Journal of Dairy Research (2011) **78** 1–8. © *Proprietors of Journal of Dairy Research 2010*
doi:10.1017/S0022029910000567

Single nucleotide polymorphisms at the imprinted bovine insulin-like growth factor 2 (*IGF2*) locus are associated with dairy performance in Irish Holstein-Friesian cattle

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Received 29 April 2010; accepted for publication 9 July 2010; first published online 8 September 2010

The imprinted insulin-like growth factor 2 gene (*IGF2*) encodes a fetal mitogenic hormone protein (IGF-II) and has previously been shown to be associated with performance in dairy cattle. In this study we assessed genotype-phenotype associations between four single nucleotide polymorphisms (SNPs) located within the bovine *IGF2* locus on chromosome 29 and a range of performance traits related to milk production, animal growth and body size, fertility and progeny survival in 848 progeny-tested Irish Holstein-Friesian sires. Two of the four SNPs (*rs42196909* and *IGF2.g-3815A>G*), which were in strong linkage disequilibrium (r^2=0·995), were associated with milk yield ($P \leqslant 0{\cdot}01$) and milk protein yield ($P \leqslant 0{\cdot}05$); the *rs42196901* SNP was also associated ($P \leqslant 0{\cdot}05$) with milk fat yield. Associations ($P \leqslant 0{\cdot}05$) with milk fat percentage and milk protein percentage were observed at the *rs42196901* and *IGF2.g-3815A>G* SNPs, respectively. The *rs42196909* and *IGF2.g-3815A>G* SNPs were also associated with progeny carcass conformation ($P \leqslant 0{\cdot}05$), while an association ($P \leqslant 0{\cdot}01$) with progeny carcass weight was observed at the *rs42194733* SNP locus. None of the four SNPs were associated with body size, fertility and progeny survival. These findings support previous work which suggests that the *IGF2* locus is an important biological regulator of milk production in dairy cattle and add to an accumulating body of research showing that imprinted genes influence many complex performance traits in cattle.

Keywords: *Bos*, cattle, genetic imprinting, *IGF2*, performance traits, single nucleotide polymorphism.

Genetic studies have shown that the paternally inherited and maternally inherited genomes in eutherian mammals are not functionally equivalent. This is most aptly illustrated by genetic (or 'genomic') imprinting, a form of epigenetic regulation that results in the preferential expression of an allele from one of the two parentally inherited chromosomes in a parent-of-origin manner (McGrath & Solter, 1984; Surani et al. 1984). To date, nearly 100 imprinted mammalian genes have been identified, many of which play important roles in fetal growth and development (Bartolomei, 2009; Feil, 2009).

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The insulin-like growth factor 2 gene (*IGF2*), which encodes a fetal mitogenic protein (IGF-II) structurally related to insulin (O'Dell & Day, 1998), has been the most extensively studied imprinted mammalian gene owing to its pivotal role in the regulation of embryonic development and relationship to disease (Reik et al. 2000; Rodriguez et al. 2007). In mammals, *IGF2* is expressed preferentially from the paternally inherited allele in most embryonic tissues and forms a conserved imprinted gene cluster with the reciprocally imprinted, non-protein coding *H19* gene, which is highly expressed in embryonic and fetal tissue but whose function remains unclear (Bartolomei et al. 1991; Rachmilewitz et al. 1992; Giannoukakis et al. 1993;

Feil et al. 1998; McLaren & Montgomery, 1999; Dindot et al. 2004a; Dindot et al. 2004b; Zhang et al. 2004; Li et al. 2008). The detection of biallelic *IGF2* expression in certain mammalian post-natal and adult tissues, however, does suggest that monoallelic expression of this gene is both tissue- and developmental-stage specific (Dindot et al. 2004b; Curchoe et al. 2005; Goodall & Schmutz, 2007; Chao & D'Amore, 2008; Li et al. 2008).

The majority of studies involving *IGF2* have focused on mouse models and human biomedical disorders (Chao & D'Amore, 2008); however, there is growing interest in the role of *IGF2* in domestic livestock. For example, DNA sequence polymorphisms within *IGF2* have been shown to contribute to variation in complex production traits, notably muscle mass and fat deposition in pigs (Jeon et al. 1999; Nezer et al. 1999; Nezer et al. 2002; Van Laere et al. 2003). Since these early studies, a range of SNPs distributed across the porcine *IGF2* gene have been shown to be associated with carcass traits, meat production, body size, fertility and survival traits in pigs (Vykoukalova et al. 2006; Stinckens et al. 2007; Heuven et al. 2009; Oczkowicz et al. 2009; Hou et al. 2010; Stinckens et al. 2010).

According to the Ensembl database (http://www.ensembl.org), the current fully annotated bovine *IGF2* gene is located at the telomeric end of chromosome 29 (BTA29) and consists of five exons, the first two of which are untranslated (Ensembl gene ID ENSBTAG00000013066; Ensembl transcript ID ENSBTAT00000017372). Two alternative RNA transcripts have also been reported from this gene (Ensembl transcript IDs ENSBTAT00000044139 and ENSBTAT00000044140). Recently, there have been a number of publications detailing associations between DNA sequence polymorphisms in the bovine *IGF2* gene and meat and milk production traits in beef and dairy cattle, respectively (Flisikowski et al. 2007; Goodall & Schmutz, 2007; Sherman et al. 2008; Bagnicka et al. 2010), hence we hypothesized that DNA sequence polymorphisms within this gene may also be associated with performance traits within a population of progeny-tested Irish Holstein-Friesian artificial insemination (AI) sires.

Previously, we validated 15 SNPs in the bovine *IGF2* gene (Magee et al. 2010). In the present study, we investigated genotype-phenotype associations between four of these SNPs distributed across a ~31 kilobase (kb) region of *Bos taurus* chromosome 29 (BTA29) encompassing the bovine *IGF2* gene and genetic merit for a range of performance traits in 848 Irish Holstein-Friesian AI sires, estimated from progeny performance.

Materials and Methods

SNP validation

The methods used to validate DNA sequence polymorphisms within, or proximal to, the bovine *IGF2* gene have been discussed in detail elsewhere (Magee et al. 2010). Briefly, high-fidelity polymerase chain reaction (PCR) amplicons spanning putative *IGF2*-associated SNPs on BTA29 (as per build BTAU_4.0 of the bovine genome in the Ensembl database: http://www.ensembl.org) were generated for a panel of 26 animals of wide geographic provenance and sequenced bi-directionally (Macrogen Inc., Seoul, Korea). The MEGA 4.0 software package (Tamura et al. 2007) was used to analyse all resulting DNA sequences, validate the SNPs reported in Ensembl and identify novel SNPs in the re-sequenced regions.

In the current study, four validated SNPs distributed across a 30 827 base pair (bp) region spanning the bovine *IGF2* gene (Ensembl gene ID ENSBTAG00000013066) were selected for high-throughput genotyping: *rs42196909*, *IGF2.g-3815A>G*, *rs42194733* and *rs42196901* (Table 1). These SNPs were selected as they are distributed across the full length of the annotated bovine *IGF2* gene and displayed minor allele frequencies (MAF) $\geqslant 0{\cdot}18$ in a panel of 138 European *B. taurus* animals screened previously by us (Magee et al. 2010). Based on the current annotation of the bovine *IGF2* gene in the Ensembl database, two SNPs were located upstream of the *IGF2* gene (*rs42196909* and *IGF2.g-3815A>G*), one SNP (*rs42194733*) was located in an intron between the 2nd and 3rd exon of the *IGF2* gene, and one SNP (*rs42196901*) was located downstream of the *IGF2* gene. All SNPs represented transitions.

It is important to note that the *IGF2.g-3815A>G* is not currently deposited within the dbSNP database (http://www.ncbi.nih.gov/projects/SNP); however, this SNP has previously been reported by us (Magee et al. 2010), where it was listed as SNP *IGF2_08*. In the current study we have recoded this SNP using the guidelines of the Human Genome Variation Society (http://www.hgvs.org). This SNP was named by first listing the nearest gene (i.e. *IGF2*) followed by '*.g*' to denote genomic DNA, '*-3815*' to denote the position of this SNP relative to the transcriptional start site (i.e. 3815 bp upstream of the *IGF2* gene based on Ensembl transcript ID ENSBTAT00000017372) and finally '*A>G*' to denote the alleles present at this locus.

DNA samples, DNA extraction, high-throughput SNP genotyping and SNP data filtering

Genomic DNA from 914 Irish Holstein-Friesian AI bulls was purified using a Maxwell™ 16 automated apparatus (Promega Corp., Madison WI, USA) according to the manufacturer's instructions. These bulls have been used to produce progeny in Ireland and are representative of the commercial germplasm used in Irish dairy herds in recent years. All four *IGF2* SNPs were genotyped in all 914 sires (together with an additional 25 independently extracted, duplicate samples that were included for genotype quality control purposes) using the MassARRAY® iPLEX™ Gold genotyping platform provided by Sequenom Inc. (San Diego CA, USA; http//:www.sequenom.com). This SNP genotyping method discriminates between SNP alleles using

Table 1 Summary statistics for the *IGF2*-associated SNPs analysed across 848 Holstein-Friesian sires analysed in this study†

SNP ID	Location of SNP relative to the bovine *IGF2* gene	SNP location on BTA29	Genotypes	Genotype frequencies	Minor allele frequency	Deviations from HWE (*P* value)
rs42196909[1]	Upstream	51 250 879	GG AG AA	0·40 0·45 0·15	0·38 (A)	0·28
IGF2.g-3815A>G‡	Upstream	51 254 122	GG AG AA	0·40 0·45 0·15	0·38 (A)	0·28
rs42194733§	Intronic	51 263 683	CC CT TT	0·55 0·37 0·08	0·26 (T)	0·06
rs42196901§	Downstream	51 281 706	AA GA GG	0·79 0·19 0·02	0·12 (G)	0·02

† Genotype and allele frequencies, and the significance of deviations from Hardy-Weinberg equilibrium (HWE) based on *P*-values obtained from χ^2-test results are shown for all four *IGF2*-associated SNPs. All SNP nucleotide positions (on BTA29) were obtained from the Build 4.0 of the *B. taurus* genome sequence on the Ensembl database (http://www.ensembl.org, release 58, as per Ensembl gene ID ENSBTAG00000013066) or the UCSC genome browser (http://genome.ucsc.edu). All SNPs genotyped are located on BTA29. The open reading frame (ORF) gene model positions for each SNP are given
‡ Details of the SNP nomenclature used is given in the Materials and Methods section of the manuscript
§ The dbSNP accession for the analysed SNP

single base primer extension technology after which primer extension products are analysed using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectroscopy (http://www.sequenom.com/iplex). Following quality control on all genotype data (Waters et al. 2010) genotypes for 848 bulls remained. The SNP genotype concordance rate between technical replicate for these SNPs was 100%.

A range of phenotypic traits were analysed in this study and were categorized into seven broad categories: (1) milk production traits (milk yield, fat yield, protein yield, milk fat and protein percentage); (2) udder health (somatic cell count, SCC); (3) carcass traits (cow carcass weight, progeny carcass weight, subcutaneous carcass fat level and carcass conformation score); (4) growth and size related traits in live animals (stature, chest width, body depth, rump width); (5) subjectively assessed subcutaneous fat level on live animals (angularity and body condition score); (6) calving traits (direct calving difficulty, maternal calving difficulty, perinatal mortality); and (7) fertility and survival (calving interval and functional survival). Sire predicted transmitting ability (PTA) was the dependent variable for all traits with the exception of the milk production traits, including SCC, which were daughter yield deviations (DYDs) expressed on a PTA scale.

Models used in genetic evaluations in Ireland, as well as variance components, have been previously described in detail by Berry et al. (2009) and summarized by Waters et al. (2010). All PTAs were de-regressed using the procedure outlined by Berry et al. (2009). Only sires with a reliability score, less parental contribution, of >60% were retained for inclusion in the association analysis. A total of 742 sires fulfilled this criterion for inclusion in the analysis of milk, fat and protein yield as well as milk fat and protein concentration and the number of sires included for calving interval and survival was 501 and 477, respectively. The numbers of sires for direct calving difficulty, maternal calving difficulty, and perinatal mortality were 575, 506 and 201, respectively. The number of sires with a reliability of >60% for the carcass traits was 446 and the number of sires with a reliability of >60% for the size linear type traits varied from 484 to 551.

The association between each SNP and performance was quantified using weighted mixed linear models in ASReml (Gilmour et al. 2009), with individual included as a random effect, and average expected relationships among individuals accounted for through the numerator relationship matrix. Year of birth (divided into five-yearly intervals) and percent Holstein of the individual sire were included as fixed effects in the model. In all instances the dependent variable was de-regressed PTA or DYD, weighted by their respective reliability, less the parental contribution. Genotype was included in the analysis as a continuous variable coded as the number of copies of a given allele. The Haploview package (Barrett et al. 2005) was used to measure r^2 and D' values of linkage disequilibrium (LD) between pair-wise combinations of segregating SNPs (Lewontin, 1964; Hill & Robertson, 1968).

Results and Discussion

Summary statistics for the four analysed IGF2 locus SNPs

Summary statistics, including genotype and allele frequencies together with deviations from Hardy-Weinberg equilibrium (HWE), for each of the four genotyped *IGF2*

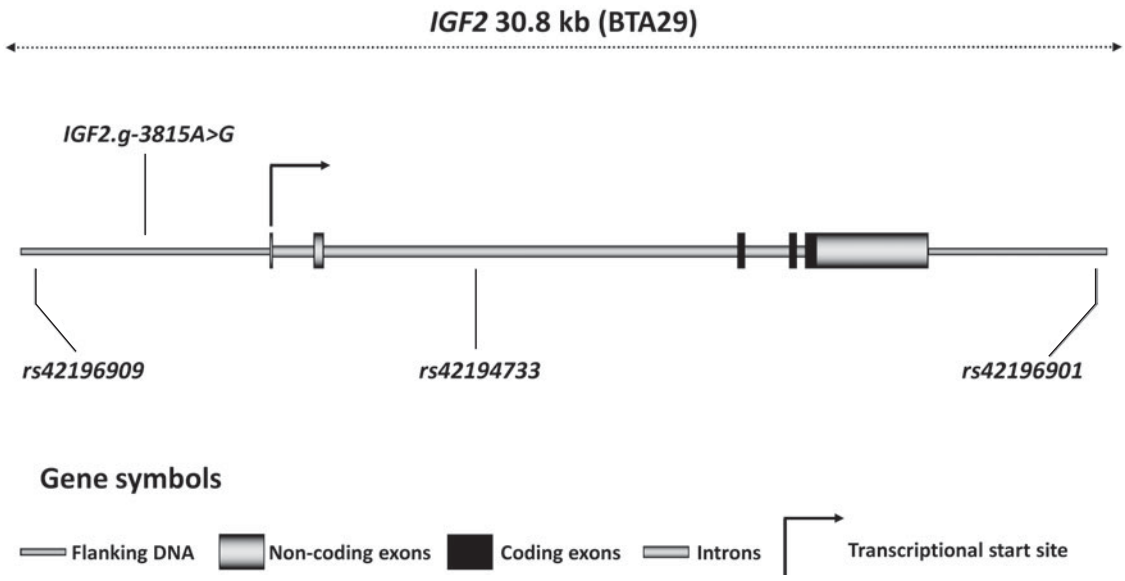

Fig. 1. Location of the four bovine *IGF2*-associated SNPs analysed in this study based on ORF model for the annotated *IGF2* gene transcript (Ensembl transcript ID ENSBTAT00000017372; BTA29) as per Ensembl release 58 of the *B. taurus* genome.

SNPs are presented in Table 1 and the location of all four SNPs is depicted graphically in Fig. 1. Minor allele frequencies (MAF) for all analysed loci were $\geqslant 0{\cdot}12$. Observed heterozygosity (i.e. the proportion of heterozygous individuals within the analysed sire population) ranged from 0·19 (*rs42196901*) to 0·45 (*rs42196909* and *IGF2.g-3815A>G*) with a mean observed heterozygosity of 0·37 across all four loci. Only one SNP (*rs42196901*) exhibited a significant deviation from HWE ($P=0{\cdot}02$). This was due to an observed excess of homozygous individuals for the 'C' allele (observed proportion of C/C individuals = 0·021; expected proportion = 0·013) and may be explained by random sampling.

Inspection of the r^2 values of LD for all pairwise SNP combinations (Table 2) demonstrated that the *rs42196909* and *IGF2.g-3815A>G* SNPs, which are separated by a total of 3243 bp, were in strong LD ($r^2=0{\cdot}995$), suggesting that they represent an *IGF2* haplotype. This is further supported by the identical allele and genotype frequencies observed at these markers (Table 1). All remaining pairwise SNP combinations displayed r^2 values $\leqslant 0{\cdot}230$ suggesting the existence of discrete haplotype blocks within the 30·8 kb genomic region analysed. LD statistics for each pairwise SNP combination are presented in Table 2.

Table 2. Pairwise SNP linkage disequilibrium (LD) statistics for the four *IGF2*-associated SNPs analysed in this study

SNP 1	SNP 1	Distance between SNPs in bp	r^2	D′
rs42196909	*IGF2.g-3815A>G*	3243	0·995	1·000
rs42196909	*rs42194733*	12 804	0·229	1·000
rs42196909	*rs42196901*	30 827	0·115	0·719
IGF2.g-3815A>G	*rs42194733*	9561	0·230	1·000
IGF2.g-3815A>G	*rs42196901*	27 584	0·116	0·731
rs42194733	*rs42196901*	18 023	0·027	0·737

Associations with carcass and body conformation traits

Significant phenotype-genotype associations for all SNPs analysed are detailed in Table 3. In the current study, one of the four bovine SNPs (*rs42194733*) within the bovine *IGF2* locus was significantly associated ($P<0{\cdot}01$) with progeny carcass weight: a T-to-C allele substitution at this intronic SNP was associated with gain in progeny carcass weight. The *rs42194733* was not associated with any of the other performance traits analysed in this study. In addition, the two SNPs located upstream of the bovine *IGF2* gene (*rs42196909* and *IGF2.g-3815A>G*), both of which were in strong LD, were associated ($P\leqslant 0{\cdot}05$) with overall carcass conformation—a visually assessed measure of animal muscularity (Butterfield, 1988). Both the G-to-A substitutions at the *rs42196909* and *IGF2.g-3815A>G* loci were associated ($P\leqslant 0{\cdot}05$) with small increases in progeny carcass conformation, which are indicative of increased muscle mass. With the exception of these associations, none of the four assayed SNPs were associated with any of the other carcass, body conformation and body size traits analysed (listed in the Materials and Methods section). Also, although a recent study reported associations between SNPs within the porcine *IGF2* gene and prolificacy and longevity in pigs (Stinckens et al. 2010), no associations with fertility or survival were observed here.

These observations suggest that the bovine *IGF2* locus may harbour a QTL for muscle mass in cattle, an assertion which is supported by previous animal genetic studies.

Table 3 Significant allele substitution effects between the four *IGF2* SNPs and milk traits and carcass traits. SE for each trait is shown in parentheses.

SNP	Allele substitution	Milk yield, kg/lactation	Milk protein yield, kg/lactation	Milk fat yield, kg/lactation	Milk fat percentage‡ (×10)	Milk protein percentage‡ (×10)	Carcass weight (kg)	Carcass conformation§ [scale 1 (low) to 15 (high)]
rs42196909	A→G	28·40** (9·89)	0·63* (0·27)	0·42 (0·35)	−0·13† (0·08)	−0·07† (0·04)	−0·37 (0·49)	−0·04* (0·02)
IGF2.g-3815A>G	A→G	30·31** (9·78)	0·66* (0·27)	0·51 (0·35)	−0·12† (0·07)	−0·07* (0·04)	−0·45 (0·48)	−0·04* (0·02)
rs42194733	C→T	11·59 (10·39)	0·31 (0·29)	0·25 (0·37)	−0·06 (0·08)	−0·02 (0·04)	−1·39** (0·52)	−0·03 (0·02)
rs42196901	G→A	1·91 (14·29)	−0·45 (0·40)	−1·03* (0·50)	−0·21* (0·11)	0·09† 0·05	0·76 (0·68)	0·04 (0·03)

‡ A value of 1 prior to multiplication by 1000 equates to 1 percentage unit
§ See Materials and Methods for details
Significance of difference from zero: †$P \leqslant 0{\cdot}10$; *$P \leqslant 0{\cdot}05$; **$P \leqslant 0{\cdot}01$

QTL mapping studies in cattle initially identified BTA29—the chromosome to which the bovine *IGF2* locus maps—as containing a QTL influencing muscle mass in cattle (Casas et al. 2003). More recently, genetic studies have revealed association between a single *IGF2* C-to-T SNP [designated *IGF2c.-292C>T* (Goodall & Schmutz, 2003)] and meat traits (including rib eye area and body fat content) and body weight in beef cattle (Goodall & Schmutz, 2007; Sherman et al. 2008). Also, Schmutz & Goodall (2005) reported that the C allele of this SNP was associated with lighter birth weight, while Sherman et al. (2008) have reported that Aberdeen Angus animals with the TT genotype for this marker displayed increased average daily weight gain.

Studies in pigs have also shown that DNA sequence variation in the porcine *IGF2* gene directly contributes to growth and carcass traits. Notably, a single G-to-A substitution within a regulatory region of the 3rd intron of the *IGF2* gene (termed '*IGF2 intron3 g.3072G>A*') which is directly responsible for a QTL influencing muscle mass and fat deposition in pigs; this SNP has since been classified as a quantitative trait nucleotide [QTN] (Van Laere et al. 2003). It is considered likely that the 'A' allele prevents binding of a transcriptional repressor protein to the *IGF2* gene; hence individuals inheriting a sire-derived 'A' allele at this locus display increased muscle growth and a corresponding reduction in body fat due to increased expression of padumnal *IGF2* mRNA (Van Laere et al. 2003; Stinckens et al. 2007). Other studies have demonstrated similar phenotypic effects attributable to this QTN in other pig populations (Jungerius et al. 2004; Estellé et al. 2005; Stinckens et al. 2007).

The observed phenotypic associations between DNA sequence polymorphisms within the *IGF2* locus and animal carcass and growth traits, as reported in the current study and elsewhere, are not surprising as it encodes an important fetal mitogen (DeChiara et al. 1991; Giannoukakis et al. 1993). While functional genetic studies have identified a causal mutation for muscle mass and fat deposition in pigs (Van Laere et al. 2003), no such causal mutations have yet been identified within the orthologous *IGF2* locus in cattle. It has been proposed that the observed associations between the *IGF2c.-292C>T* SNP and muscle mass and fat content could be due to the location of the SNP in a regulatory region of the *IGF2* gene, which may alter the efficiency of *IGF2* mRNA translation and stability (Goodall & Schmutz, 2007). Initially, this SNP was reported within the untranslated exon 2 of the bovine *IGF2* gene (Goodall & Schmutz, 2003; Goodall & Schmutz, 2007); however, inspection of the currently annotated bovine *IGF2* gene in the Ensembl database (Ensembl gene ID ENSBTAG00000013066; Ensembl release 58 of the *B. taurus* genome) locates the *IGF2c.-292C>T* SNP 54 bp before the start of the 5′ UTR of the *IGF2* gene (Ensembl transcript ID ENSBTAT00000017372). As per Build 4.0 of the *B. taurus* genome (Ensembl release 58), the *IGF2c.-292C>T* SNP is located at nucleotide position 51 257 871 on BTA29 and has not yet been deposited within the bovine dbSNP database (http://www.ncbi.nih.gov/projects/SNP). Despite the relocation of this SNP from an untranslated exon sequence to 54 bp upstream of the gene, it is possible that this polymorphism influences, or is linked to a polymorphism that regulates *IGF2* expression, thereby accounting for the observed associations with bovine carcass traits.

Notably, none of the SNPs analysed in this study were located in amino-acid coding exons of the *IGF2* gene or within untranslated *IGF2* exonic sequences. Indeed, only one SNP [(*rs42194733*; located in *IGF2* intron 2 based on the annotation of the bovine *IGF2* gene (Ensembl ID ENSBTAG00000013066)] was located within the *IGF2* gene region; all other SNPs were located upstream or downstream of the gene. As with the intronic *IGF2* QTN described in pigs by Van Laere et al. (2003), it is possible that the intronic *rs42194733* SNP has a similar function

in regulating *IGF2* expression, possibly through interference with repressor proteins; however, functional genetic studies are required to confirm this. It is more plausible, however, that this SNP is itself associated with a regulatory SNP (or set of SNPs) located proximal to, or within the *IGF2* locus.

Associations with milk performance traits

In the current study, significant associations between all four assayed SNPs and milk performance traits were detected (Table 3). The A to G allele substitution at the *IGF2.g-3815A>G* SNP was positively associated with both milk yield and milk protein yield, and was negatively associated with milk protein percentage. A tendency to be associated ($P \leqslant 0{\cdot}10$) with milk fat percentage was also evident at this locus; however, this SNP did not display any association with milk fat yield. Similar phenotypic associations with milk yield were also observed at the *rs42196909* locus, where an A-to-G substitution was associated ($P \leqslant 0{\cdot}05$) with improved milk yield and milk protein yield and tended to be associated ($P \leqslant 0{\cdot}10$) with reduced milk protein percentage. Again, the similar phenotypic associations support the existence of an upstream *IGF2* haplotype incorporating both the *IGF2.g-3815A>G* and *rs42196909* SNP loci. Finally, the A-to-G allele substitution at the *rs42196901* SNP, located downstream of the *IGF2* gene, was the only SNP associated ($P \leqslant 0{\cdot}05$) with increases in milk fat yield and milk fat percentage. A tendency to be associated ($P \leqslant 0{\cdot}10$) with increased milk protein percentage was also observed for this marker; however, this SNP was not associated with milk yield or milk protein yield.

Recently there have been a number of publications indicating that the bovine *IGF2* locus may also harbour a QTL for milk production traits. Ashwell et al. (2004) detected a QTL associated with milk production traits on BTA29; however, this QTL was localized to the centromeric region of BTA29, whereas *IGF2* maps to the telomeric region of this chromosome. More recently, Flisikowski et al. (2007) observed associations between an insertion/deletion polymorphism in the non-translated exon 6 of the bovine *IGF2* gene and estimated breeding values (EBV) for milk yield, milk fat yield and milk protein yield in Holstein-Friesian bulls. Furthermore, associations with milk traits including milk yield and milk constituents have been reported at haplotypes constructed from the C-to-T SNP located at position 51 257 871 and a G-to-T non-amino acid changing substitution in the translated exon 10 of the *IGF2* gene (nucleotide position 51 273 733 on BTA29) in Polish Holstein-Friesian cows (Bagnicka et al. 2010).

The open reading frame gene model location of the SNPs analysed here (two upstream, one intronic and one downstream) does not immediately suggest that these polymorphisms are functional. Instead, our results suggest that these *IGF2* SNPs are associated with a causal regulatory mutation (or set of mutations) located proximal to, or within the *IGF2* locus that has not yet been identified. It is noteworthy that previous studies have implicated *IGF2* as playing a role in mammary gland development. For example, local infusion of the IGF-II protein has been shown to increase milk production in goats (Prosser et al. 1994) while studies in mice have shown that locally secreted IGF-II mediates the effect of prolactin on mammary gland development (Hovey et al. 2003). Another possible candidate gene harbouring a QTL for milk production on BTA29 is the insulin (*INS*) gene, which encodes the insulin hormone peptide and is located $\sim 9{\cdot}3$ kb upstream of the bovine *IGF2* gene. It is possible that the observed associations reported here between SNPs within the *IGF2* locus may in fact be due to strong LD between the SNPs described here and unidentified functional polymorphisms associated with the *INS* gene. This hypothesis is supported by the role of the insulin protein in mediating mammalian gland development and lactation in dairy cattle (Akers, 2006).

Conclusion

The identification of DNA sequence variations associated with body composition and milk performance traits make the imprinted bovine *IGF2* gene an attractive target for future dairy cattle breeding programmes. The detection of association between a single genotyped SNP in the current study and traits related to animal growth also support this locus as harbouring a potentially important QTN(s) for beef breeding as observed in other cattle genetic studies and studies in other domestic species, most notably pigs. Furthermore, the results presented here add to an accumulating body of research showing that imprinted genes contribute to many complex performance traits in cattle and other livestock species. These findings, together with the documented biological roles of mammalian imprinted genes in mediating growth and development suggest that they represent an important reservoir of molecular markers for future genetic improvement of dairy and beef cattle populations.

This work was supported by a Research Stimulus Grant from the Irish Department of Agriculture, Fisheries and Food (project number: RSF-06-0406, RSF-06-0353; RSF-06-0409) and Investigator Programme Grants from Science Foundation Ireland (SFI/01/F.1/B028 and SFI/08/IN.l/B2038). MPM was funded by Science Foundation Ireland research grant number 07/SRC/B1156.

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