

Trait-Based Analysis in Dairy Cattle Using Blood Group Polymorphisms

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ABSTRACT

The potential of trait-based analysis to detect quantitative trait loci was investigated using blood group polymorphisms as the marker systems and milk and type traits in Holstein cattle as the quantitative traits. Within large half-sib families, animals were ranked on their predicted transmitted abilities or phenotypes, and blood group allele frequencies were compared between the upper and lower 5% tails of the distributions. Genotype frequencies within large families were also examined for evidence of selection. All of the major effects that had previously been detected using linear model analyses were identified by the trait-based analyses of a C blood group effect on rump angle, an L effect on milk yield and composition traits, an S effect on milk fat yield, and a direct effect of the M locus on milk and protein yields. These results provide additional support for the biological validity of these associations and also demonstrate the utility of trait-based analysis for the detection of quantitative trait loci within existing dairy breeding programs. However, just as in the linear model analyses, an analytical strategy should be utilized that allows the identification of the effects that are consistent across environments and genetic backgrounds.

(**Key words:** trait-based analysis, selective genotyping, quantitative trait loci, blood groups)

Abbreviation key: HWE = Hardy-Weinberg equilibrium, QTL = quantitative trait locus, SG = selective genotyping, TBA = trait-based analysis.

INTRODUCTION

Trait-based analysis (TBA) has been proposed by Lebowitz et al. (12) as an alternative approach for the detection of quantitative trait loci (QTL) (4).

Traditionally, analyses for the detection of QTL have relied on the statistical contrasting of certain of the marker-genotype means within the experimental design (19, 20) after all available individuals have been marker-genotyped (marker-based analyses). However, most of the information regarding linkage between a marker and QTL is contained in the tails of the progeny distributions, and savings in resources can be accomplished (with only modest losses in statistical power) by selectively genotyping only the individuals within the distribution tails (2, 10). The potential for improving efficiency is greatest when the study focuses on only a few traits and when the cost of obtaining trait measurements is negligible compared with the cost of marker genotyping individuals.

Lebowitz et al. (12) coined the term "trait-based analysis" to represent the statistical comparison of marker allele frequencies between opposing tails of a distribution using the normal approximation to the binomial distribution. Subsequently, Lander and Botstein (10) and Darvasi and Soller (2) developed the concept of selective genotyping (SG) in which the tails are marker genotyped and the means of the progeny groups inheriting alternate parental alleles are contrasted within an analysis based on maximum likelihood. These approaches have not been widely utilized for QTL detection (6, 9, 13, 15). Unfortunately, the article by Lebowitz et al. (12) contains an error in the test statistic that results in the underestimation of the statistical power of the test [Rocha (17); M. Soller, personal communication], which might have contributed to the limited interest in the approach.

Darvasi and Soller (3) have recently demonstrated that almost all of the "power for QTL detection with selective genotyping derives from the allelic frequency difference at the extremes." Therefore, TBA and SG are essentially equivalent in their statistical power. Consequently, the purpose of this study was to examine the potential of TBA for QTL detection against the results of a larger study that was conducted to bring into perspective over 40 yr of research into the associations between blood group polymorphisms and milk

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and type traits of dairy cattle and to assess the utility of blood group polymorphisms as selection aids in current Holstein breeding schemes (17, 18). Specifically, we were interested in whether TBA could identify marker-QTL associations that had been detected and validated by other statistical approaches (17, 18). In addition to the TBA, the chi-square test proposed by Mackinnon and Georges (14) was also applied within large half-sib families from heterozygous sires to assess whether trait-based selection had resulted in any deviations from the expected marker genotype frequencies under a model of no QTL effects.

MATERIALS AND METHODS

The data this study used for quantitative trait and blood group were described by Rocha et al. (18).

Statistical Analyses

Within-family trait-based analyses. For each blood group, families of male and female half-sibs with at least 300 members from heterozygous sires were identified (Table 1). Within each of these families and for each trait, blood group allele frequencies between the upper and lower 5% tails of the sibs were compared using a normal approximation to the binomial distribution (the least frequent alleles were pooled into a single allele class) as proposed by Lebowitz et al. (12). The 5% proportion in each of the selected tails was chosen because Lander and Botstein (10) and Darvasi and Soller (3) recommend against using tails of less than 5% of the total sample and because many of the sib families were very large (18), permitting the detection of quantitative effects of a moderate magnitude (3, 12) with this proportion.

Contrary to Lebowitz et al. (12), the appropriate denominator for the TBA test statistic is $(m_1 m_2 / PT)^{0.5}$ where m_1 and m_2 = observed allele frequencies within the family, P = proportion of individuals selected within each tail, and T = total number of sibs in the family. Comparisons of genotype (rather than allele) frequencies were performed for the L blood group in which the effects of selection prevented the use of the square root method for the estimation of allele frequencies under the assumption of Hardy-Weinberg equilibrium (**HWE**) (utilized for the dominant, two-allele L, M, T', and Z systems). Analyses were conducted within each family to prevent different phase relationships in different families from canceling the QTL effects associated with each linked blood group allele. To control the experimental Type I error rate, a stringent level of significance ($P < 0.005$) was imposed because a large number of simul-

TABLE 1. Number of families for the trait-based analyses.

Blood group	Male families ¹	Female families
	(no.)	
A	7	6 ²
B	8	8 ²
C	9	12 ²
F	2	6
L	5 ³	10
M	1 ³	1
R'	1	2 ³
S	9	8 ²
T'	2	6
Z	3	8

¹Male and female half-sib families with ≥ 500 members with at least 1 allele known and out of heterozygous sires.

²Because of the high locus polymorphism content, the family size requirement was increased to ≥ 2500 .

³Because of the low locus polymorphism content, the family size requirement was decreased to ≥ 300 .

taneous comparisons were performed. However, if consistency of results with those previously reported (18) was evident, $P = 0.05$ was used.

Finally, the presence of null alleles in the complex systems (A, B, C, and S) caused the estimates of tail allele frequencies to be biased in families of sires that were heterozygous for the null allele. In these families, tail allele frequencies were estimated only using progeny with completely ascertained genotypes that had been determined by the Holstein Association (Brattleboro, VT) from appropriate information about grandprogeny blood group. Because no difference between tail frequencies remained when the null hypothesis was true, this bias only affected the statistical power of the test in these families.

Analyses to detect the impact of selection within families. Within each of the half-sib families in Table 1 and following the suggestion of Mackinnon and Georges (14), the expected genotype (or phenotype) numbers were computed under the assumption of an equal probability of inheritance of each of the sire alleles and using the allele frequencies for the overall female sample reported by Rocha (17). For the male half-sib families, the use of estimates for allele frequency from the overall female sample led to the undesirable consequence of not being able to discriminate between selection acting upon alternate sire chromosomal segments and selection occurring on the dams of sires pathway. The first type of selection should not be evident in these data, because all young bulls are blood typed before genetic evaluation. However, this does not preclude the selection of young bulls within families based on conformation and

potentially other traits. The female half-sib families reflect selection operating on both the sire and dam pathways and selection acting upon Mendelian segregation effects (phenotypic selection).

In the tests for the codominant systems (F and R'), the expected genotypic proportions are $1/2p$, $1/2$, and $1/2(1 - p)$ for homozygotes, heterozygotes, and alternate homozygotes, respectively (p is the allele frequency estimate from the female sample) (17). For the dominant systems, genotypes were pooled into three (A, B, C, and S systems) or two (L, M, T', and Z) classes. In the first case (multiple allele systems), if the sire genotype was A_iA_j ($i \neq j$) for a particular blood group, the three progeny genotypic classes compared were $A_iA_$, $A_jA_$, and A_iA_j where $A_$ = any allele $\neq A_j$. The expected frequencies for these three classes are $1/2(1 - p_j)$, $1/2(1 - p_i)$, and $1/2(p_i + p_j)$, respectively, where p_i and p_j = the estimated frequencies of A_i and A_j , respectively, in the population of dams. Within-family numbers of the expected progeny genotype were obtained by multiplying these frequencies by the total number of sibs (with at least one allele determined) in the family. When the sire genotype was A_iA_n (A_n being the recessive null allele), only two classes were considered (the first and third classes were pooled), and expected frequencies were $1/2(1 + p_i)$ and $1/2(1 - p_i)$, respectively. Similarly, only two genotypic classes are present for the two-allele dominant systems (L, M, T', and Z), homozygous dominant pooled with heterozygotes and homozygous recessive, with expected frequencies of $1/2(1 + p)$ and $1/2(1 - p)$ where p is the frequency of the dominant allele in the female sample. A chi-square goodness-of-fit test was conducted within each of the families to test for departures from expected values. Departures from expected values were interpreted as indications of the impact of selection on blood group genotypic frequencies because of the presence of QTL linked to the blood group loci (14).

RESULTS AND DISCUSSION

Within-family Trait-based Analyses

Results ($P < 0.05$) for the B, C, L, and S blood groups are presented in Tables 2 and 3. A comparison of these results with those of Rocha et al. (18) shows that the major element of consistency to emerge concerns the effects of the L blood group on milk yield and composition traits (especially fat and protein percentages). The null allele of the L blood group is predominantly associated with negative effects on yield traits (milk, fat, and protein) and positive effects on fat and protein percentages. Historical selec-

tion for increased milk production should, therefore, have selected against the null allele, and its frequency has decreased over time for both males and females (males: 0.78 in the 1950s to 0.73 in the 1980s; females: 0.80 in the 1960s to 0.75 in the late 1980s). Segregating families detected in the TBA were among the families detected in the linear model analyses (18), and there was agreement in the direction of the effects estimated under both analytical approaches.

Because the results reported by Rocha et al. (18) indicated the existence of linkage disequilibrium in the population, TBA were also performed across families under the assumption of direct effects of the L blood group on milk traits. The frequencies of L blood group alleles were compared between the extreme 0.1% tails of the overall male and female samples. No significant differences were found in the comparisons of males, but results from the females concurred with previous findings. The L allele was found at higher frequencies in the upper tail for milk yield (0.314 vs. 0.188; $P = 0.003$) and for milk fat yield (0.344 vs. 0.238; $0.05 > P > 0.01$), which further substantiates that linkage disequilibrium is present in the population. As expected, the magnitude of these differences in allele frequencies were smaller than were detected in the within-family TBA (Tables 2 and 3; 18).

The results in Tables 2 and 3 suggest some conflict between yield and type traits for this system. In a few of the families segregating for effects on milk traits, the frequency of the L allele was higher among sires with the lowest PTA values for udder depth (Table 2) and among cows (Table 3) with the deepest udders [lowest udder depth scores (18)], and selection is against these udder types. However, the linear model analyses reported by Rocha et al. (18) found no associations between the L blood group and udder depth.

Another consistent effect that emerges is the association between the S system and milk fat yield (18). Results in Table 3 suggest this association to be because of a linked QTL rather than a direct effect of S system alleles. The C system effect on rump angle is the only blood group effect on a type trait that was consistently detected in all designs [Tables 2 and 3 and Rocha et al. (18)], and this effect appears to be due to a linked QTL (Tables 2 and 3).

An effect of the B system on milk fat percentage that was discussed by Rocha et al. (18) is also suggested by the results in Table 3. In order to examine further the possibility of a direct positive effect of the BO_1Y_2D' phenogroup on milk fat percentage (18), TBA were conducted across families but failed to

reveal any direct effects of this allele. However, a related B phenogroup, G₂O₁Y₂(I'') (which shares the linear O₁Y₂ subarray present in the BO₁Y₂D' haplo-

type), was at a higher frequency in the upper tails for PTA for fat yield (0.172 vs. 0.021; $P < 0.002$) and milk fat yield (0.056 vs. 0.012; $P = 0.008$), confirming

TABLE 2. Results from the trait-based analyses in male families (B, C, L, and S groups).

PTA Trait	Families ¹ (no.)	Mean P	Δf^2	Effect ³
B Group				
Fat yield	2	0.05 > $P > 0.01$	0.188	Linked QTL ⁴
Protein yield	1	0.004	0.105	I ₂ ⁵ upper tail
Protein dollars	1	<0.002	0.111	I ₂ ⁵ upper tail
Thurl width	1	0.002	0.220	G ₂ Y ₂ E' ₁ Q', ⁵ lower tail D'E' ₃ F'G'O', ⁵ upper tail
Body depth	2 ⁶	<0.002	0.150	I ₂ ⁵ lower tail
Strength	1	<0.002	0.288	G ₂ Y ₂ E' ₁ Q', ⁷ lower tail I ₂ ⁵ lower tail
C Group				
Fore udder attachment	1	0.003	0.180	EC'', ⁵ upper tail
Foot angle	1	0.004	0.209	C ₁ E, ⁷ lower tail
Rump angle	3 ⁶	0.05 > $P > 0.01$	0.225	C ₁ E, ⁸ lower tail
			0.169	C ₁ E, ⁸ upper tail
			0.117	EC'', ⁵ upper tail
L Group				
Milk yield	3	0.01	0.150 ⁹	Null, ¹⁰ lower tail; L ¹⁰ - upper tail
Fat yield	1	0.009	0.063 ¹¹	Null, ⁷ lower tail; L ⁷ - upper tail
Protein yield	1	0.003	0.077 ¹¹	Null, ⁷ lower tail; L ⁷ - upper tail
Protein dollars	1	<0.002	0.115 ¹¹	Null, ⁷ lower tail; L ⁷ - upper tail
Udder depth	1	0.004	0.200	L, ⁷ lower tail; null ⁷ - upper tail
Dairy form	1	<0.002	0.216	Null, ⁷ lower tail; L ⁷ - upper tail
S Group				
Milk yield	2	<0.002	0.208	H', ¹⁰ upper tail
Fat yield	1	0.05 > $P > 0.01$	0.140	H', ⁷ upper tail
Protein yield	2 ⁶	<0.002	0.186	H', ⁷ upper tail
		0.01	0.114	UH'H', ⁵ upper tail
Protein dollars	2 ¹²	0.004	0.204	H', ¹⁰ upper tail
		0.008	0.117	SH', ⁵ lower tail
Udder depth	1	0.003	0.298	H', ⁷ lower tail
Dairy form	3 ⁶	<0.002	0.130	UH'H', ⁵ lower tail
		0.004	0.292	H', ⁷ upper tail
		0.009	0.179	SH', ⁵ lower tail

¹Number of families (of those in Table 1) for which a significant difference in allele frequency between opposite tails was detected.

²Difference in allele frequency between tails of the PTA distribution within a family.

³Allele or alleles yielding the significant effect and the tail in which the higher frequency was observed.

⁴Different paternal alleles in different families suggest a linked quantitative trait locus (QTL).

⁵Allele inherited from the dams suggests a direct effect.

⁶Effect was not consistent across families; results for individual families are described separately.

⁷Paternal allele. Because only one family is involved, this result is compatible with the existence of a linked QTL or with a direct effect.

⁸Paternal allele. The direction of effect varied among families, suggesting a linked QTL.

⁹In two families, selection against homozygous null was so intense that frequency differences were measured for genotypes. The average difference in genotype frequency between tails for the two families was 0.060.

¹⁰Paternal allele. Because the results were consistent among families, either a direct effect or the existence of linked QTL and linkage disequilibrium are indicated.

¹¹Difference in genotype frequency between tails.

¹²In one family two effects were detected, one with a paternal allele and the other with a maternal allele. The effect that was associated with the paternal allele was consistent across both families, and results from both families were pooled.

the results reported by Rocha et al. (18) using a linear model.

The TBA were also applied across families assuming direct effects of the M blood group alleles (18) and were found to support the existence of this remarkably consistent QTL effect that has been reported in different environments, breeds, and countries (1, 5, 7, 8, 16, 17, 18, 21, 22). The null allele was at fixation in the upper tail and at a frequency of 0.71 in the lower tail of the distribution for PTA for milk yield ($P < 0.002$). No differences in allele frequency were detected in the milk yield data for females. Other significant results that were obtained from the TBA, but that did not fit the patterns of consistency discussed by Rocha et al. (18), are presented in Tables 4 and 5.

Within-family Chi-square Tests

Results ($P < 0.05$) for the B, C, L, M, and S blood groups are presented in Table 6. Eleven half-sib families deviated from the genotypic frequencies expected under the assumption of no selection acting on the chromosomal segment marked by the L blood group. Selection in favor of the L allele was confirmed by the changes in allele frequency that were reported by Rocha et al. (18) and also, in part, by the results in Table 6. In general, those families identified as segregating for L-marked quantitative effects in the TBA (Tables 2 and 3) also provided supporting evidence for within-family selection in Table 6, and the direction of the effects in both analyses was according to expectations. However, in two of the female families (Tables 3 and 6), the detected effects were of a

TABLE 3. Results from the trait-based analyses in female families (B, C, L, and S groups).

Phenotypic trait	Families ¹ (no.)	Mean <i>P</i> value	Δf^2	Effect ³
B Group				
Fat percentage	2	0.05 > <i>P</i> > 0.01	0.169	Linked QTL ⁴
Protein percentage	1	0.005	0.087	E ₃ G ⁵ , upper tail
C Group				
Fat yield	2 ⁶	<0.002	0.155	X ₂ C ⁵ , upper tail
		<0.002	0.060	C ₁ X ₂ ⁵ , lower tail
Rump angle	3	0.05 > <i>P</i> > 0.01	0.234	Linked QTL ⁴
L Group				
Milk yield	2	0.05 > <i>P</i> > 0.01	0.207	Null, ⁷ lower tail; L, ⁷ upper tail
Fat yield	1	<0.002	0.060 ⁸	Null, ⁹ lower tail; L, ⁹ upper tail
Fat percentage	1	0.05 > <i>P</i> > 0.01	0.233	Null, ⁹ upper tail; L, ⁹ lower tail
Protein yield	1	0.05 > <i>P</i> > 0.01	0.169	Null, ⁹ upper tail; L, ⁹ lower tail
Protein percentage	2	0.007	0.257	Null, ⁷ upper tail; L, ⁹ lower tail
Udder depth	3	0.01	0.235 ^{8,10}	Linked QTL ¹¹
S Group				
Fat yield	2	0.05 > <i>P</i> > 0.01	0.192	H ⁷ , linked QTL ¹¹

¹The number of families (of those in Table 1) for which a significant difference in allele frequency between opposite tails was detected.

²The difference in allele frequency between tails of the within-family phenotype distribution.

³Allele or alleles yielding the significant effect and the tail in which the higher frequency was observed.

⁴Different paternal alleles in different families suggest a linked quantitative trait locus (QTL).

⁵Allele inherited from the dams suggests a direct effect.

⁶Effect not consistent across families; results for individual families are described separately.

⁷Paternal allele; results are consistent across families, indicating either a direct effect or a linked QTL under linkage disequilibrium.

⁸In one family, selection against null homozygotes was so intense that frequency differences were measured for genotypes rather than alleles.

⁹Paternal allele. Because only one family is involved, this result is compatible with either the existence of a linked QTL or with a direct effect.

¹⁰The average genotypic frequency difference between tails for this family was 0.057.

¹¹Paternal allele. Direction of effect varied among families suggesting a linked QTL.

TABLE 4. Other results from the trait-based analyses in male families.

PTA Trait	Families ¹	Mean P value	Δf^2	Effect ³
	(no.)			
			A Group	
Udder cleft	1	0.003	0.173	H, ⁴ upper tail
Strength	1	0.005	0.272	Null, ⁵ lower tail; A, ⁵ upper tail
			R' Group	
Rump angle	1	0.005	0.250	R', ⁵ lower tail; S', ⁵ upper tail
			Z Group	
Milk yield	1	<0.002	0.397	Null, ⁵ lower tail; Z, ⁵ upper tail
Protein dollars	1	<0.002	0.272	Null, ⁵ lower tail; Z, ⁵ upper tail
Udder depth	1	<0.002	0.317	Z, ⁵ lower tail; null, ⁵ upper tail
Body depth	1	<0.002	0.370	Null, ⁵ lower tail; Z, ⁵ upper tail
Strength	1	<0.002	0.370	Null, ⁵ lower tail; Z, ⁵ upper tail

¹Number of families (of those in Table 1) for which a significant difference in allele frequency between opposite tails was detected.

²Difference in allele frequency between tails of the within family PTA distribution.

³Allele or alleles yielding the significant effect and the tail in which the higher frequency was observed.

⁴Allele inherited from the dams suggests a direct effect.

⁵Paternal allele. As only one family is involved, this result is compatible with either the existence of a linked quantitative trait locus or with a direct effect.

higher frequency of the null allele in the lower tails for yields and in the upper tails for percentages. There was a slight within-family excess of null alleles (Table 6). Results from the linear model analyses were similar (18). This finding is difficult to explain unless other QTL are present in this chromosomal segment and different phase relationships among families lead to conflicting selection pressures on the loci.

Not all of the families in Table 6 exhibit an advantage to the L allele, confirming that the effect is not a

direct effect of the blood group, but involves at least one QTL linked to the L blood group, and that different phase relationships occur in different families (18). However, when the deviation is in favor of the L allele, the effect is large; when the deviation is in favor of the null allele, the effect is slight. This relationship is also illustrated in Table 7 in which observed and expected HWE genotype counts for the 5 families with an L allele advantage reveal a dramatic departure from equilibrium. Assuming HWE, the mean frequency of the null allele estimated from

TABLE 5. Other results from the trait-based analyses in female families.

Phenotypic Trait	Families ¹	Mean P value	Δf^2	Effect ³
	(no.)			
			A Group	
Milk yield	1	0.003	0.131	H, ⁴ upper tail
			Z Group	
Protein yield	1	<0.002	0.353	Null, ⁴ lower tail; Z, ⁴ upper tail

¹Number of families (of those in Table 1) for which a significant difference in allele frequency between opposite tails was detected.

²Difference in allele frequency between tails of the within-family PTA distribution.

³Allele or alleles yielding the significant effect and the tail in which the higher frequency was observed.

⁴Paternal allele. Because only one family is involved, this result is compatible with either the existence of a linked quantitative trait locus or with a direct effect.

TABLE 6. Significant results of chi-square tests within families (B, C, L, M, and S groups).

Locus	Families ¹ (no.)	Mean χ^2	Mean <i>P</i>	Effect
Male families				
C	1	12.64	<0.001	Excess of X ₂ C''; deficit of C ₁ X ₂
L ²	2	396.62	<0.001	Extreme excess of L; extreme deficit of null
L ²	1	6.34	0.05 > <i>P</i> > 0.01	Slight excess of null; slight deficit of L
M	1	423.51	<0.001	Extreme excess of null; extreme deficit of M ₂ M'
S ²	2	21.74	<0.001	Excess of null; deficit of H'
S ²	1	68.44	<0.001	Excess of H' phenotypes ³
Female families				
B	1	7.09	0.01 > <i>P</i> > 0.001	Excess of G ₂ Y ₂ E' ₁ Q'; deficit of G ₂ O ₁ Y ₂ (I'')
C ²	1	12.29	<0.001	Excess of X ₂ C''; deficit of C ₁ X ₂
C ²	1	9.64	<0.001	Excess of WX ₂ C''; deficit of X ₂ C''
C ²	1	7.61	0.01 > <i>P</i> > 0.001	Excess of EC''; deficit of EWC''
L ²	3	1001.70	<0.001	Extreme excess of L; extreme deficit of null
L ²	5	19.63	0.05 > <i>P</i> > 0.01	Slight excess of null; slight deficit of L
M	1	982.20	<0.001	Extreme excess of null; extreme deficit of M ₂ M'
S ²	2	57.88	<0.001	Excess of null; deficit of H'
S ²	4	61.84	0.01 > <i>P</i> > 0.001	Excess of H' phenotypes ³

¹Number of families (of those in Table 1) with a significant chi-square test.

²Each detected situation is described separately.

³Pooled class including homozygous dominant and heterozygotes for the recessive null allele.

these families was 0.02, and an estimate of 0.77 was obtained from the overall cow population (17). Also assuming HWE, the frequency was 0.85 of the null allele estimated from the 6 families possessing a small advantage for this allele (Tables 6 and 7). The consistency of estimates for allele frequency across families (0.010 to 0.026 in the 5 families with an excess of L alleles; 0.827 to 0.873 in the 6 families with an excess of null alleles) supports the biological

validity of this effect. Families with a large excess of L alleles (Tables 6 and 7) tended not to yield significant contrasts within families in the linear models (18) in agreement with the impacts of truncation selection on QTL detection modeled by Mackinnon and Georges (14).

Although the results in Tables 6 and 7 support all of the previous findings related to the L blood group [TBA and Rocha et al. (18)], a few questions remain

TABLE 7. Results of chi-square tests for the L group for individual families.

Family	L_ Counts ¹		Homozygous recessive counts	
	Expected	Observed	Expected	Observed
Male half-sib families				
1	396.7	639	248.3	6
2	429.9	690	269.1	9
3	931.7	884	583.3	631
Female half-sib families				
4	856.7	1376	536.3	17
5	1220.2	1967	763.8	17
6	1008.0	1631	631.0	8
7	641.4	591	401.6	452
8	4843.7	4502	3032.3	3374
9	1115.6	1064	698.4	750
10	1410.2	1322	882.8	971
11	260.8	239	163.2	185

¹Counts for LL homozygotes and heterozygotes for the null allele.

TABLE 8. Results of chi-square tests for the M group for individual families.

Family	M ₂ M' counts ¹		Homozygous recessive counts	
	Expected	Observed	Expected	Observed
	Male half-sib families			
1	227.5	9	223.5	442
	Female half-sib families			
2	522.1	18	512.9	1017

¹Homozygous dominant and heterozygotes for the null allele.

to be addressed. If selection on alternate chromosomal segments inherited from the sire should not be evident within these paternal half-sib families, then some of the results in Tables 6 and 7 are difficult to explain because selection could only have occurred on the dams of sires path, which would be evident only if direct effects were associated with the L blood group. Effects from linked QTL would not be detected unless considerable linkage disequilibrium was present. However, the occurrence of families with the direction of effects reversed and the enormous difference in magnitude of effects in different families (Tables 6 and 7) lack a satisfactory explanation. Different mating strategies for different bulls (based on their PTA) might explain some of these findings. The occurrence of a lethal or extremely deleterious allele linked to the null allele in some families does not seem likely. Consequently, we concluded that, at least in some male families, the data reflect selection on alternate chromosomal segments inherited from the sire and that there is more than one QTL linked to the L blood group, there are multiple alleles at one QTL and

different alleles segregate in different families [see Rocha et al. (18)], or both.

Another remarkable effect (Tables 6 and 8) concerns the highly significant deviations from HWE for the M blood group. The frequency of the M₂M' allele was 0.015 in males and 0.009 in females (17). The frequency of the null allele has increased over time (17, 18), and previous research has established that the M₂M' allele is associated with a reduction in milk yield and with an increase in mastitis prevalence (1, 5, 7, 8, 11, 16, 17, 18, 21, 22). Both associations would result in strong selection against this allele, which is supported by results in Tables 6 and 8. Other results from the within-family chi-square tests that were significant but that did not fit patterns of consistency discussed by Rocha et al. (18) are presented in Table 9.

CONCLUSIONS

All of the important and consistent QTL effects that were detected from the linear model analyses

TABLE 9. Significant results for chi-square tests within families.

Locus	Families ¹ (no.)	Mean χ^2	Mean <i>P</i>	Effect
Male families				
A	2	43.70	<0.001	Excess of null; deficit of H
F	2	11.27	0.01 > <i>P</i> > 0.001	Deficit of VV genotypes
R'	1	11.69	0.01 > <i>P</i> > 0.001	Excess of S'; deficit of R'
Z	3	14.35	0.05 > <i>P</i> > 0.01	Excess of null; deficit of Z
Female families				
A ²	5	100.97	<0.001	Excess of null
A ²	1	42.29	<0.001	Excess of AH; deficit of H
F	2	24.09	<0.001	Deficit of VV genotypes
R'	2	101.48	<0.001	Excess of S'; deficit of R'
T'	4	262.46	0.01 > <i>P</i> > 0.001	Excess of null; deficit of T'
Z	1	4.74	0.05 > <i>P</i> > 0.01	Excess of null; deficit of Z

¹Number of families (of those in Table 1) with a significant chi-square test.

²Each detected situation is described separately.

and reported by Rocha et al. (18) were identified by the trait-based analyses. Thus, the TBA approach has the potential to become a useful method of QTL detection in situations in which only a limited number of traits are to be analyzed, in which family sizes are large, and in which the cost of phenotyping is considerably less than the cost of genotyping. However, an analytical strategy should be used that allows the identification of the effects that are consistent across environments and genetic backgrounds. The advantage of the TBA approach could be considerably enhanced if the allele frequencies for the marker systems could be determined from pooled DNA samples (3). The chi-square test proposed by Mackinnon and Georges (14) also offers the potential to provide important insights in some QTL detection studies. The quantitative effects associated with the C, L, M, and S blood groups that were reported by Rocha et al. (18) were supported by the results of these analyses, which further emphasizes their biological relevance and potential usefulness for marker-assisted selection.

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