initiation factor 1B) gene, was associated with calving interval. The *EIF1B* gene codes for a eukaryotic translation initiation factor, ultimately also involved in growth processes. In addition to SNPs within regions of the genome involved in growth processes, we also identified two SNPs involved in fatty acid and lipid metabolism and transport to be associated with calving difficulty and calf mortality in Limousin cows. Rs110879087 which is located downstream of the fatty acid binding protein gene; *FABP6* (Fatty acid binding protein 6), was significantly associated with beef heifer calving difficulty, whilst a separate SNP (rs1107567766) downstream of the *NR5A2* (Nuclear receptor subfamily 5 group A member 2) gene was associated with calf mortality. Additionally although not significantly associated within the Charolais population, the rs110879087 (*FABP6*) SNP was also significantly commonly associated with beef heifer calving difficulty between Charolais and Limousin cattle from out meta-analyses. These results indicate a relationship between genomic regions coding for genes involved in growth as well as lipid metabolism with reproductive related traits in the two populations tested, with the relationship particularly evident within the Limousin population.

Results from this well powered study provide evidence for genetic variants that are associated with traits involved in both reproductive efficiency and end-point production efficiency. The identification of SNPs associated with reproductive traits in two beef cow populations shows that there is potential for genomically assisted genetic selection to improve reproductive performance in beef cows. Our results clearly show genetic variants that are associated with both reproductive and production efficiency traits, it is important though that SNPs identified in this study be validated in independent populations of cows. This is of particular relevance given the heterogeneity of genetic variants and their respective impact across breed. Further validation and confirmation of the impact of the statistically significant SNPs identified in this study will facilitate their subsequent incorporation into genomic selection breeding programs for enhanced reproductive and productive efficiency of beef cattle.

Ethics approval

The data used in the present study originated from a pre-existing national database managed by the Irish Cattle Breeding Federation (ICBF); therefore it was not necessary to obtain animal care and use committee approval in advance. All animal information is routinely submitted by herdowners and other industry stakeholders as part of the national beef cattle breeding program in Ireland.

Data and model availability statement

None of the data were deposited in an official repository

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Author Contributions

DAK and MCMcC conceived the study and oversaw manuscript preparation. KK and TRC conducted the genetic analysis. SMW assisted with manuscript preparation.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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