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Associations between the K232A polymorphism in the diacylglycerol-O-transferase 1 (DGAT1) gene and performance in Irish Holstein-Friesian dairy cattle

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Selection based on genetic polymorphisms requires accurate quantification of the effect or association of the polymorphisms with all traits of economic importance. The objective of this study was to estimate, using progeny performance data on 848 Holstein-Friesian bulls, the association between a non-conservative alanine to lysine amino acid change (K232A) in exon 8 of the diacylglycerol-O-transferase 1 (DGAT1) gene and milk production and functionality in the Irish Holstein-Friesian population. The DGAT1 gene encodes the diacylglycerol-O-transferase microsomal enzyme necessary to catalyze the final step in triglyceride synthesis. Weighted mixed model methodology, accounting for the additive genetic relationships among animals, was used to evaluate the association between performance and the K232A polymorphism. The minor allele frequency (K allele) was 0.32. One copy of the K allele was associated ($P < 0.001$) with 77 kg less milk yield, 4.22 kg more fat yield, 0.99 kg less protein yield, and 1.30 and 0.28 g/kg greater milk fat and protein concentration, respectively; all traits were based on predicted 305-day production across the first five lactations. The K232A polymorphism explained 4.8%, 10.3% and 1.0% of the genetic variance in milk yield, fat yield and protein yield, respectively. There was no association between the K232A polymorphism and fertility, functional survival, calving performance, carcass traits, or any conformation trait with the exception of rump width and carcass conformation. Using the current economic values for the milk production traits

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in the Irish total merit index, one copy of the K allele is worth €5.43 in expected profitability of progeny. Results from this study will be useful in quantifying the cost-benefit of including the K232A polymorphism in the Irish national breeding programme.

Keywords: allele substitution; dairy; DGAT1; Holstein

Introduction

The gene diacylglycerol-O-transferase 1 (DGAT1) is mapped to the centromeric end of BTA14 and encodes the diacylglycerol-O-transferase microsomal enzyme which catalyses the final step in triglyceride synthesis (Grisart *et al*., 2002). There is a general consensus in the literature that the alanine to lysine amino acid change (K232A) in exon 8 of the DGAT1 gene is associated with reduced milk production (Spelman *et al*., 2002; Thaller *et al*., 2003a; Banos *et al*., 2008), reduced milk protein yield (Spelman *et al*., 2002; Thaller *et al*., 2003a) and increased milk fat yield (Spelman *et al*., 2002; Thaller *et al*., 2003a). Less is known about the association between the K232A polymorphism (i.e., the presence or absence of the K232A mutation) and traits not related to milk production. Although there is limited evidence to suggest that there is no association between this polymorphism and body size (Spelman *et al*., 2002; Kaupe *et al*., 2007; Banos *et al*., 2008), some studies have reported an association with animal conformation (Spelman *et al*., 2002; Kaupe *et al*., 2007). The association between the K232A polymorphism (Grisart *et al*., 2002) at the DGAT1 locus and performance in Irish Holstein-Friesians has never been quantified. Accurate quantification of allele substitution effects on traits of economic importance facilitates their inclusion in genomic evaluation.

The objective, therefore, of this study was to quantify the association between the K232A polymorphism and milk production and functionality in Irish Holstein-Friesian dairy cattle.

Materials and Methods

DNA was isolated from semen samples of 848 Holstein-Friesian AI bulls with progeny in Ireland using the Maxwell 16 Instrument and the Maxwell 16 Tissue DNA Purification Kits from Promega (Southampton, UK). The automated procedure is preceded by an overnight incubation of the washed semen in lysis buffer (Heyen *et al*., 1997). Prior to genotyping, the quality and quantity of all DNA samples were assessed using a Nanodrop spectrophotometer and agarose gel electrophoresis. The bases underlying the K232A dinucleotide polymorphism are located at positions 10,433 and 10,434 of the DGAT1 gene (GenBank accession AJ318490). SNP genotyping was performed by Sequenom Inc. using their iPLEX® Gold technology.

In the present study, the description of the different DGAT1 alleles is based on the amino acids the genetic polymorphism encodes: lysine (K) or alanine (A). For comparative purposes across studies, the K allele, as defined in the present study, corresponds with the dinucleotide AA which is sometimes referred to as the Q allele (Spelman *et al*., 2002; Moore *et al*., 2003; Banos *et al*., 2008).

Data editing

Domestic predicted transmitting abilities (PTA), and associated reliabilities, for a

range of performance traits evaluated by the Irish Cattle Breeding Federation in January 2009 were used in the analysis. Models used in genetic evaluations in Ireland as well as the variance components used were summarised in detail by Berry *et al*. (2007). Predicted transmitting ability for predicted 305-day milk, fat and protein yield as well as geometric mean somatic cell score ($\log_e$ somatic cell count) are estimated in Ireland using a repeatability animal model across the first five lactations. Predicted transmitting ability for calving interval and survival are estimated using a multi-trait animal model, including data from the first three lactations. Predicted transmitting ability estimates for milk yield, predicted in a multi-trait analysis which includes survival and calving interval across the first three parities are used to adjust survival for differences in genetic merit for milk yield. Therefore, this survival trait, which is the measure used in the present study, is functional survival (i.e., genetically independent of milk yield). Genetic evaluations for calving ease are undertaken using a bivariate animal-dam model so that PTAs for direct and maternal calving ease are both generated. In the bivariate model the goal trait is calving ease scored by commercial Irish farmers and the predictor trait is calving ease scored prior to 2002 in progeny test and pedigree herds. A similar approach is used to estimate breeding values for gestation length and perinatal mortality with the exception that an animal model is used. Genetic evaluations for linear type traits are undertaken as part of a joint evaluation in the UK and Ireland. The estimated breeding values are standardised to the mean and standard deviation of the base population. Within trait, only bulls with a reliability (excluding parental contribution) of > 60% were retained for the association analysis.

A total of 742 bulls had a reliability of > 60% for milk, fat and protein yield as well as milk fat and protein concentration. The number of bulls exceeding this threshold for SCS, calving interval and survival was 703, 501, and 477, respectively. The number of bulls with a reliability > 60% for direct calving difficulty, gestation length, calf mortality and maternal calving difficulty were 575, 614, 201 and 506, respectively. The number of bulls with a reliability of > 60% for the linear type traits varied from 484 to 551.

Association analyses

The association between the K232A polymorphism and performance was quantified using weighted mixed 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. The following model was used:

$$\tilde{Y}_{ij} = \mu_j + YOB_i + \beta_1 Hol_i + \beta_2 K232A_i + a_i + e_{ij}$$

Where $\tilde{Y}_{ij}$ is the deregressed PTA of bull i for trait j; μ_j is the population mean for trait j; YOB_i is the year of birth of bull i (divided into 5 yearly intervals and included as a class effect); β_1 is the regression on Hol_i (Holstein proportion of bull i); β_2 is the regression coefficient on $K232A_i$ (representing the number of copies of the K allele); a_i is the random animal (i.e., bull) effect and e_{ij} is the random residual. Significance of the association with K232A was based on the Wald F-statistic with one degree of freedom. The pedigree of each bull was traced back at least four generations and the relationship matrix included 3205 non-founder animals. The

proportion of genetic variance in the trait under investigation explained by K232A was calculated as:

$$\frac{2p(1-p)a^2}{\sigma_a^2}$$

Where p is the frequency of the K allele in the population, a is the estimated allele substitution effect, and σ_a^2 is the genetic variance of the trait used in national genetic evaluations (Berry *et al*., 2007) on the PTA scale.

Results and Discussion

Population statistics

The frequency of the K/K, K/A, and A/A genotypes was 0.11, 0.42 and 0.47, respectively, and did not deviate from Hardy-Weinberg equilibrium (P = 0.32); the minor allele frequency (K) was 0.32. These genotype frequencies differ from those documented for Holstein-Friesian sires in New Zealand (Spelman *et al*., 2002). Animals of New Zealand ancestry are less related to animals of North American ancestry than Holstein-Friesians in Ireland (Dillon *et al*., 2007) and therefore differences in genotype frequencies are expected. This is substantiated by Spelman *et al*. (2002) reporting a greater frequency of the A allele as the proportion of "overseas" ancestry (i.e., genetic contributions originating from outside of New Zealand) increased. The genotype frequencies in the present study are consistent with those for Holstein-Friesian populations in the UK (Banos *et al*., 2008), Greece (Oikonomou *et al*., 2008), France (Gautier *et al*., 2007) and The Netherlands (Schennink *et al*., 2007). The difference in allele frequency among populations is likely a reflection of differences in past breeding goals. In New Zealand greater milk fat yield has been selected for and subsequently the frequency of the K allele in these animals is expected to be greater (Spelman *et al*., 2002; Thaller *et al*., 2003a). In most other countries milk volume has been selected for more aggressively with little or no emphasis on milk composition, thereby increasing the frequency of the A allele (Spelman *et al*., 2002; Thaller *et al*., 2003a; Banos *et al*., 2008).

Allele substitution effects on milk production

The K allele was associated with reduced milk yield and protein yield but greater milk fat yield, milk fat concentration and milk protein concentration (Table 1). Based on the substitution effects and allele frequencies in the current sample, the K232A polymorphism explained 4.8%, 10.3% and 1.0% of the genetic variance in 305-day milk, fat, and protein yield, respectively. Gautier *et al*. (2007) in their analysis of French Holstein cattle reported that the K232A polymorphism explained 13.1, 14.8 and 2.4% of the genetic variance in milk yield, fat yield and protein yield, respectively, while the corresponding values in Dutch Holstein-Friesian cattle were 22, 22 and 14% (Schennink *et al*., 2007). These estimates, including estimates from the present study, however, are likely to be the upper limit since the associations may include the effects of other loci in linkage disequilibrium with the K232A polymorphism. Other polymorphisms in the region of the K232A polymorphism with possible associations with milk production have been reported (Bennewitz *et al*., 2004; Gautier *et al*., 2007).

The allele substitution effects of the K232A polymorphism are consistent with most international studies (Spelman *et al*., 2002; Thaller *et al*., 2003a; Sanders *et al*., 2006) in Holstein-Friesian cattle. The size of the reported allele substitution effects in the present study are approximately half those from some other studies such

Table 1. Genetic standard deviation (on the scale of breeding values) as well as the allele substitution effect (A being substituted by K) and associated standard error of the K232A polymorphism

Trait	Genetic s.d.	Effect	s.e.
Milk yield (kg)	464	–77.08	10.21
Fat Yield (kg)	17.4	4.22	0.34
Protein yield (kg)	13.3	–0.99	0.29
Fat concentration (g/kg)	3.1	1.30	0.06
Protein concentration (g/kg)	1.5	0.28	0.04
Somatic cell score (log_e units)	0.205	–0.004	0.006
Calving interval (days)	8.5	–0.10	0.27
Survival (%)	2.8	2.34	1.47
Stature (s.d. units)	1	–0.018	0.092
Body depth (s.d. units)	1	–0.003	0.086
Chest width (s.d. units)	1	–0.113	0.098
Angularity (s.d. units)	1	0.062	0.095
Body condition score (s.d. units)	1	–1.688	1.155
Rump angle (s.d. units)	1	0.044	0.091
Rump width (s.d. units)	1	–0.195	0.095
Cow carcass weight (kg)	17.6	0.02	0.07
Carcass weight (kg)	21.6	–0.07	0.05
Carcass conformation (Scale 1 [poor] to 15 [excellent])	0.98	–0.089	0.022
Carcass fat (Scale 1 [thin] to 15 [fat])	0.70	–0.024	0.020

as Spelman *et al*. (2002), but the difference can be attributed to the dependent variable of Spelman *et al*. (2002) being daughter yield deviations on the estimated breeding value scale while the dependent variable in the present study was deregressed predicted transmitting ability (i.e., half the estimated breeding value). Even after accounting for this difference in scale, the allele substitution effects obtained in the present study were less than reported in other studies (Gautier *et al*., 2007; Kaupe *et al*., 2007). Possible reasons for differences among studies include 1) different allele frequency in the populations involved; 2) the statistical models used to undertake the association analysis as well as the statistical models and data (e.g., number of parities) used to estimate the dependent variables, 3) the mean performance of the animals or the environment to which they were exposed (i.e., genotype by environment interaction); and 4) the genetic background of the animals in the study and possible interactions between the K232A polymorphism and background genes.

Fewer studies have attempted to relate the K232A polymorphism to somatic cell count but, consistent with the results from the present study, both Näslund *et al*. (2008) and Kaupe *et al*. (2007) reported no association in Swedish or German Holsteins, respectively. Even after accounting for dilution (i.e., genetic merit for milk yield included as a covariate in the model) there was no association with SCS in the present study.

Allelic substitution effects on non-production traits

Despite the strong association between the K232A polymorphism and milk production, the lack of an association between the polymorphism and calving interval is in direct contrast to expectations based on the known antagonistic genetic correlations between milk production and fertility (Olori, Meuwissen and Veerkamp, 2002). Predicted transmitting ability for survival

in Ireland is for survival from lactation *i* to lactation *i+1* after post-hoc adjustment for genetic merit for milk yield. Therefore, PTA for survival in the present study is independent of PTA for milk yield. Few studies have attempted to quantify the association between the K232A polymorphism and fertility or survival. Kaupe *et al*. (2007) reported an unfavourable association between the K allele and 90-day maternal non-return rate in a granddaughter design with 18 paternal half-sib Holstein families. However, Oikonomou *et al*. (2008) reported no association between the K232A polymorphism and a similar trait, first service conception rate, in Greek Holsteins, but they did report a greater number of services per conception and lower conception rate in the first 305-days of lactation in animals with a second copy of the K allele, although in that study there were apparently no homozygous AA animals despite the sample consisting of 76% heterozygotes.

Corroborating the results from the present study, Kaupe *et al*. (2007) also reported an association between the K232A polymorphism and rump width; a wider rump was associated with the A allele in both studies. This association with the K232A polymorphism may be explained by the positive genetic correlation (0.46; s.e. = 0.058) between milk yield and rump width in Irish Holsteins (Berry *et al*., 2004) suggesting that either DGAT1 has a pleiotropic effect on rump width or is in linkage disequilibrium with a locus that affects rump width. The only other type trait reported previously to be associated with the K232A polymorphism was "strength" where the K allele was associated with reduced strength (Kaupe *et al*., 2007).

No association was evident between the K232A polymorphism and either direct or maternal calving difficulty. Similarly there was no association between the K232A polymorphism and either calf mortality or gestation length. This is in agreement with Kaupe *et al*. (2007) who also found no association between the K232A polymorphism and similar calving performance traits although they did not include direct gestation length in their analysis. There was no association between the K232A polymorphism and fore-udder attachment, teat length, teat placement viewed from the rear, udder depth or udder support in the present study.

The lack of an association between the K232A polymorphism and traits reflecting body fat (i.e., angularity, body condition score, carcass fat) is contrary to expectations since DGAT1 is involved in triglyceride synthesis (Cases *et al*., 1998) and there is indirect evidence from expressed sequence tag analysis, of its transcription in adipose tissue (Fries and Winter, 2002). Thaller *et al*. (2003b) reported an association between the K allele of DGAT1 and increased intramuscular fat in the *M. semitendinosus* in German Holsteins. However, it was not clear from the study what type of Holstein animal (bull, steer or heifer) was used, the number of animals included in the study was small (n = 28), and pedigree structure was not taken into account in the analysis. Furthermore, there was no association between the DGAT1 polymorphism and intramuscular fat levels of the *M. longissimus dorsi*. Kennedy, Quinton, and van Arendonk (1992) concluded that using ordinary least squares without accounting for relationships among animals, as undertaken by Thaller *et al*. (2003a), would inflate the F-test statistic and may thus increase the risk of Type I error. Agreeing with the present study, Cases *et al*. (2005), in a population of almost 500 Brahman (i.e., *Bos indicus*) steers and heifers, failed to find an association between the K232A polymorphism in DGAT1 and a range of

carcass traits including carcass backfat thickness and hot carcass weight.

Although none of the 497 primiparous Greek Holstein cows included in the association study of Oikonomou *et al*. (2008) appeared to be A/A at the DGTA1 locus, they reported a significant association between the K232A polymorphism and body condition score, where the second K allele was associated with lower body condition score. In agreement with the present study, however, others have failed to detect an association between the K232A polymorphism in the DGAT1 gene and body condition score (Banos *et al*., 2008), dairy conformation (Spelman *et al*., 2002) or dairy character (Kaupe *et al*., 2007); the latter two are traits similar to angularity. Similarly, Moore *et al*. (2003), using backfat measures on almost 500 male calves from three different selection lines, failed to identify an association between the K232A polymorphism in DGAT1 and backfat thickness.

Economic implications

While knowledge of the association between polymorphisms and performance can increase the accuracy of selection, the economic impact of selecting for a given polymorphism can be useful in quantifying the cost-benefit analyses of alternative breeding schemes. In the present study the economic effect of the K232A mutation was determined by summing the significant ($P < 0.05$) allele substitution effects times their respective economic values in the Irish total merit index (Berry *et al*., 2007). The association between the K232A polymorphism and carcass conformation was ignored as it has not been verified in an independent study.

Based on the current economic values, the allele substitution effect (allele A substituted by allele K) was €5.43 which decreased by €0.01 when the association with carcass conformation was included. If the milk price included in the bioeconomic model for the estimation of the economic values (Shalloo *et al*., 2004) was reduced by 10 c/L from the current 30 c/L, the value of the allele substitution would decrease (linearly) by €1.46.

Not considered, however, in the economic analyses is the impact of the K232A polymorphism on traits not included in the total merit index. Schennink *et al*. (2007), substantiating the predictions from a mathematical model of Shorten, Pleasants, and Upreti (2004), reported that the K allele is associated with an increased proportion of palmitic acid in milk fat and that the polymorphism explained 40% of the genetic variation in this component. The K allele was also associated with lower proportions of myristic acid, unsaturated fat and conjugated linoleic acid. No account is taken of the potential implications of milk fat composition for the risk of coronary heart disease (Mozaffarian *et al*., 2006), type II diabetes (Parillo and Riccardi, 2004), obesity or cancer (Bartsch, Nair and Owen, 1999; Zock, 2001; Willett and Leibel, 2002) in the determination of total merit index for Irish dairy cattle; this should not be ignored in deriving long term economic implications.

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