Journal of Genetics, Vol. 96, No. 6, December 2017, pp. 1027–1031
https://doi.org/10.1007/s12041-017-0870-z

RESEARCH ARTICLE

Dominance effects estimation of *TLR4* and *CACNA2D1* genes for health and production traits using logistic regression

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Received 4 February 2017; revised 8 April 2017; accepted 12 April 2017; published online 8 December 2017

Abstract. Knowledge of nonadditive variance and genetic effects can be helpful in explaining the total genetic variation for most of the traits. The objective of this study was to estimate dominance effects of several single-nucleotide polymorphism (SNP) genotypes for the production traits and clinical mastitis residual (CMR), in Holstein dairy cattle in a case–control study. Records of 305 days lactation were obtained for production traits and CMR. Animals were selected based on extreme values for CMR from mixed model analyses. Samples were genotyped for four SNP-single genotypes and their associations with production traits (breeding values for protein and fat yield, and protein and fat percentage) were estimated by applying logistic regression analyses. Calculation of contrast between both homozygous and heterozygous genotypes permitted to estimate dominance effects, which ranged from −0.49 to 0.35 standard deviation units for the production traits and clinical mastitis (CM), respectively. Results showed that the dominance effects may be important in contribution of total genetic effects for production traits and CM. Therefore, evaluation of animals based on additive variance alone and disregarding nonadditive effects may lead to failure in selection programmes and exactly estimating the genetic variation. The method that we used would help breeders in accurately estimation of genotypic values in a new genomic selection scenario including dominance effects.

Keywords. dominance effects; selective genotyping; production traits; dairy cattle.

Introduction

Keeping milk producers competitive in dairy products is economically related to reducing mastitis losses. There are some methods to reduce mastitis incidence such as therapeutic and prophylactic measures, but they are not completely effective. Natural genetic resistance to udder pathogens is another approach to improve mastitis resistance (Detilleux 2002). Activation of neutrophils is the most important way to induce innate immunity, which is related to expression of several genes. Neutrophils migrate from blood to milk and affect the mammary gland immunity. Therefore, genes associated with neutrophil functions can be used as mastitis genetic markers (Paape *et al.* 2000). Toll-like receptors play crucial roles as pattern recognition receptors (*PRRs*). Molecules broadly shared by pathogens, but distinguishable from host molecules, are recognized by toll-like receptors (TLRs). Identified candidate genes such as toll like receptor4 (*TLR4*) and calcium channel, voltage dependent, alpha-2/deltasubunit1 (*CACNA2D1*) play one or multiple roles in the host's immune system to improve mastitis incidence. Reinhardt and Lippolis (2006) suggested an essential role for mammary gland parenchyma in pathogen detection due to the identification of TLR4 on milk fat globule membranes. Besides, TLR4 is involved in a signal transduction pathway to mediate *Escherichia coli* pathogenesis of mastitis (De Schepper *et al.* 2008). Also *CACNA2D1* is an important gene that is involved in resistance to clinical mastitis (CM) which is described by Buitkamp *et al.* (2003).

For low-heritable traits such as health and reproduction, genetic variation amount of nonadditive effects is unclear in dairy cattle. As we know, dominance as a nonadditive genetic effect arises due to the interactions between alleles at the same locus. Also, following the

theoretical background of genetics, dominance higher values are supposed to be for low-heritable traits such as mastitis. Dominance appears whenever the effects of alleles are additive and interact at a locus. Thus, the value of the heterozygous genotypes deviates from the mean values of homozygous genotypes (Falconer and Mackay 1996).

Until recently, there have not been a sufficient survey on dominance deviations and also the informative data have been sparse. Since having a large dataset with sufficient proportions of individuals with nonnull dominance effect relationships, such as full-sibs, is necessary to estimate dominance component, studies have been based on species like fish, poultry and pig (Misztal 2001; Dufrasne *et al.* 2014; Nagy *et al.* 2014). But, recently, estimation and use of dominance effects are encouraged by using DNA marker approaches in other species (Su *et al.* 2012; Vitezica *et al.* 2013; Ertl *et al.* 2014; Bagheri and zahmatkesh 2017).

In the present study, to estimate the effects of dominance for health and production traits, we combined the logistic regression analysis and selective genotyping data. In this way, the heterozygous genotype was contrasted to both homozygous variants.

Materials and methods

Data

The data were collected from 3823 Holstein dairy cattle based on CM. For the case of lactation per cow, a complete lactation of 305 days (lactations 2, 3, 4 and 5 were used, and due to the lack of previous data, first lactation was excluded) was used. Observation of obvious infections in the udder and presence of flakes in the milk were considered as CM (Gernand *et al.* 2012). More description about the data structure has been presented in Bagheri *et al.* (2013). Finally, 1647 CM cases were recorded and two extreme groups including 135 cows per group were used for selective genotyping based on values of clinical mastitis residuals (CMR). A multiple-trait animal model including 305-day lactation yields of the first lactation was used for fat and protein yield. For fat and protein percentage, estimated breeding values (EBVs) were calculated using deterministic equations. More information has been presented in Bagheri *et al.* (2013).

Genotyping and statistical analysis

Genomic DNA was extracted by an optimized salting-out method (Miller *et al.* 1988). Genotyping was performed using PCR-SSCP for *TLR4(1)* (chromosome 8, exon 2, 316 bp) and PCR-RFLP by *Alu*1, *Rsa*1 and *Taq*1 restriction enzymes, respectively for *TLR4(2)* (chromosome 8, partial fragment of exon 3, 382 bp), *CACNA2D1(1)* (chromosome 4, exon 5, 322 bp) and *CACNA2D1(2)* (chromosome 4, exon 15, 386 bp). More information on experimental conditions can be found in Bagheri *et al.* (2013). To estimate the SNP effects, selective genotyping analysis was carried out using the approach proposed by Henshall and Goddard (1999). Differences between the genotypes in case and control groups were determined by two-sample *t*-test with $\alpha \leq 0.05$. Estimation of SNP effects were carried out by logistic regression analyses using SAS Glimmix macro (Schabenberger 2007). Briefly, a statistical model was applied to estimate the probability of genotype GC versus genotype CC and genotype GC versus genotype GG. The model is as follows:

$$\text{logit}(\pi_r) = \log\left[\frac{\pi_r}{1-\pi_r}\right] = a + bY_r,$$

with π_r denoting the probability of the genotype, e.g. AG of a cow (r), 'a' is the intercept, 'Y_r' is EBVs for production traits or CMR and 'b' is the linear regression coefficient of the genotype, e.g. AG on EBVs or on CMR.

The contrast (α) of the heterozygous genotype and homozygous genotypes was estimated as described by Sharma *et al.* (2006) using below equation:

$$\alpha = \frac{-1 + \sqrt{1 + b^2\sigma^2 X}}{b},$$

where $\sigma^2 X$ is the variance of the EBV or CMR in the unselected base population.

Dominance effects estimation

Both homozygous genotypes (e.g., GG and CC) were contrasted to the heterozygous genotype, i.e. GC in two consecutive runs. With knowledge of both contrasts, we were able to estimate the effects of dominance (*d*) (Falconer and Mackay 1996) for EBVs of protein and fat yield, protein and fat percentage, and also for CMR.

Results and discussion

All four loci were biallelic and resulted in three different genotypes. Determined SNPs were as follows: NG_011475.1:g.13843A>G for TLR4(1), NG_011475.1:g.14143C>T for TLR4(2), AC_000161.1:g.367400C>T for CACNA2D1(1) and AC_000161.1:g.496561A>G for CACNA2D1(2). Both homozygous genotypes for all SNPs were contrasted to the heterozygous genotypes in two consecutive runs. Details of contrasts for all four SNPs have been given in Bagheri *et al.* (2013). The dominance effects were estimated based on differences in EBVs of production traits and in CMR, resulting from the comparison of the heterozygous genotype to both homozygous genotypes and the results are presented in tables 1 and 2. The effects of dominance for EBVs of fat percentage for *TLR4(1,2)* and *CACNA2D1(2)* were negative, but for *CACNA2D1(1)* was positive. For *TLR4(2)*, all

Table 1. Estimates of dominance effects for the EBV in protein and fat yield, and protein and fat percentage and in CMR for the SNP genes *TLR4(1)* and *TLR4(2)*.

Trait	Dominance effect
TLR4(1)	
Protein yield EBV (kg)	7.22 (0.35)
Fat yield EBV (kg)	0.51 (0.01)
Protein % EBV	0.03 (0.2)
Fat % EBV	−0.35 (−0.49)
CMR*	0.09 (0.10)
TLR4(2)	
Protein yield EBV (kg)	−2.4 (−0.11)
Fat yield EBV (kg)	−1.52 (−0.06)
Protein % EBV	−0.03 (−0.26)
Fat % EBV	−0.3 (−0.42)
CMR	0.04 (0.04)

*CMR, 1/4 residuals for the cases of CM per lactation after accounting for effects of herd, parity, and production level in milk yield (Bagheri *et al.* 2013).

Table 2. Estimates of dominance effects for the EBV in protein and fat yield (kg) and protein and fat per cent and in CMR for the SNP genes *CACNA2D1(1)* and *CACNA2D1(2)*.

Trait	Dominance effect
CACNA2D1(1)	
Protein yield EBV (kg)	0.81 (0.03)
Fat yield EBV (kg)	0.49 (0.01)
Protein % EBV	0.02 (0.16)
Fat % EBV	0.06 (0.08)
CMR*	−0.18 (0.19)
CACNA2D1(2)	
Protein yield EBV (kg)	0.64 (0.01)
Fat yield EBV (kg)	−1.95 (−0.07)
Protein % EBV	0.015 (0.14)
Fat % EBV	−0.12 (0.09)
CMR	0.13 (0.14)

*CMR, 1/4 residuals for the cases of clinical mastitis per lactation after accounting for effects of herd, parity, and production level in milk yield (Bagheri *et al.* 2013).

values were negative ranging from −0.03 to −2.4, while for *TLR4(1)*, values were positive except fat percentage EBV. The dominance effects for protein yield were d1/4 0.31 kg and d1/4 −1.52 kg for *TLR4(1)* and *TLR4(2)*, respectively. For fat yield, these values were 7.22 kg and −2.4 kg for *TLR4(1)* and *TLR4(2)*, respectively. For *CACNA2D1(1)* and *CACNA2D1(2)*, EBV of protein yield for the heterozygous genotype was lower than the expected value (0.81 kg and −2.4 kg, respectively).

In addition to the availability of phenotypes, we also should know the genotype of each individual for dominance estimation. Further estimation of EBVs represents additive genetic values and it seems that it is not useful for estimation of dominance, but in our study, we were able to estimate the dominance effects for production traits and CMR using selective genotyping approach. Results showed that dominance effects existed for some traits. For example, differences between genotypes GG and AA for *TLR4(1)* were 12.4 and 5.99 kg for protein and fat yield, respectively. This indicates an expected value of 6.2 and 3 kg for these traits. In this case, for the heterozygous genotype of AG, the protein yield decreased but the fat yield increased compared to both homozygous genotypes (GG and AA). Also it is assumed that low-heritable traits such as CM have higher dominance values. Our investigations indicated that the estimated values of dominance effects for production traits and CMR ranged from −0.49 to 0.35 SD units. One of the most suitable strategies for studying the genetic background of complex traits is the candidate gene approach. This approach is explained by association tests that select loci with biological actions involved in the physiology of trait, as functional candidates, or chromosomal regions containing previously identified QTLs as positional candidates (Szyda and Komisarek 2007). Dense SNP panels can create new opportunities for detection and use of dominance at individual loci (Lopes *et al.* 2014).

For yield production traits such as protein and fat, positive dominance effects are favourable and show an excess in yield for heterozygous genotype compared to expected values (1/4 average of homozygous genotypes). However, for health trait, the values which are lower than the expected cases of CM for the heterozygous genotype are favourable. In this case, lower and negative cases are favourable.

Since alleles pass from parents to offspring, selection acts only on additive substitution effects. We should keep in mind that dominance is of theoretical and practical interest because of its application in crosses of animal breeds such as in poultry (Toro and Varona 2010). Till date, reported dominance variance for various traits in farm animal carried out by pedigree information has varied from 1 to 34% contribution (Fuerst and Sölkner 1994). To detect additive effects of SNPs in association studies, genomewide SNP information have been used in dairy cattle (Cole *et al.* 2009). There are large numbers of SNP genotype studies focussing on the action of nonadditive genetic effect at an individual level. Sun *et al.* (2014) have estimated the effects of dominance for eight traits in Holstein and Jersey breeds using SNPs genotypes and their results showed 5 to 7% of the total phenotypic variance. The variance of dominance for yield traits could explain their results. Also, they showed that the contribution of dominance effects in the variance of nonyield traits were very small to zero in both breeds. Further, the accounted model for both additive and dominance effects fitted with data better than that of only additive effects in yield traits. Aliloo *et al.* (2016) investigated that 3.8% and 3.2% of the phenotypic variance was

of dominance variance for fat and protein yield, respectively in Holstein dairy cattle. It is reported that variance of dominance was up to 5% for production traits in Holstein cows in the USA (Van Tassell *et al.* 2000). Also in Canada, Miglior *et al.* (1995) showed that variance of dominance was up to 3% for milk production traits in Holstein cows. In contrast to these studies, Tempelman and Burnside (1990) reported a considerable dominance variance for fat yield (24%).

Estimation of additive and dominance variance components can be used to estimate additive and dominance effects of SNPs (Wang *et al.* 2007; Zhang *et al.* 2009; Yuan *et al.* 2011). Dominance action at the locus appears if $d \neq 0$, thus in this case the genotypic value at the locus will be completely additive. As it is obvious, genetic correlations between yield traits and CM are generally antagonistic. Therefore, in small but influential selection groups of animals, we can apply selection genes or genetic markers carrying alleles which can improve both antagonistic traits simultaneously and favourably (Gernand *et al.* 2012).

Based on the impact of both candidate genes, *TLR4* and *CACNA2D1*, on udder health, linear or generalized linear mixed models was applied for testing the associations of SNPs located in the genes and CM. As we know, in harsh production systems, the dominance effect can be more important for health traits than other traits such as production traits (milk and protein yield, etc.). Also it can be beneficial for mate selection programmes (Henderson 1989).

In this study, the value of inbreeding was not considered since it has been reported that animals with larger positive dominance effects are less inbred because dominance deviations for production traits are moderate in size and negatively correlated with inbreeding level and also define the proportion of homozygous SNPs. Further, it has been indicated that negative dominance deviations are associated with lower values of animal inbreeding (Aliloo *et al.* 2016).

Selective genotyping and contrasting both homozygotes to heterozygote is a new approach for estimation of dominance effects. In the present study we used selective genotyping and logistic regression to estimate dominance effects of four SNPs for production traits related to mastitis. The method that we used would help breeders in accurately estimation of genotypic values in a new genomic selection scenario including dominance effects. Based on these findings, we can suggest that it would be useful to evaluate dominance values using selective genotyping and consider them in estimation of genetic effects.

Acknowledgements

Authors thank Prof. Sven Konig and Dr Eduardo Pimentel, University of Kassel, for their assistance throughout numerous consultations.

References

Aliloo H., Pryce J. E., González-Recio O., Cocks B. G. and Hayes B. J. 2016 Accounting for dominance to improve genomic evaluations of dairy cows for fertility and milk production traits. *Genet. Sel. Evol.* **48**, 1–11.

Bagheri M., Miraie-Ashtiani R., Moradi-Sharhrbabak M., Nejati-Javaremi A., Pakdel A., Von Borstel U. U. *et al.* 2013 Selective genotyping and logistic regression analyses to identify favorable SNP-genotypes for clinical mastitis and production traits in Holstein dairy cattle. *Livest. Sci.* **151**, 140–151.

Bagheri M. and Zahmatkesh A. 2017 Estimation of dominance effects related to mastitis and production traits for CXCR1 gene using logistic regression analysis in dairy cattle. *Agric. Gene.* **3**, 63–66.

Buitkamp J., Ewald D., Masbanda J., Bishop M. D. and Fries R. 2003 FISH and RH mapping of the bovine alpha (2)/delta calcium channel subunit gene (CACNA2D1). *Anim. Genet.* **34**, 309–310.

Cole J. B., Van Raden P. M., O'Connell J. R., Van Tassell C. P., Sonstegard T. S., Schnabel R. D. *et al.* 2009 Distribution and location of genetic effects for dairy traits. *J. Dairy Sci.* **92**, 2931–2946.

De Schepper H. U., De Man J. G., Ruyssers N. E., Deiteren A., Van Nassauw L. and Timmermans J. P. 2008 TRPV1 receptor signaling mediates afferent nerve sensitization during colitis-induced motility disorders in rats. *Am. J. Physiol.* **294**, 245–253.

Detilleux J. C. 2002 Genetic factors affecting susceptibility of dairy cows to udder pathogens. *Vet. Immunol. Immunopathol.* **88**, 103–110.

Dufrasne M., Faux P., Piedboeuf M., Wavreille J. and Gengler N. 2014 Estimation of dominance variance for live body weight in a crossbred population of pigs. *J. Anim. Sci.* **92**, 4313–4318.

Ertl J., Legarra A., Vitezica Z. G., Varona L., Edel C., Emmerling R. and Götz K. U. 2014 Genomic analysis of dominance effects on milk production and conformation traits in Fleckvieh cattle. *Genet. Select. Evol.* **46**, 40.

Falconer D. S. and Mackay T. F. C. 1996 *Introduction to quantitative genetics*, 4th edition. Pearson Education, Harlow.

Fuerst C. and Sölkner J. 1994 Additive and non-additive genetic variances for milk yield, fertility, and lifetime performance traits of dairy cattle. *J. Dairy Sci.* **77**, 1114–1125.

Gernand E., Rehbein P., von Borstel U. U. and König S. 2012 Incidences of and genetic parameters for mastitis, claw disorders, and common health traits recorded in dairy cattle contract herds. *J. Dairy Sci.* **95**, 2144–2156.

Henshall J. M. and Goddard M. E. 1999 Multiple-trait mapping of quantitative trait loci after selective genotyping using logistic regression. *Genetics* **151**, 885–894.

Henderson C. R. 1989 Prediction of merits of potential mating from sire-maternal grandsire models with non-additive genetic effects. *J. Dairy Sci.* **72**, 2592–2605.

Lopes R. T., Gonçalves M. M., Fassnacht D. B., Machado P. P. P. and Sousa I. 2014 A comparative study of narrative therapy and cognitive-behavioral therapy. *J. Affect. Disord.* **167**, 64–73.

Miller S. A., Dykes D. D. and Polesky H. F. 1988 A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16**, 1215.

Miglior F., Burnside E. B. and Kennedy B. W. 1995 Production traits of Holstein cattle: estimation of non additive genetic variance components and inbreeding depression. *J. Dairy Sci.* **78**, 1174–1180.

Misztal I. 2001 Estimation of variance components with large-scale dominance models. *J. Dairy Sci.* **80**, 965–974.

Nagy I., Farkas J., Curik I., Gorjanc G., Gyovai P. and Szendrő Zs. 2014 Estimation of additive and dominance variance for

litter size components in rabbits. *Czech J. Anim. Sci.* **59**, 182–189.

Paape M. J., Shafer-Weaver K. and Capuco A V. 2000 Immune surveillance of mammary tissue by phagocytic cells. *AEM Biol.* **480**, 259–277.

Reinhardt T. A. and Lippolis J. D. 2006 Bovine milk fat globule membrane proteome. *J. Dairy Res.* **73**, 406–416.

Schabenberger O. 2007 Growing up fast: SAS 9.2 enhancements to the GLIMMIX procedure SAS Global Forum Citeseer SAS Institute Cary.

Sharma B. S., Jansen G. B. and Karrow N. A. 2006 Detection and characterization of amplified length polymorphism markers for clinical mastitis in Canadian Holsteins. *J. Dairy Sci.* **89**, 3653–3663.

Su G., Christensen O. F. and Ostersen T. 2012 Estimating additive and non-additive genetic variances and predicting genetic merits using genome-wide dense single nucleotide polymorphism markers. *PLoS One* **7**, e45293.

Sun C., Van Raden P. M., Cole J. B. and O'Connell J. R. 2014 Improvement of prediction ability for genomic selection of dairy cattle by including dominance effects. *PLoS One* **9**, e103934.

Szyda J. and Komisarek J. 2007 Statistical modeling of candidate gene effects on milk production traits in dairy cattle. *J. Dairy Sci.* **90**, 2971–2979.

Tempelman R. J. and Burnside E. B. 1990 Additive and non-additive genetic variation for production traits in Canadian Holsteins. *J. Dairy Sci.* **73**, 2206–2213.

Toro M. A. and Varona L. 2010 A note on mate allocation for dominance handling in genomic selection. *Genet. Sel. Evol.* **42**, 33.

Van Tassell C. P., Misztal I. and Varona L. 2000 Method R estimates of additive genetic, dominance genetic, and permanent environmental fraction of variance for yield and health traits of Holsteins. *J. Dairy Sci.* **83**, 1873–1877.

Vitezica Z G., Varona L. and Legarra A. 2013 On the additive and dominant variance and covariance of individuals within the genomic selection scope. *Genetics* **195**, 1223–1230.

Wang X., Shangzhong X., Hongyan R. and Jinbao C. 2007 Genetic polymorphism of TLR4 gene and correlation with mastitis in cattle. *J. Genet. Genomics* **34**, 406–412.

Yuan Z. R., Li J., Zhang L. P., Zhang L. M., Chen C., Chen X. J. *et al*. 2011 Novel SNPs polymorphism of bovine CACNA2D1 gene and their association with somatic cell score. *Afr. J. Biotechnol.* **10**, 1789–1793.

Zhang L. P., Gan Q. F., Ma T. H., Li H. D., Wang X. P., Li J. Y. *et al*. 2009 Toll-like receptor 2 gene polymorphism and its relationship with SCS in dairy cattle. *Anim. Biotechnol.* **20**, 87–95.

Corresponding editor: Rajiva Raman