Detection of QTL for milk production traits in cattle by application of a specifically developed marker map of BTA6

Ch Kühn, G Freyer, R Weikard, T Goldammer, M Schwerin

Summary

Interval mapping was carried out to identify quantitative trait loci (QTL) for milk production traits in five granddaughter design families of the German Holstein population. Fourteen randomly generated markers spanning the whole of BTA6 and six targeted microsatellite markers from BTA6q21–31 were included in the analysis. In one family a QTL with effects on milk fat yield and milk protein yield was mapped to the interval TGLA37-FBN13 (3 cm proximal to FBN13, lodscore 3.22) in the middle part of the chromosome. Although there are several reports about QTL with effects on milk production traits on BTA6 in the literature, a QTL with effects on milk fat and milk protein yield has not been previously described.

Keywords: QTL, chromosome 6, milk production traits, cattle, chromosome fragment specific markers

Introduction

Livestock breeding schemes based on quantitative genetics have facilitated an enormous improvement of production traits such as milk production in cattle. Molecular genetic methods may be able to dissect these complex oligo-or polygenic traits into single components. The detection of genetic markers tightly linked to such loci influencing quantitative traits (QTL) would offer tools for the application of marker assisted selection and is a prerequisite for the detailed molecular genetic analysis of traits. For milk production traits, analyses of the casein gene polymorphisms and their direct effects on milk production have directed an early interest in bovine chromosome 6 (BTA6) where the casein genes are located. The results, summarised by Bovenhuis et al. (1992), however, were rather contradictory regarding allele effects, especially when investigations were performed at the population level. Recently, the application of a new experimental design (daughter or granddaughter design, Weller et al. 1990) resulted in several reports, which showed, that there are QTL for milk production traits segregating on BTA6. The reports mainly focused on the casein locus and gave the first indications, that there might be more than one QTL in this chromosomal area. Velmala et al. (1995) and Georges et al. (1995) agreed on a QTL near the casein locus with negatively correlated effects on milk yield and milk fat percentage in the Finnish Ayrshire and US-American Holstein populations, respectively, while Lien et al. (1995) found a QTL in this area for milk and milk protein yield in Norwegian cattle. Recently, Spelman et al. (1996) found evidence for a QTL for milk protein percentage in a Dutch Holstein family on BTA6 about 40 cm proximal of the casein locus. The limited number of informative genetic markers in these studies (concentrated in the area of the casein complex) handicapped detailed analyses of the QTL. We have developed methods to directly isolate microsatellite markers for specific chromosome areas of interest from chromosome fragment specific libraries produced by chromosome microdissection, DOP-PCR (Goldammer et al. 1996), cloning and screening for microsatellite sequences (Weikard et al. 1997). This approach enabled us to develop a set of such targeted markers on BTA6 for dissecting QTL for milk production traits in selected families of the German HF breed. In our study, we describe and map a new QTL with effects on milk fat and protein yield in the middle of BTA6. Additionally, in our data set we found indications of the previously published QTL for milk yield and milk fat and milk protein percentage in the same chromosomal region.

Materials and methods

Animals

Five families (families 13–16, 18) following a granddaughter design were included from the...
German Black and White Holstein population (comprising 53–219 sons/family). Four families were selected, because they displayed marked variance in estimated breeding values (EBVs) of sons for milk production traits compared to all grandsires, which had > 20 sons with EBV. Family 18 was selected for the analysis because of its large number of unselected sons prior to first calculation of their estimated breeding values (EBVs) (Table 1). Additionally, those grandsires fulfilled the conditions of having a homogeneous mating population over all sons, of homogeneous age of sons (born 1986–1990) and availability of semen samples for at least 15 sons. All sons had their first progeny test for milk production traits calculated in Germany (Vereinigte Information systeme Tierhaltung (VIT), Verden). EBVs for sons were calculated by applying an animal model for milk yield (MKG), milk fat yield (FKG), milk protein yield (PKG), milk fat percentage (FP) and milk protein percentage (PP) (VIT 9/95). The analysis included records of 107,909 daughters (based on full 305 day lactations) with an average of 231 daughters/son.

**Genetic markers**

Twelve commercially (Research Genetics, Huntsville, AL) available microsatellite markers distributed over the whole of chromosome 6, one microsatellite located within intron III of the κ-casein gene (CSN3) (Moore et al. 1992) and the cosmid derived marker FBN3 (Kühn et al. 1996a) were initially tested in the five grandsires. All markers, which were heterozygous in at least one grandsire, were genotyped in all informative families. After an initial indication of a QTL for FKG and PKG in the middle of chromosome 6 (Kühn et al. 1996b) six additional markers were included. These markers were isolated directly from a chromosome fragment-specific library of BTA6q21–31, that was constructed by microdissection (Goldammer et al. 1996; Weikard et al. 1997). Five of these six targeted markers (Fig. 1) were heterozygous in at least one grandsire and were genotyped in all informative families.

### Table 1

Number of sons (n), mean value and standard deviation (in brackets) of estimated breeding values (EBVs) of three paternal halfsib families available for this study (MKG, milk yield; FP, milk fat percentage; PP, milk protein percentage; FKG, milk fat yield; PKG, milk protein yield. Additionally, mean reliability (Rel.) of these EBVs (All, all sons; Typed, sons available for genotyping) and average heterozygosity (Het.) of the grandsires at 16 marker loci are provided.

<table>
<thead>
<tr>
<th>Family</th>
<th>13</th>
<th>14</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>Typed</td>
<td>All</td>
</tr>
<tr>
<td>n</td>
<td>219</td>
<td>73</td>
<td>126</td>
</tr>
<tr>
<td>Rel.</td>
<td>0.92</td>
<td>0.94</td>
<td>0.93</td>
</tr>
<tr>
<td>Het.</td>
<td>0.69</td>
<td>0.69</td>
<td>0.38</td>
</tr>
<tr>
<td>MKG</td>
<td>449.7</td>
<td>639.9</td>
<td>513.9</td>
</tr>
<tr>
<td>[kg]</td>
<td>(448.6)</td>
<td>(418.0)</td>
<td>(471.5)</td>
</tr>
<tr>
<td>FP</td>
<td>−0.072</td>
<td>−0.088</td>
<td>−0.129</td>
</tr>
<tr>
<td>[%]</td>
<td>(0.302)</td>
<td>(0.289)</td>
<td>(0.268)</td>
</tr>
<tr>
<td>FKG</td>
<td>14.0</td>
<td>21.1</td>
<td>13.0</td>
</tr>
<tr>
<td>[kg]</td>
<td>(16.4)</td>
<td>(16.3)</td>
<td>(18.2)</td>
</tr>
<tr>
<td>PP</td>
<td>−0.033</td>
<td>−0.040</td>
<td>−0.029</td>
</tr>
<tr>
<td>[%]</td>
<td>(0.123)</td>
<td>(0.125)</td>
<td>(0.115)</td>
</tr>
<tr>
<td>PKG</td>
<td>12.8</td>
<td>18.6</td>
<td>15.3</td>
</tr>
<tr>
<td>[kg]</td>
<td>(11.9)</td>
<td>(10.1)</td>
<td>(13.7)</td>
</tr>
</tbody>
</table>
QTL detection

A genetic marker map was generated using CRIMAP options (Donis-Keller et al. 1987) including the genotypes established in this study. FBN markers were mapped in addition using the International Bovine Reference Panel (Barendse et al. 1997) families. For multipoint map construction a threshold of difference of log odds of 3 was applied. Markers not entering the map at this threshold were placed according to their most likely position, but were not included in further linkage analyses between markers and phenotypic traits. For each grandsire the most likely chromosomal haplotypes were determined by analysing genotypes of sons.

To screen for the presence of putatively segregating QTL in marked chromosome sections variance analyses were performed (GLM, SAS). The effect of alternative paternal alleles at each marker locus inherited from the grandsire was calculated within each family separately:

\[ Y_{ij} = \mu + A_i + e_{ij} \]

where \( Y_{ij} \) is the EBV of son \( j \) with paternal allele \( i \) and \( A_i \) is the effect of paternal allele \( i \). The term \( e_{ij} \) represents the respective residuals. For the within-family analyses the EBVs of sons were not weighted, because the reliability of the EBVs was homogeneously very high (88–94%) and all sons with reliabilities < 85% were excluded from the analysis, with the exception of one family. Only one marker was fitted in each analysis.

After indications of the presence of a QTL by ANOVA, interval mapping was performed to give further information on the position and the effect of these QTL. For this purpose the program package ANIMAP (D. Nielsen, see Georges et al. 1995) was applied, which is a

---

Fig. 1. Map of informative markers of BTA6. Closed arrows indicate markers in a multipoint map (threshold: log odds difference > 3) at their respective position (cm, Kosambi) on BTA6. Markers indicated by open arrows are placed according to their most likely position.
maximum likelihood approach (Lander & Botstein 1989) for interval mapping. In the program \( \sigma^2 \) (additive genetic variance of the considered trait) and \( \alpha \) (difference in average effect of QTL alleles) were calculated maximising the likelihood of the pedigree at a given QTL position. This likelihood is divided by the likelihood of the pedigree maximised with respect to \( \sigma^2 \), but with \( \alpha \) fixed at 0 (indicating no segregating QTL at the corresponding map position). Each family was analysed separately. Allele frequencies for the markers were calculated from the allele transmission of the son’s dams. Separate segregation analyses by the programs MACSA (Liu 1994) and LINKAGE (Terwilliger & Ott 1994) were performed to confirm estimation of QTL effects.

Results

Genetic markers

Of 20 markers tested, two showed PCR patterns unsuitable for genotyping and two were monomorphic in all five grandsires. The average heterozygosity of the remaining 16 was 0.53 (0.2–1.0/marker). The average heterozygosity of grandsires over all loci was 0.38–0.69. The mean informativity of markers in informative families (proportion of sons with genotypes different from grandsire) was 0.65, varying between markers from 0.49 to 0.85. Ten of the 16 informative markers entered a marker map spanning 118 cm (Kosambi) (Fig. 1). The map is in good agreement with the maps of Barendse et al. (1997), Kappes et al. (1997) and Ma et al. (1996). All five informative FBN markers mapped to the targeted area of BTA6.

Variance analysis

Families 15, 16 and 18 had no or only weak significant effects (\( P < 0.05 \)) of alternative paternal alleles at all informative marker loci. However, when looking at individual marker loci in family 13 (Fig. 2), the effects of FBN12 marker alleles on MKG and percentage traits (especially PP) appeared to be highly significant, whereas FKG and PKG were not significantly affected. For neighbouring markers located towards the centromeric direction the differences between sons with alternative paternal marker alleles almost vanished. At loci IL97 and FBN14, however, the significant effects as for FBN12 were found again, with stronger influence on PKG. While in family 13 there seems to be a QTL with negatively correlated effects on MKG and percentage traits, in family 14 (Fig. 2) the marker alleles with superior effects on MKG also showed higher FP and PP and highly superior FKG and PKG. These differences were greatest and had the highest significance in the chromosome region between TGLA37 and FBN13 (\( P < 0.0001 \) for FKG, \( P = 0.007 \) for PKG). Additionally, in the centromeric region of the chromosome, there are strong differences between groups of sons inheriting alternative paternal alleles regarding FP and PP, while MKG is not affected. At the casein locus the proportion of sons with alternative paternal alleles was very unequal in family 14 (31 sons with observed transmission of allele 1, two sons with allele 2), which strongly influenced the variance analysis, so that the differences between paternal alleles are not statistically significant (for all traits \( P < 0.30 \)).

Linkage analysis

In family 14 a QTL for FKG was located between TGLA37 and FBN13 (3 cm proximal FBN13) with a lodscore of 3.22 (Fig. 3). The size of the effect between QTL alleles was calculated as \( a = 17.22 \) kg corresponding to 1.06 \( \sigma_{\text{EBV}} \) (standard deviation of EBV within family). The large size of the allele effect was confirmed by calculations with the LINKAGE program (\( a = 19.88 \) kg) and MACSA (\( a = 21.1 \) kg). A putative QTL with a lodscore of 2.83 was detected at position FBN13 for PKG with \( a = 12.59 \) kg. The lodscore maxima of the curves for FP, PP and for MKG were also located in the interval between TGLA37 and FBN13. For the percentage traits the lodscore maxima were located about 25 cm proximal of locus TGLA37.

In family 13 the results of the linkage analysis reflect the results of the variance analysis in that there are two peaks for yield and percentage traits in the area of FBN12 and IL97-FBN14, respectively. However, none of them exceeded lodscore 2.0. The lodscore curves in families 15, 16 and 18 did not exceed a value of 1 for any of the traits considered.

Discussion

After initial indications of at least two QTL for milk production traits (Kühn et al. 1996b) located in the middle of BTA6, microsatellite markers specific for the region of interest were developed (Weikard et al. 1997). The addition of five informative markers in the region BM143-CSN3 decreased the average marker interval from 12.5 cm to 5.5 cm and more than
Fig. 2. Difference (A-B) in EBV between paternal halfsib groups inheriting alternative paternal alleles at marker loci on BTA6 in families 13 and 14. Within each family, the FBN13 allele associated with superior MKG is denoted A. Denotation is maintained for all marker alleles of the same paternal haplotype and all other traits. *P < 0.05; **P < 0.01; ***P < 0.001.

Detection of QTL for milk production traits in cattle

doubled the number of informative genotypes.

In our data set, the big differences in heterozygosity of grandsires (0.33–0.69) and informativity of markers (0.49–0.85) generated big differences in marker density and information between the families. For both ends of BTA6, QTL analyses were hampered by the low number of informative meioses, as was the study of Spelman et al. (1996). But as variance analysis showed no strong indication of a QTL in these chromosomal regions we did not attempt to generate chromosome fragment specific markers for these regions.

Only 10 of 16 informative markers could be ordered in a marker map at a threshold LOD 3 for support of locus order. We insisted on this stringent level of significance, because simulations showed, that QTL linkage mapping by the ANIMAP program was very sensitive to variation of the underlying genetic map.

In one medium sized family (grandsire 14), which displayed high variance of EBVs, indication on a segregating QTL was found by linkage analysis. Calculations replacing EBVs by daughter yield deviations (DYDs) (VanRaden & Wiggins 1991) did not show deviating results in variance analysis or linkage analysis either in families 13 or 14, or in those families without significant QTL effects (families 15, 16 and 18) (data not shown).

In contrast to family 13, in family 14 the paternal chromosomal haplotype, which is superior for MKG is also associated with higher FP and PP. Therefore, in family 14 highly significant, large differences between paternal chromosomal haplotypes for FKG and PKG could be detected. The opposite effects in families 13 and 14 which are unrelated for four generations showed a constant tendency at almost all marker loci over the whole chromosome (Fig. 2). Therefore, it is unlikely that these principal differences are random observations at single marker loci, but probably display the effects of at least two different QTL for milk production traits. Therefore, across family variance analyses of our data set is strongly influenced by the distribution of QTL and informative markers over all families (data not shown).

We report of a QTL with effects on both FKG and PKG on BTA6 in cattle. Results of Lien et al. (1995) indicate a similar QTL with effects on PKG in the Norwegian cattle population at the casein locus on BTA6, but without localisation by linkage analysis. The superior haplotype 5 in their study contained the CSN3 microsatellite allele, which also turned out to be superior in our investigation. The small number of sons with confirmed inheritance of one of the alternative paternal CSN3 alleles disables a sensible analysis of the casein region in family 14. However, MKG and PKG tend to differ in this region (see also second peak of lodscore.

Fig. 3. Lodscore curves for linkage analysis of milk production traits with microsatellite markers on BTA6 (marker position as indicated) in family 14. Genetic distances are given in cm (Haldane) as calculated by the ANIMAP program. (●) milk yield (MKG), (●) milk fat yield (FGK), (▲) milk protein yield (PKG), (+) milk fat percentage (FP), (X) milk protein percentage (PP).
Detection of QTL for milk production traits in cattle

Therefore, it cannot be decided, if this tendency is due to the QTL in interval TGLA37-FBN13 or due to a second independent effect at the CSN3 locus.

Analysis in family 13 was handicapped by selection effects (see Table 1). This was outlined by the fact, that at the majority of the marker loci the group of sons with the inferior marker allele had a higher mean than all sons of grandsire 13 in the data set (genotyped and not genotyped) taken together. Nevertheless, in the variance analysis targeted markers FBN12 and FBN14 gave indication on a putative QTL with negatively correlated effects on MKG and percentage traits in this family. This kind of effect had also been found in single families by Georges et al. (1995), Velmala et al. (1995) and Spelman et al. (1996).

Several aspects regarding the relatively large size of the estimated QTL effect in family 14 have to be considered. First of all, due to the given family size only QTL with such strong effects were likely to be detected. Other maximum likelihood (ML) estimates (MACSA, LINKAGE) of our data set confirmed the magnitude of QTL effects in family 14. On the other hand Georges et al. (1995) showed in their study, that high lodscore thresholds like 3 resulted in an overestimation of QTL effects, especially in smaller pedigrees when using ML approaches. Large QTL effects for milk production traits were also observed in other studies investigating milk production traits in cattle: In a within-family study considering DYDs Georges et al. (1995) found QTL effects of 0.84±1.7σDYD (standard deviation within family) compared to 1.06σEBV QTL effect for milk fat yield within family 14 in our study. Spelman et al. (1996) published a QTL effect in the magnitude of 0.68±1.12σCG (genetic standard deviation within population calculated for DYDs.

Compared to the previously published QTL with negatively correlated effects on MKG and FP/PP the QTL with effects on FKG and PKG described in this study promises a higher potential for application in dairy cattle breeding. The application of markers TGLA37 and FBN13 in marker assisted selection, however, requires exact estimates of QTL effects and position to select for the desired positive effect on FKG and PKG. Ruane & Colleau (1996) showed, that overestimation of QTL effect reduced genetic response compared to correctly estimated QTL effect and leads to only marginal advantage of MAS compared to conventional selection even under conditions which favour MAS such as multiple ovulation and embryo transfer (MOET). Therefore, further studies on the described QTL concerning their effect as well as the underlying genes are necessary before they can be applied in cattle selection schemes.

Acknowledgements

We would like to thank the German A.I. stations for providing semen samples, Dr S. Grupe for providing DNA of family 18 and the VIT, Verden for providing phenotypic data of bulls. The excellent technical assistance of O. Haufft and A. Kuhl is thankfully acknowledged. We are indebted to D. Nielsen for the ANIMAP program. This work was supported by grants from the Deutsche Forschungsgemeinschaft Ku 771/2–2 and We 1786/1–1.

References


