Genetic variation in *PLAG1* associates with early life body weight and peripubertal weight and growth in *Bos taurus*


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**Summary**

Variation at the *pleiomorphic adenoma gene 1* (*PLAG1*) locus has recently been implicated in the regulation of stature and weight in *Bos taurus*. Using a population of 942 outbred Holstein–Friesian dairy calves, we report confirmation of this effect, demonstrating strong association of early life body weight with *PLAG1* genotype. Peripubertal body weight and growth rate were also significantly associated with *PLAG1* genotype. Growth rate per kilogram of body weight, daily feed intake, gross feed efficiency and residual feed intake were not significantly associated with *PLAG1* genotype. This study supports the status of *PLAG1* as a key regulator of mammalian growth. Further, the data indicate the utility of *PLAG1* polymorphisms for the selection of animals to achieve enhanced weight gain or conversely to aid the selection of animals with lower mature body weight and thus lower maintenance energy requirements.

**Keywords** bovine, genetics, growth, *PLAG1*

In agriculture, optimizing the growth of livestock species is of key economic interest. In *Bos taurus*, selection of animals with enhanced growth rate and size is desirable in the case of beef production. In some dairy-farming systems, this may be undesirable when, for a given lactation output, increased animal size costs extra maintenance energy. The availability of tools to permit the selection of animals on this basis would therefore be of economic benefit. To this end, the identification of the genetic determinants of animal growth and related traits has been the focus of numerous genetic studies.

Karim *et al.* (2011) recently reported a genomic interval on *Bos taurus* chromosome 14 with a major effect on stature and weight. This observation builds on previous studies reporting quantitative trait loci (QTL) for growth and stature on chromosome 14 in taurine and mixed ancestry beef cattle (Mizoshita *et al.* 2004; Takasuga *et al.* 2007; Pryce *et al.* 2011). Karim *et al.* (2011) identified several tightly linked polymorphisms as potential causative genetic elements underlying this QTL and, using gene expression analysis, demonstrated functional consequences to two of these polymorphisms. These variants located to the bidirectional promoter of the *PLAG1* and *CHCHD7* genes. Given that *Plag1* knock-out mice display marked growth retardation (Hensen *et al.* 2004), these results suggest that differential expression of the *PLAG1* gene, under the control of *PLAG1* promoter variation, likely underlies the chromosome 14 stature and weight QTL.

The aim of this study was to test for *pleiomorphic adenoma gene 1* (*PLAG1*) genetic effects on animal size and growth in an independent, outbred population of dairy cattle. Furthermore, we aimed to assess whether growth effects mediated by variation in *PLAG1* translated to differences in feed intake, growth rate per kilogram of body weight and feed use efficiency in order to give further insight into the phenotypic consequences of *PLAG1* variation.

Animals used in this study were part of a purpose-bred trial designed to discover genetic markers for feed conversion efficiency and related traits. Female calves with a minimum of 15/16 New Zealand Holstein–Friesian (NZHF) parentage were selected at birth from the New Zealand national herd, descended from a total of 47 sires. Ninety-five per cent of the daughter population was represented by 25 sires only, and 50% was represented by only five sires. Calves were acquired at 4–7 days of age, with post-partum calf weights recorded at an average of 8 days after birth. Calves were raised by a commercial calf rearer in groups of 50 and, following weaning (approximately 90 kg), were
grazed on pastures until placement in the feeding facility at 6–8 months old. To mimic the nutritional profile of forage diets associated with grazing, animals were fed alfalfa (*Medicago sativa*) cubes. Animals entering the facility were pre-pubertal, and weight gain and feed intake were assessed over an interval of 50 days. The feeding facility consisted of 28 pens, each holding eight calves, with a single feed bunker per pen that restricted feed access to a single animal at a time. Calf weights were recorded three times a week during the trial, with feed intakes of animals measured per meal using an electronic monitoring station (Gallaghers Ltd).

Body weight mid-trial was calculated by linear regression of body weights measured through the course of the trial, taking the fitted value at 25 days (trial mid-point). Regression coefficients were calculated from the same equations to derive the daily growth rates of each animal. Daily body weight gain per kilogram of body weight (Kleiber ratio) was calculated by dividing the daily growth rates of animals by mid-trial body weight. Gross feed efficiency was calculated by dividing the trial-mean daily feed intakes by daily growth rates. Residual feed intake (RFI) was determined by multiple regression of daily feed intakes against the median metabolic body weight and daily body weight gain (independent variables), with individual RFI calculated from the difference between actual and fitted intakes derived from the model (residuals). A total of 942 calves with robust phenotypic variance explained by the ss319607402 SNP was calculated for each trait using \( (2p(1-p)\bar{l})/\bar{t} \) where \( p \) is the frequency of the A allele, \( a \) is the estimated allele substitution effect and \( \bar{t} \) is the total phenotypic variation.

High-quality genotypes were obtained for 921 animals and used for association analysis. The PLAG1 ss319607402 SNP was in Hardy–Weinberg equilibrium \((P > 0.01)\) and had a minor allele frequency of 0.15, consistent with the frequency predicted in outbred NZHP sires (Karim et al. 2011). PLAG1 ss319607402 genotype was strongly associated with body weight of new-born calves \((P = 1.5 \times 10^{-10})\), with the ‘A’ allele of this SNP over-represented in animals of increased size (Table 1), consistent with the direction of allelic association observed previously (Karim et al. 2011). Peripubertal body weight and daily growth rate were also significantly associated with PLAG1 ss319607402 genotype during the feeding trial period \((P = 1.9 \times 10^{-7} \) and \( P = 0.035 \) respectively; Table 1). Daily feed intake. Kleiber ratio, gross feed efficiency and RFI were not significantly associated with PLAG1 ss319607402 genotype \((P > 0.05; \) Table 1).

This study confirms the results of Karim et al. (2011), who showed that variation in the PLAG1 gene is predictive of animal weight in early life, and in growing, peripubertal animals. The growth rate of calves was also found to differ by genotype. Weight differences between genotype groups increased with age, with a 17.7-kg difference between adjusted means for homozygote classes in peripubertal animals versus a 6.9-kg difference in newborn calves. Although body weight differences appeared greater in peripubertal animals, differences as a percentage of total body weight were striking in newborn animals. Percentage increases in total body weight (relative to GG animals) were +18.8% (AA) and +10.4% (AG). It is noteworthy that there was no association found between Kleiber ratio and PLAG1 genotype. The Kleiber ratio describes the growth rate of animals relative to body weight; the lack of association may indicate that differences in growth rates derive partly from differences in animal sizes between PLAG1 genotype groups. These findings suggest that, in relative terms, much of the PLAG1 effect occurs during foetal development. This seems a reasonable proposition given that in humans and mice, PLAG1 is expressed most strongly in foetal tissues (Kas et al. 1997; Kas 1998; Hensen et al. 2004).
Allelic effects of \textit{PLAG1} in NZHF animals may not be strictly additive, with the differences between genotype groups suggesting possible partial dominance. This differs from the observations of Karim et al. (2011), although it could be a function of the limited number \((n = 12)\) of \(G\) allele homozygotes in the NZHF population. The low frequency of the \textit{PLAG1} ss319607402A\(\rightarrow\)G allele may have limited our power to detect the associations for intake-related effects. Some effect of \textit{PLAG1} genotype on intake, gross feed efficiency, RFI or all three of these traits was related effects. Some effect of \textit{PLAG1} genotype on intake, limited our power to detect the associations for intake.

\textit{PLAG1} has been implicated in the aetiology of human tumour formation (Hibbard et al. 2000; Martins et al. 2005), specifically through the dysregulation of gene expression (Kas et al. 1997). Homozygous \textit{Plag1} knock-out mice are viable but are 30\% smaller than wild-type litter mates at birth (Hensen et al. 2004). Subsequent growth rate is also lower in knock-out mice (Hensen et al. 2004), and recent genomewide association studies in humans have implicated the \textit{PLAG1} syntenic region in human height regulation (Gudbjartsson et al. 2008; Lango Allen et al. 2010). Gene expression changes arising as a result of bovine \textit{PLAG1} promoter variation, as demonstrated by Karim et al. (2011), could thus result in the growth modulation observed by Karim et al. (2011) and in the current study. Although some evidence suggests \textit{IGF2} as an effector molecule for \textit{PLAG1} (Voz et al. 2000), the precise molecular pathways through which \textit{PLAG1} regulates growth in \textit{Bos taurus} remain to be elucidated.

This study suggests robust association of \textit{PLAG1} genetic variation in determining bovine growth rates and animal size and further implicates \textit{PLAG1} as a major regulator of mammalian growth across species. These data support the utility of animal selection using \textit{PLAG1} markers, presenting the opportunity for the selection of faster-growing, heavier animals in beef production systems. For dairy populations such as the one assessed in the current analysis, selecting cows with reduced overall size may be preferred, owing to an anticipated corresponding reduction in energetic maintenance requirements.

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\textbf{References}


