GWAS and eQTL analysis identifies a SNP associated with both residual feed intake and GFRA2 expression in beef cattle

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Residual feed intake (RFI), a measure of feed efficiency, is an important economic and environmental trait in beef production. Selection of low RFI (feed efficient) cattle could maintain levels of production, while decreasing feed costs and methane emissions. However, RFI is a difficult and expensive trait to measure. Identification of single nucleotide polymorphisms (SNPs) associated with RFI may enable rapid, cost effective genomic selection of feed efficient cattle. Genome-wide association studies (GWAS) were conducted in multiple breeds followed by meta-analysis to identify genetic variants associated with RFI and component traits (average daily gain (ADG) and feed intake (FI)) in Irish beef cattle (n = 1492). Expression quantitative trait loci (eQTL) analysis was conducted to identify functional effects of GWAS-identified variants. Twenty-four SNPs were associated (P < 5 × 10−5) with RFI, ADG or FI. The variant rs43555985 exhibited strongest association for RFI (P = 8.28E-06). An eQTL was identified between this variant and GFRA2 (P = 0.0038) where the allele negatively correlated with RFI was associated with increased GFRA2 expression in liver. GFRA2 influences basal metabolic rates, suggesting a mechanism by which genetic variation may contribute to RFI. This study identified SNPs that may be useful both for genomic selection of RFI and for understanding the biology of feed efficiency.

Feed can account for more than 75% of variable costs of beef enterprises1. Consequently, selection of cattle that efficiently convert feed to carcass growth would improve farm profits due to reducing expenditure on feed while maintaining protein output2. Moreover, there is pressure on the agricultural industry to reduce methane emissions and improve its environmental footprint, while simultaneously increasing beef output to meet the growing demand for protein worldwide3. Selection for feed efficient cattle could increase beef output while concurrently decreasing methane production, as it has been suggested that low residual feed intake (RFI) (feed efficient) animals emit less methane than their high RFI counterparts4.

RFI is a measure of feed efficiency, defined as the difference between actual and predicted feed intake (FI)5. RFI has been shown to be moderately heritable, with an estimated heritability of 0.332,6, making it an ideal trait for selection as any genetic gain will be maintained and propagated through the cattle herd5. However, calculation of RFI is currently impeded by both the expense and logistics associated with its measurement, involving recording of both FI and body weight gain for each individual animal for a period of 70 days7. Identification of genetic markers for RFI and component traits, such as FI and average daily gain (ADG), and their incorporation into genomic assisted breeding programmes would enable more rapid and cost effective selection of feed efficient cattle8. Indeed, RFI has been incorporated into the Australian dairy industry’s genomic breeding programme9. Unlike the situation that predominates for dairy production systems worldwide, effective identification

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of selection markers of RFI for beef cattle must take into account a multiplicity of breeds and the mainly crossbred nature of cattle typically utilised within the global beef industry through employing multi-breed populations in order to identify variants of interest\(^{10,11}\). Differences in linkage disequilibrium (LD) between breeds may impact the association of markers and quantitative trait loci across breeds. The use of multiple breeds in a reference population is important to account for this variation in LD between breeds\(^{10}\).

In Ireland, the genomic assisted breeding programme is administered by the Irish Cattle Breeding Federation (ICBF)\(^{12}\). Markers for selection are included on the International Dairy and Beef (IDB) custom genotyping chip, which is based on the Illumina BovineSNP50 genotyping chip providing the IDB chip with genome-wide coverage\(^{13,14}\). As well as single nucleotide polymorphisms (SNPs) that are used for the genomic selection programme, the IDB chip contains SNPs for parentage verification and SNPs included for research purposes only\(^{15}\). This includes a selected subset of SNPs associated with RFI in cattle populations outside of Ireland\(^{16-21}\) which have been added to the IDB chip for research purposes to validate their use as biomarkers of RFI and associated traits in Irish beef cattle (n = 102, Supplementary Table S1).

Genetic markers for RFI can be identified via genome-wide association studies (GWAS). Several GWAS have identified SNPs associated with feed-efficiency-related traits in cattle populations, both purebred and crossbred, from North America, South America and Australia\(^{16,20-23}\). Despite considerable interest in identifying markers for RFI, ADG and FI, no published GWAS has been carried out to test for associations between SNPs and these traits in Irish beef cattle. In Ireland, commercial beef cattle are mainly crossbreds, with Charolais (CH), Limousin (LM), Aberdeen Angus (AA), Belgian Blue (BB) and Simmental (SI) breeds predominating genetically\(^{17}\). This breed heterogeneity coupled with the challenges in obtaining sufficient numbers of RFI phenotypes for GWAS are primary reasons for the difficulty in applying GWAS on a large-scale basis for RFI to beef cattle in Ireland and most other beef producing nations.

It is important to identify genetic variants that underlie phenotypic variation. One method to identify SNPs that are implicated in observed variation is by carrying out expression quantitative trait loci (eQTL) studies. eQTL analysis enables investigation of the effect of genotype on gene-expression levels which may in turn affect phenotype\(^{24}\). Previous eQTL analysis carried out in mammary tissue of dairy cattle has identified several eQTLs for milk production traits enabling the identification of genes such as PLAG1 and MGST1 as potentially functional in the development of divergent milk production traits\(^{25,26}\). eQTLs have been identified for temperament in the adrenal cortex of crossbred German cattle\(^{27}\). Despite the ability of eQTL analysis to identify potentially causative genes for complex traits, to the best of the authors’ knowledge no eQTL analysis has been carried out for RFI in beef cattle, or in any other livestock species. Liver and muscle are key tissues of interest with regards to feed efficiency as they are both large, metabolically active tissues accounting for approximately 24% and 25% of basal energy expenditure, respectively\(^{28,29}\). Thus, investigation into the presence of eQTLs in these tissues may aid in unravelling the biology underlying divergence in feed efficiency.

Due to multiple breeds of cattle present in beef production systems, it is important to identify markers for traits that have effects across multiple breeds\(^{30}\). Thus, the objectives of this study were to: (i) perform GWAS for RFI, and its component traits, namely FI and ADG, in different breeds of Irish beef cattle and combine results in order to identify associated SNPs in a mixed breed cohort, (ii) validate a selection of internationally identified markers of RFI present on the IDBv3 chip for utility as selection markers for RFI in Irish cattle and (iii) to investigate the effects of associated variants on gene expression in metabolically important tissues using eQTL analysis, in order to understand the biological mechanisms underlying divergence in RFI and component traits.

**Methods**

All biological sampling and procedures involving animals within this study were reviewed by the Teagasc Animal Ethics Committee and/or the UCD Animal Research Ethics Committee. All procedures carried out prior to 2013 were licensed by the Irish Department of Health, all procedures carried out since 2013 were licenced by the Irish Health Products Regulatory Authority in accordance with the cruelty to Animals Act 1876 and the European Communities (Amendment of Cruelty to Animals Act 1876) Regulations 2002 and 2005.

**Phenotypic data collation.** Data were collated for this study from growing bulls (n = 1823), steers (n = 459) and heifers (n = 164), which had previously undergone phenotypic measurement testing in Ireland between 2006 and 2017. The average ages and standard deviations for cattle included in the phenotypic data file were available were available on a group-by-group basis (Supplementary Table S2). Throughout each phenotypic measurement trial, the health of the animals was monitored. Any animal that required treatment was noted and excluded from further analysis. Phenotypes were gathered at the national beef research centre in Teagasc Grange; UCD Lyons Research Farm, University College Dublin and the ICBF national beef performance test station, Tully, Co. Kildare, Ireland. Phenotypes were collected from both purebred and crossbred beef cattle. For crossbred animals to be included in the phenotypic dataset the proportion of genetic material from a single parental breed had to be greater than 50%. Prior to further analysis cattle were grouped by breed. Predominant breeds were LM, CH, SI, BB and AA (n = 737, 499, 413, 191 and 174, respectively), other breeds were represented at smaller numbers. The RFI range for LM, CH, SI, BB and AA was 2.69 to −2.52, 2.70 to −2.48, 2.75 to −2.82, 1.63 to −2.00, and 2.87 to −2.64 respectively.

This resulted in the generation of a phenotypic file consisting of 2,446 cattle. For 429 of these animals, data relating to breed, diet, and methods used to calculate RFI, ADG and FI has been previously described\(^{4,28,30-33}\). Records for remaining cattle were made available by the ICBF from the national beef performance test centre at Tully, Co. Kildare, Ireland.

The management protocol of the ICBF animals is described briefly here. Animals were housed in pens for the duration of their test period which was between 70 and 105 days. A Calan gate system (American Calan, Northwood, NH, USA) was used to record individual animal FI. Bulls were individually offered *ad libitum*...
concentrates and 3 kg fresh weight of hay, while steers were offered 8 kg concentrates and 5 kg fresh weight of hay. Hay was offered in order to maintain healthy rumen function and to reflect an Irish commercial high concentrate based dietary regimen. Refused feed was weighed weekly and subtracted from total feed offered in order to calculate total feed consumed. Dry matter intake was calculated in order to determine FI, which was used for calculation of RFI. Cattle were weighed at the beginning and end of the test period, and every 21 days during the test period. ADG was calculated as the coefficient of linear regression of body weight on time, computed in the software package R34. Mid-test metabolic bodyweight (body weight0.75, MBW) was calculated as body weight0.75 in the middle of the RFI measurement period, which was estimated from the intercept and slope of the regression line after fitting a linear regression through all MBW observations. RFI was calculated for each animal as the difference between actual and predicted FI. Predicted FI for each animal was computed by regressing FI on MBW and ADG. Calculation of predicted FI was calculated for each contemporary group individually.

Genotyping. DNA was isolated for genotyping from one of two tissue types sourced from 429 cattle described previously. Muscle was used when blood was unavailable. Blood samples were obtained by jugular venipuncture at the end of the RFI measurement period, as per Fitzsimons et al.35, and stored at −80 °C prior to use1. Muscle samples were obtained via biopsy of the M. longissimus dorsi following the RFI measurement period, as per Kelly et al.35, and stored at −80 °C before DNA extraction. DNA from blood samples was extracted using the Maxwell 16 Blood DNA kit (Promega, Madison, WI, USA) as per manufacturer’s instructions. DNA was extracted from muscle samples using a phenol-chloroform extraction method. Briefly, 0.1 g of frozen muscle tissue was immersed in 1 mL of Trizol and homogenized using a Precellys 24 homogeniser. 200 µl RNA was extracted from muscle samples using a phenol-chloroform extraction method. Two volumes of ice cold ethanol were added to the aqueous phase and this mixture was centrifuged at 16,000 g for 15 minutes at 4 °C resulting in the formation of a DNA pellet. The supernatant was removed and the pellet was washed by the addition of 1 ml 70% ethanol and centrifugation the aqueous phase was transferred to a new tube. Two volumes of ice cold ethanol were added to the aqueous phase and this mixture was centrifuged at 16,000 g for 15 minutes at 4 °C resulting in the formation of a DNA pellet. The supernatant was removed and the pellet was washed by the addition of 1 ml 70% ethanol and centrifuged at 16,000 g for 5 minutes at 4 °C. Washing was carried out twice. Following washing, any remaining supernatant was removed and the pellet was left to air-dry. The DNA was then re-suspended in 150 µl RNase/ Dnase free H2O.

Once DNA was isolated, samples were analysed for quality and quantity using a Nanodrop spectrophotometer. DNA of sufficient quality for genotyping was available for 422 samples. All DNA samples were normalised to a concentration of 50ng/µl for genotyping analysis. Genotyping was carried out on the IDBv3 chip37 by Weatherby’s Scientific Ltd. (Johnstown, Naas, Co. Kildare, Ireland). The ICBF provided a further genotypes for 1,262 cattle that had been genotyped on the IDBv3 chip by Weatherby’s Scientific.

In addition to the 1,684 animals genotyped directly on the IDBv3 chip, 338 cattle were genotyped on the Illumina Bovine HD genotyping chip. These 338 cattle were imputed to IDBv3 density using Fimpute version 2.2.4. The reference population used for Fimpute was 50,000 Irish cattle with genotyped parents. Imputation of all 338 cattle was conducted across breed type to reflect the Irish national cattle population.

Preparation of files for analysis. Quality control (QC) was carried out on genotypes imported into the SVS environment. SNPs were removed from analysis if they had a call rate of less than 0.80 or a minor allele frequency of less than 0.05. Cattle were removed from analysis if they had a call rate of less than 0.95. Following QC, 2,008 cattle and 44,338 markers remained for analysis. LD pruning was carried out at r2 threshold of 0.5 and 7,841 markers were discarded following pruning37. The remaining 36,496 SNPs that passed all QC measures were acceptable for further analysis (Supplementary Table S3).

The collated phenotypic data were merged with the genotype data, creating a dataset containing 1,822 cattle eligible for analysis. A genomic kinship matrix was computed from the population, which was included as a covariate in the GWAS in order to account for relatedness. From this dataset, cattle from five beef breeds were analysed (n = 1492). The breeds included in the analysis were AA, BB, CH, LM and SI (n = 102, 177, 387, 537 and 289, respectively).

Genome-wide association studies. GWAS were carried out in the SVS environment of Golden Helix using a mixed linear model method, EMMAX38, for each breed individually. GWAS resulted in the generation of summary statistics for each trait of interest, i.e. RFI, ADG and FI, for each breed (AA, BB, CH, LI, and SI).

Meta-Analysis. Following initial breed specific GWAS, meta-analyses were carried out for each trait using the software package METAL39. METAL combines P-value and direction of effect from each GWAS to conduct Z-score method meta-analysis. METAL analysis results in two outputs, the Z-score for each SNP and a P-value for each SNP. A large positive Z-score results in a small P-value providing evidence that the allele positively associated with the trait under test. Conversely, a large negative Z-score results in a small P-value, showing an allele is negatively associated with the trait39. A P-value of less than 5 × 10−8 was used to denote genome-wide significance as per recent GWAS studies42.

Validation of internationally identified RFI SNPs in Irish beef cattle. The inclusion of internationally identified RFI SNPs (n = 102, Supplementary Table S1) in the current study enabled investigation of their role as markers for feed efficiency in an Irish population of beef cattle.

Functional annotation of genes. Functional annotation of candidate genes was carried out to gain insight into the underlying biology of RFI, ADG and FI. Database for Annotation, Visualisation and Integrated Discovery
(DAVID, version 6.8) was used for functional annotation. From the meta-analysis, a list of candidate genes was generated using Ensembl's Variant Effect Predictor. The list contained the nearest gene within a 500 kb window to each nominally significant SNP ($P < 0.05$). DAVID assigned genes to pathways as per the Kyoto Encyclopaedia of Genes and Genomes (KEGG), and determined enrichment of pathways using Fisher's exact test. In order to account for multiple testing, a Benjamini-Hochberg correction was applied. Pathways were deemed to be significant if they obtained a corrected $P$-value of $< 0.05$. Pathways specifically addressing human diseases and disorders were not included in further analysis of DAVID identified pathways, as these were not relevant to this study.

**eQTL analysis.** Samples for eQTL analysis were obtained from CH and Holstein-Friesian cattle that had been genotyped as part of the current study and for which RNA-Seq data were available within our group. The RFI range of the CH and Holstein-Friesian cattle were 1.48 to $-0.98$ and 1.48 to $-1.41$ respectively. RNA-Seq raw read counts were collated from liver and muscle tissue analyses carried out by our group in published studies and studies in preparation (Higgins et al., McKenna et al.) related to feed efficiency traits. Forty-two liver samples and 39 muscle samples were brought forward to eQTL analysis. For eQTL identification, liver and muscle samples were analysed separately.

Raw read counts were filtered and genes with more than 10 instances of zero expression were removed from analysis, resulting in 14,588 and 14,309 genes with expression in the liver and muscle, respectively, remaining for eQTL analysis. Filtered raw read counts were normalised using DESeq2's variance stabilizing transformation (VST) command. Covariates included in DESeq2 were batch, RFI status (i.e. high or low RFI) and breed. VST normalised counts were brought forward for eQTL analysis.

eQTL analysis was carried out using the R package Matrix eQTL. Only SNPs that reached genome-wide significance after meta-analysis ($n = 24$) and their nearest gene were considered for eQTL analysis. If a nearest gene was greater than 100 kilobases away from a SNP, this combination was not included in eQTL analysis. As part of eQTL analysis RFI, breed and sex were included as covariates. SNPs with a MAF of less than 0.1 or with known functions, i.e. missense mutations, were excluded, as were genes that were not expressed in either tissue of interest, i.e. liver or muscle. This resulted in 11 SNPs in the analysis. A Bonferroni correction was applied to account for the 11 SNPs. If an eQTL reached a $P$-value of $0.0045$ or less, it was considered significant after multiple test correction.

**Results**

**GWAS and meta-analysis for RFI.** GWAS results generated for RFI by meta-analysis are plotted in Fig. 1. Seven SNPs achieved genome-wide significance for RFI (Table 1). The SNP most associated with RFI was rs43555985, located at chromosome 8 position 69,658,202, a non-coding region 53.4 kb upstream from GFRA2. Two variants were within the start-stop coordinates of genes; intronic variant rs110418027 in SMC1B and 3′ untranslated region (UTR) variant rs43691372 in DIS3. The remaining four associated SNPs were located upstream or downstream of genes as indicated in Table 1. Functional annotation of genes containing or near to nominally significant SNPs for RFI using DAVID did not identify any enriched pathways that survived Benjamini-Hochberg correction.

**GWAS and meta-analysis for ADG.** GWAS results for ADG are illustrated in Fig. 2. A total of 14 SNPs reached genome-wide significance for ADG. The most significantly associated SNP was rs386023985 which is located at chromosome 19 position 48,916,589, 7.9 kb upstream from the ERN1 gene. One missense variant, rs136457441 in RPL26, was associated with ADG. One associated variant was synonymous, rs382426807 in STAT5A. Five intronic variants were associated with ADG in the genes: CSFRA2, ITFG1, TBC1D16, TLL1 and BCAS3. The remaining 5 associated SNPs were located upstream or downstream of genes as indicated in Table 1.
Discussion

Despite the economic and environmental benefits of RFI, the trait or indeed any measure of feed efficiency, is not widely adopted within breeding programmes for beef cattle due to the difficulty and expense associated with measuring feed intake. The identification of robust genetic markers of RFI applicable to several breeds, as well as crossbred cattle, would enable the traits inclusion in genomic breeding programmes. This study sought to identify
SNPs associated with RFI that could be applicable to Irish beef production enterprises as well as uncovering novel markers of potential use to international beef producers. To unravel the underlying biology causing phenotypic variation in feed efficiency related traits, we carried out eQTL analysis of GW AS-identified variants to study their effect on local gene expression.

| Trait | SNP ID         | Meta-analysis P-value | Direction of Effect | AA P-value | BB P-value | CH P-value | LM P-value | SI P-value |
|-------|----------------|-----------------------|---------------------|------------|------------|------------|------------|------------|
| RFI   | rs43555985     | 8.28E-06              | −−−−                | 2.14E-01   | 1.18E-01   | 5.30E-01   | 1.24E-03   | 2.39E-03   |
|       | rs41638273     | 1.08E-05              | ++++                | 1.65E-03   | 3.14E-03   | 5.79E-03   | 9.03E-02   | 1.74E-01   |
|       | rs109695205    | 1.61E-05              | ++++                | 3.02E-02   | 4.12E-02   | 1.09E-01   | 4.04E-02   | 2.35E-02   |
|       | rs110161277    | 2.76E-05              | ++++                | 1.68E-01   | 5.24E-03   | 1.41E-02   | 1.50E-01   | 8.58E-02   |
|       | rs110418027    | 4.34E-05              | −−−−                | 7.77E-02   | 8.29E-02   | 4.74E-02   | 3.92E-02   | 7.50E-02   |
|       | rs43691372     | 4.47E-05              | ++++                | 1.74E-01   | 5.36E-02   | 6.36E-01   | 8.67E-03   | 4.65E-03   |
|       | rs42820242     | 4.48E-05              | ++++                | 1.49E-01   | 2.74E-02   | 1.38E-02   | 1.86E-02   | 4.41E-02   |

Table 2. Individual breed GWAS results for all genetic variants that reached genome-wide significance following meta-analysis. SNP: single nucleotide polymorphism; RFI: residual feed intake; ADG: average daily gain; Direction of effect: direction of effect of the Illumina A allele; FI: feed intake; AA: Aberdeen Angus; BB: Belgian Blue; CH: Charolais; LM: Limousin; SI: Simmental.

Figure 2. Manhattan plot of meta-analysis results for ADG. Meta-analysis was carried out on GWAS results generated for five breeds of Irish beef cattle. The blue line indicates $P$-value $< 5 \times 10^{-5}$.

SNPs associated with RFI that could be applicable to Irish beef production enterprises as well as uncovering novel markers of potential use to international beef producers. To unravel the underlying biology causing phenotypic variation in feed efficiency related traits, we carried out eQTL analysis of GWAS-identified variants to study their effect on local gene expression.

rs43555985 was associated with RFI and is an eQTL of the GFRα2 gene in liver tissue. The minor allele of this SNP was associated lower RFI within each of the individual breed GWAS and following meta-analysis. The
minor allele of rs43555985 was also associated with increased expression of *GFRA2* following eQTL analysis. *GFRA2* is a cell-surface receptor that facilitates binding of a member of the glial cell-derived neurotrophic factor family. *GFRA2* knock-out mice are unable to digest food correctly, have impaired salivary secretion and gut motility and exhibit a slower growth rate than wild-type mice while having an increased basal metabolic rate. If increased *GFRA2* expression is associated with improved feed efficiency, the mechanism may involve lowering metabolic rates. Increased metabolic rate leads to increased energy requirements to carry out biological processes and to maintain physiological homeostasis, resulting in less consumed energy being used for growth. It has been illustrated previously that high-RFI lambs have a higher basal metabolic rate than their low-RFI (more desirable) counterparts and low-RFI heifers exhibited lower metabolic rates than their high-RFI (inefficient) counterparts. Further investigation and validation of rs43555985 prior to inclusion in genomic breeding programmes is required. Furthermore, rs43555985 is also located 79.9 kb upstream from *XPO7*. Post-hoc eQTL analysis for this gene illustrated that there is no statistically significant relationship between rs43555985 genotype and *XPO7* expression.

The second most statistically significant SNP for RFI, rs41638273, maps to a region of chromosome 2 that contains the *SLC40A1* gene. This region is also the site of a QTL for RFI which contains the myostatin gene. Specific mutations in the myostatin gene have been linked with increased muscle growth traits. Improved feed efficiency was associated with double muscled Angus steers by Cafe et al. when compared to lesser muscled...
**counterparts**\(^52\). \(\text{DIS}3\), a gene nearby to a variant associated with RFI in the current study, encodes a protein involved in RNA metabolism\(^53\) and has been linked with feed conversion efficiency in pigs\(^54\).

The minor allele of \(\text{rs386023985}\) was negatively associated with ADG following GWAS meta-analysis in the current study. Similarly, this variant was negatively associated with ADG within each individual breed GWAS conducted, this SNP also reached genome-wide significance within the LM individual breed GWAS. \(\text{rs386023985}\) has not previously been associated with ADG or other growth traits in cattle. The gene nearest to \(\text{rs386023985}\) is \(\text{ERN1} (\text{IRE1})\), a sensor of metabolic stress, is involved in the unfolded protein response\(^55\).

Copy number variation in \(\text{RPL26}\), a ribosomal protein gene, has been linked to RFI divergence in Holstein cows\(^56\). A variant identified in this study, \(\text{rs136457441}\), is a missense variant in \(\text{RPL26}\) causing an isoleucine to threonine change at amino acid position 67 in the \(\text{RPL26}\) protein. This variant, associated with ADG following meta-analysis in the current study and reached genome-wide significance for ADG within the CH individual breed GWAS, is located in exon 3 of \(\text{RPL26}\). \(\text{rs136457441}\) has been designated as tolerated by the Sorting Tolerant from Intolerant (SIFT) algorithm, which predicts whether amino acid substitutions effect protein function\(^57\). Further investigation into the functional effect of this mutation is required to elucidate its biological role in ADG.

### Table 4. Results from eQTL analysis of genome-wide significant SNPs in liver and muscle.

| SNP          | Nearest Gene | Trait | Liver P-value | Muscle P-value |
|--------------|--------------|-------|---------------|----------------|
| rs43555985   | \(\text{GFRA2}\) | RFI   | 0.0038\(^*\)  | 0.25           |
| rs109695205  | \(\text{NFAM1}\) | RFI   | 0.95          | 0.55           |
| rs110418027  | \(\text{SMC1B}\) | RFI   | 0.15          | Not expressed  |
| rs43691372   | \(\text{DIS3}\) | RFI   | 0.96          | 0.16           |
| rs386023985  | \(\text{ERN1}\) | ADG   | 0.99          | 0.23           |
| rs110780286  | \(\text{ITFG1}\) | ADG   | 0.83          | 0.23           |
| rs382426807  | \(\text{STAT5A}\) | ADG   | 0.94          | 0.72           |
| rs41592667   | \(\text{FRK}\) | ADG   | 0.22          | 0.19           |
| rs41630180   | \(\text{TLI1}\) | ADG   | Not expressed | 0.33           |
| rs137576435  | \(\text{BCAS3}\) | ADG   | 0.25          | 0.93           |
| rs109691080  | \(\text{MAP3K7CL}\) | FI    | Not expressed | 0.80           |

*\(\text{eQTLs were designated as significant if they reached } P < 0.0045.\)*

**Figure 4.** Boxplot representing the relationship between \(\text{rs43555985}\) genotypes and normalised liver gene-expression of \(\text{GFRA2}\). Presence of the minor allele of \(\text{rs43555985}\) is correlated with increased expression of \(\text{GFRA2}\). 0: GG; 1: GA; 2: AA.
An exonic variant associated with ADG is rs382426807, a synonymous variant in STAT5A. This gene encodes a transcription factor that can be activated as part of the somatotropic axis, which is the pathway involved in the secretion of growth hormone and skeletal muscle growth. STAT5A has been associated with increased live weight gain in Polish Black-and-White bulls and increased expression of the growth hormone receptor, an upstream activator of STAT5A, has been previously demonstrated in efficient beef heifers by Kelly et al. Five variants associated with ADG were located in introns of the following genes: TLL1, CSF2RA, ITFG1, TBC1D16 and BCAS3. TLL1 encodes a member of the tolloid family metalloproteases that have been previously implicated in the cleavage and development of myostatin in humans. Myostatin in its normal state negatively regulates muscle growth. The production of aberrant myostatin protein isoforms results in the development of the double muscle phenotype.

CSF2RA encodes for a granulocyte/macrophage colony stimulating factor. ITFG1, the gene within which rs110780286 is located, is involved in T-cell differentiation and may induce the production of anti-inflammatory cytokines. It has been previously illustrated that immune genes and immune pathways are associated with variation in feed efficiency and ADG in cattle. Several groups have suggested that the immune system plays a key role in weight gain and feed efficiency in cattle. For example, Reynolds et al. found that steers with higher ADG have lower immunity related gene expression and it has been demonstrated that cattle with poor feed efficiency had increased activation of their immune system. It is possible that cattle with poor feed efficiency and low ADG are experiencing chronic inflammation which results in poor feed efficiency which has been suggested previously by Alexandre et al. following analysis of beef cattle divergent in RFI and by Mani et al. upon investigation of inflammation in RFI divergent pigs.

rs41592667 is upstream of FRK which encodes for tyrosine-protein kinase FRK. Gene sets enriched for cell cycle-related genes, similar to FRK, have previously been shown to be associated with feed intake and feed efficiency in beef cattle. TBC1D16, a gene which encodes for a GTPase and contains the intronic variant rs109252082, has been associated with growth rate in pigs. Despite these SNPs not being associated with feed efficiency or component traits prior to the current study, they are near to, or within, genes that have been associated with feed efficiency related traits previously.

HNF1B, a transcription factor that can be activated as part of the somatotropic axis, which is the pathway involved in the secretion of growth hormone and skeletal muscle growth, has been associated with increased live weight gain in Polish Black-and-White bulls and increased expression of the growth hormone receptor, an upstream activator of STAT5A, has been previously demonstrated in efficient beef heifers by Kelly et al. Five variants associated with ADG were located in introns of the following genes: TLL1, CSF2RA, ITFG1, TBC1D16 and BCAS3. TLL1 encodes a member of the tolloid family metalloproteases that have been previously implicated in the cleavage and development of myostatin in humans. Myostatin in its normal state negatively regulates muscle growth. The production of aberrant myostatin protein isoforms results in the development of the double muscle phenotype.

Conclusion
In this study we illustrate genome-wide associations between SNPs and RFI and its component traits in beef cattle. In total, we identified 24 SNPs as reaching statistical significance for RFI, ADG and FI in a multi-breed cohort of beef cattle. Several of the SNPs identified in this study are located nearby or within genes related to immune function, muscle growth and development, and neurological pathways. The identification of a novel eQTL for RFI at GFRα2 also represents an insight into the biology of feed efficiency.

Due to the small sample size of our individual breed GWAS, which we used meta-analysis to overcome, all identified SNPs and the eQTL must be validated, both in larger Irish and international populations before
incorporation into genomic assisted beef cattle breeding programmes. Furthermore, validation is required in larger reference populations to account for the LD and genetic heterogeneity which exists between breeds of cattle.

An additional method which may have been employed to increase sample size could have been single step GWAS (ssGWAS)88–90. ssGWAS incorporates genotypes, phenotypes and pedigree information to calculate genomic estimated breeding values for animals with or without genotypes.88

It is important to ensure that the SNPs influence these traits and have no negative impact on other economically important production traits. SNPs with a validated desirable effect can be included in Irish and international genomic assisted breeding programmes to facilitate the rapid and cost effective selection of more feed efficient beef cattle.

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Author Contributions
Conceived and designed experiments: S.W., D.M., M.McG., D.K. Collated data: M.H., C.F. Performed analyses: M.H. Contributed to data/analyses: S.W., D.M., C.F., D.K., M.McG., M.McC., S.C., C.M. Results interpretation and preparation of the paper: M.H., S.W. All authors aided with manuscript edits. All authors agree to be accountable for all aspects of the work.

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