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Fine Mapping of Quantitative Trait Loci on Bovine Chromosome 6 Affecting Calving Difficulty

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ABSTRACT

Calving difficulty is an economically and ethically important trait for dairy cattle breeding. The aim of the present paper was to refine the position of a previously detected quantitative trait locus (QTL) affecting calving difficulty (direct effect) in Norwegian Red dairy cows. A granddaughter design consisting of 18 elite sire families and a total of 713 sons was genotyped for 154 markers spanning the QTL region, and the trait data were analyzed by using a combined linkage and linkage disequilibrium approach. A highly significant QTL was detected in a 150-kb interval between the markers LAP3_281 and BTA-114677. Additionally, there were some indications of a second QTL between the markers BTA-75776 and BTA-75780 located less than 500 kb apart. Several candidate genes may be identified close to these QTL. Of these, a cluster of genes expected to affect bone and cartilage formation may be of particular interest for follow-up studies.
Key words: calving difficulty, cattle, fine mapping, linkage disequilibrium

INTRODUCTION

Good calving performance is of major importance in dairy cattle breeding, from both an economic and an animal welfare point of view. Veterinary assistance may be needed during a difficult parturition, and the cow may later experience reduced health, fertility, and milk production. A difficult calving may also substantially reduce the calf's viability, may result in morbidity or mortality, and, in the worst cases, may result in both animals dying or having to be culled.

Calving difficulty, or dystocia, arises because of factors related to the calf, the cow, or both, and is also affected by the environment. The main factors related to the calf are birth weight and viability. Calves lighter or heavier than average tend to have more difficult births (Berger et al., 1992) than average-sized calves, and male calves often experience more difficult births than females because of their larger size at birth (Johanson and Berger, 2003; Steinbock et al., 2003). Factors related to the cow include the shape of the birth canal, the size of the pelvis, and the cow's ability to nourish the fetus. Environmental factors, such as the cow's age at parturition (first-parity cows have a greater risk than cows in later parities) and calving season (more difficulties during the winter months), are also important (Johanson and Berger, 2003; Steinbock et al., 2003).

Calving difficulty has been a part of the total merit index used for selection of Norwegian Red sires since 1978 (http://www.geno.no). For calving difficulty, bulls are genetically evaluated as sire of the calf (direct effect, $\mathbf{CD_{dir}}$) and sire of the dam (maternal effect, $\mathbf{CD_{mat}}$). The trait is recorded on a 3-level scale consisting of the categories 1 (easy calving), 2 (slight problems), and 3 (difficult calving). The frequency of calving difficulty is relatively low in the Norwegian Red breed. During the period from 1991 to 2001, the mean frequency of "slight problems" increased from 4 to 7% for first calving, and from 2 to 3% for second and later calvings (Heringstad et al., 2007). The frequency of "difficult calving" was 2 to 3% for heifers and 1% for cows during the same period (Heringstad et al., 2007). Heritability estimates for Norwegian Red vary from 0.02 to 0.03 (Svendsen and Andersen-Ranberg, 2000) to 0.07 for a direct effect and 0.13 for a maternal effect (Heringstad et al., 2007), depending on the model. Heringstad et al. (2007) used a threshold model that accounted for the categorical nature of the data, whereas Svendsen and Andersen-Ranberg (2000) used a linear model.

The scoring system for calving difficulty varies among countries; thus, it is difficult to compare frequencies among cattle populations. However, the frequency found for the Norwegian Red is clearly lower than those for several other breeds. In Sweden, the mean frequency of calving difficulty was 4% for Swedish Red heifers and 8% for Swedish Holstein heifers (Philipsson et al., 2006). In Danish Holsteins, 11.2%

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of calvings were considered difficult (Hansen et al., 2004). Gevrekçi et al. (2006) reported that in American Holsteins, 13.2% of the parturitions fell in the "needed assistance" category and 13.7% fell in the "considerable force" category. In a study of first-parity Canadian Holstein cows, which included the categories of "hard pull" or "surgery needed," 19% of the male calves and 13% of the female calves were born with difficulty (Luo et al., 1999).

We previously performed a genome scan for QTL affecting calving difficulty in Norwegian dairy cattle (our unpublished results). The scan detected a QTL on bovine chromosome 6 (**BTA6**) affecting the direct effect of calving difficulty. The most likely position was between the markers FBN13 and BMS470, but the 95% confidence interval for the position spanned almost the entirety of BTA6. In a follow-up study using 399 markers on BTA6 (Nilsen et al., 2008), the QTL position was narrowed to an interval between the markers LAP3_581 and HCAP-G_119 (unpublished results). In the present study, we have constructed a very dense marker map spanning the QTL position, and have aimed to refine the position of this QTL even further by using a combined linkage and linkage disequilibrium (**LD**) approach.

MATERIALS AND METHODS

Data

All animals in the study belonged to the Norwegian Red breed. Sires and sons from 18 families were used. The total number of sons in the study was 713, ranging from 24 sons for the smallest family to 68 sons for the largest family. The total number of daughters was approximately 300,000, with an average of 418 daughters per son. The pedigree of each animal was traced back as far as known. Performance information was obtained in the form of daughter yield deviations for a direct effect (CD_{dir}) and a maternal effect (CD_{mat}) of calving difficulty of the sons of the 18 grandsires. Calving difficulty was subjectively scored on a 3-level scale consisting of 1) "easy calvings," 2) "slight problems," and 3) "difficult calvings." The 2 latter categories were subsequently combined into one group by the breeding organization for genetic evaluations. Only the first calving of each cow was included because incidence of "slight problems" and "difficult calvings" was very sparse for subsequent calvings, and records from multiple births, abortions, or stillbirths more than 20 d before expected calving date were excluded. The model used for evaluation of calving difficulty included the fixed effects of sex of the calf, the cow's age in months at calving, and month × year of calving, and the random effects of herd × year of calving, sire of the cow, and sire of the calf. The solutions for sire of the cow and sire of the calf effects were transformed into direct and maternal effects according to their expectations (Wilham, 1963; Van Vleck, 1978).

Marker Map

A dense marker map consisting of 154 single nucleotide polymorphisms (**SNP**) was developed. The map consisted of SNP detected by PCR resequencing of bulls from the Norwegian Red population (Nilsen et al., 2008) or selected from the list of "between breed" SNP produced in the Bovine Genome Sequencing Project (ftp://ftp.hgsc.bcm.tmc.edu/pub/data/Btaurus/). The SNP identification, Reference SNP (rs) numbers (http://www.ncbi.nlm.nih.gov/projects/SNP/), physical positions, and SNP allele frequencies are given in Table 1. Because very few recombinations were found between the closely linked markers, genetic distances based on recombination rates could not be obtained. Instead, distances in morgans were approximated by setting 1 cM equal to 1 Mb. Because the QTL mapping methods required some recombination between markers, all small marker distances were increased to 0.0001 M.

Statistical Analyses

Single-QTL Analysis Using Linkage and LD. The CD_{dir} and CD_{mat} were analyzed separately by using the combined linkage and LD method of Meuwissen et al. (2002). Briefly, the method consists of the following 3 steps. First, the linkage phases of all sires and sons were estimated based on marker information. Second, the identical by descent (**IBD**) probabilities of pairs of haplotypes were calculated at predefined positions on the basis of the similarity of the marker alleles carried by the haplotypes. Hayes et al. (2003) defined a measure of LD called chromosome segment homozygosity as the probability that random chromosome segments sampled from a population are IBD. Meuwissen and Goddard (2007) used chromosome segment homozygosity to calculate IBD probabilities at putative QTL positions based on the marker information at neighboring positions. These IBD probabilities were calculated at the midpoint of each marker bracket, which was regarded as the putative position for a QTL. Only the bracket midpoints were considered, because for a dense marker map, individual positions within the bracket would have similar probabilities. The IBD probability depends on the effective population size, which was assumed to equal 100. The matrix of IBD probabilities between haplotypes at position i is denoted $\mathbf{G}_i$. The last

Table 1. Marker names, reference single nucleotide polymorphism (rs) numbers, positions in base pairs, Hardy-Weinberg *P*-value (HWpval), percentage genotyped (%Geno), minor allele frequency (MAF), and alleles

No.	Name	rs no.	Position	HWpval	%Geno	MAF	Allele
1	AAFC02103536_75785	rs43711498	0	0.7911	96.0	0.273	C:A
2	AAFC02103536_75787	rs43711499	3441	0.9066	91.4	0.382	A:G
3	BTA-06585	rs29020944	31633	4.1764E-6	81.0	0.45	G:A
4	BZ954148_412	rs43711500	33450	0.6733	95.5	0.065	G:C
5	BZ954148_329	rs43711501	33533	0.5877	99.6	0.041	G:A
6	AAFC02063094_75788	rs43711502	121071	0.2673	98.1	0.146	G:A
7	AAFC02063094_75789	rs43711503	143186	0.9012	96.0	0.303	C:T
8	BTA-21842	rs41627896	318570	0.0546	94.9	0.37	A:G
9	AC149783_251	rs43711504	379623	1.0	98.8	0.079	T:A
10	BTA-15187	rs29026552	456857	0.3695	95.8	0.382	A:G
11	BTA-15186	rs29026551	457010	0.338	95.8	0.383	C:T
12	FAM13A1_124	rs43711506	489464	0.0528	66.1	0.273	C:A
13	FAM13A1_365	rs43711505	489958	0.7034	96.7	0.311	A:G
14	BTA-75976	rs41595968	518659	0.5749	99.6	0.105	T:C
15	BTA-75979	rs41595970	645752	0.8785	98.5	0.175	G:T
16	BTA-07293	rs29026959	648316	1.0	98.8	0.181	G:A
17	AAFC02162650_75814	rs43711507	901073	0.9437	97.8	0.476	A:G
18	AAFC02162650_75815	rs43711508	901138	0.7207	94.1	0.48	A:G
19	AAFC02162650_75817	rs43711509	901365	0.0271	89.2	0.304	T:C
20	AAFC02162650_75822	rs43711510	904384	0.3188	92.2	0.484	G:A
21	AAFC02162650_75821	rs43711511	904407	0.1128	86.7	0.484	T:C
22	AAFC02162650_75820	rs43711512	904520	0.3879	92.6	0.48	A:G
23	AAFC02162650_75819	rs43711513	904610	0.329	92.5	0.429	G:A
24	AAFC02162650_75818	rs43702331	904690	0.363	95.5	0.438	C:T
25	PPM1K_309	rs41256834	953640	0.4483	98.6	0.37	C:A
26	BZ916464_39	rs43702332	985757	0.4622	92.6	0.212	G:T
27	BZ916464_145	rs43702333	985863	0.9145	98.5	0.215	G:A
28	BZ916464_311	rs43702334	986029	1.0	100.0	0.051	C:T
29	BZ916464_404	rs43702335	986122	0.6548	86.7	0.208	G:A
30	BZ916464_460	rs43702336	986178	0.8399	99.9	0.051	G:C
31	BTA-22850	rs41577868	1044881	0.1982	97.9	0.367	T:G
32	ABCG2_49	rs43702337	1088080	1.0	99.3	0.05	A:C
33	ABCG2_256	rs43702338	1088699	0.792	95.2	0.213	G:A
34	BTA-03130	rs29010896	1102803	1.0	99.6	0.072	A:G
35	AAFC02144624_75784	rs29010896	1102808	0.5454	98.6	0.147	A:G
36	BTA-03129	rs29010895	1103078	0.0801	95.5	0.412	G:A
37	BTA-03128	rs29010894	1103353	0.5928	99.0	0.148	G:A
38	AAFC02144624_03128	rs29010894	1103358	0.2865	95.9	0.142	G:A
39	PKD2_746	rs29010894	1103358	0.2872	95.3	0.138	G:A
40	PKD2_1175	rs43702339	1104296	0.1534	99.9	0.073	A:G
41	PKD2_1451	rs43702340	1107052	0.8526	96.3	0.266	A:G
42	PKD2_1349	rs43702341	1107154	0.2935	77.7	0.071	T:A
43	PKD2_650	rs43702342	1107853	0.3788	100.0	0.086	C:T
44	PKD2_353	rs43702343	1108150	0.889	99.5	0.219	C:T
45	PKD2_611	rs43702344	1109358	0.4813	99.2	0.073	G:A
46	PKD2_610	rs43702345	1109359	0.1808	99.9	0.074	T:A
47	PKD2_349	rs43702346	1109620	0.1658	100.0	0.073	G:T
48	PKD2_383	rs43702347	1111932	1.0	100.0	0.023	G:A
49	PKD2_901	rs43702348	1116104	0.089	99.6	0.076	A:G
50	PKD2_377	rs43702349	1118841	0.4649	99.7	0.073	T:A
51	PKD2_447	rs43702350	1118911	0.1656	99.6	0.073	A:G
52	PKD2_1241	rs43702351	1119705	0.1798	100.0	0.074	C:T
53	PKD2_2256	—[1]	1120720	1.0	100.0	0.0	T:T
54	PKD2_2759	rs43702352	1121223	0.1798	100.0	0.074	T:C
55	PKD2_3610	rs43702353	1122074	0.2115	99.9	0.075	T:C
56	PKD2_3909	rs43702354	1122373	0.117	99.7	0.078	A:T
57	PKD2_97141	rs43702355	1122654	0.9056	99.5	0.098	G:A
58	PKD2_1013	rs43702356	1130519	0.2065	98.1	0.447	G:A
59	PKD2_953	rs43702357	1130579	0.5107	99.6	0.072	C:T
60	PKD2_597	rs43702358	1130935	0.3451	99.9	0.073	C:T
61	OPN_607	rs43702359	1189874	0.3429	98.6	0.132	T:C
62	AAFC02100954_75783	rs43702360	1217882	1.0	100.0	0.107	C:T
63	BTA-09065	rs29025232	1278411	0.9404	98.2	0.487	A:G
64	BTA-09066	rs29025233	1278814	1.0	99.7	0.074	A:G
65	BTA-75776	rs41650767	1292669	0.2294	98.1	0.302	G:A

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Table 1 (Continued). Marker names, reference single nucleotide polymorphism (rs) numbers, positions in base pairs, Hardy-Weinberg *P*-value (HWpval), percentage genotyped (%Geno), minor allele frequency (MAF), and alleles

No.	Name	rs no.	Position	HWpval	%Geno	MAF	Allele
66	BTA-75780	rs41650771	1354692	0.0874	96.4	0.285	C:T
67	BTA-02519	rs29010073	1365361	1.0	100.0	0.0	G:G
68	BTA-75829	rs41650806	1463408	0.2965	98.8	0.131	G:A
69	IBISS4snp1070	rs41255598	1632601	1.0	98.6	0.224	C:T
70	LAP3_581	rs43702364	1651481	0.8405	98.1	0.408	C:A
71	LAP3_572	rs43702363	1651490	0.9702	98.2	0.409	G:A
72	LAP3_529	rs43702362	1651534	1.0	93.0	0.408	G:A
73	LAP3_281	rs43702361	1651781	0.8995	98.4	0.408	A:G
74	BTA-114677	rs41616921	1798262	0.0864	98.5	0.348	A:G
75	HCAPG_387	rs43702368	1818909	5.666E-6	92.7	0.128	C:T
76	HCAPG_339	rs43702367	1818953	0.5228	99.5	0.092	T:C
77	HCAPG_318	rs43702366	1818978	0.9512	98.6	0.325	A:C
78	HCAPG_1119	rs43702365	1819252	0.0385	94.8	0.193	G:A
79	BZ946302_585	rs43702369	2036043	0.5039	98.6	0.153	T:C
80	BTA-09967	rs29026123	2265759	0.1862	99.3	0.116	C:T
81	bI0963H01_349	rs43702370	2947349	0.4141	99.7	0.134	A:G
82	BTA-75850	rs41650820	3076685	0.1411	97.8	0.189	A:G
83	BTA-75840	rs41567012	3280669	1.0	100.0	0.0	A:A
84	BTA-75882	rs41595946	3338686	0.1451	98.4	0.387	T:C
85	BTA-75859	rs41650828	3421418	0.5831	99.6	0.058	T:C
86	bI0389B10_424	rs43702371	3603059	1.0	99.3	0.092	T:C
87	BTA-114459	rs41615673	3640002	0.1163	94.0	0.298	G:T
88	BTA-88373	rs41665044	3778083	0.7771	99.6	0.053	C:T
89	bI0571A05_84	rs43702372	3866100	0.4741	95.3	0.136	T:C
90	BTA-11857	rs29017603	4023129	0.7412	98.1	0.301	C:T
91	BTA-84933	rs41656414	4121153	1.0	99.0	0.071	G:T
92	BTA-75992	rs41651298	4241177	0.8211	99.5	0.084	A:T
93	BTA-07405	rs29027071	4290284	0.3606	98.1	0.286	G:A
94	BTA-04099	rs29014464	4305493	0.0262	97.1	0.447	A:G
95	BTA-04100	rs29014465	4305701	1.0	99.3	0.097	G:A
96	BTA-04095	rs29014460	4312757	0.5901	97.7	0.181	G:T
97	BTA-04097	rs29014462	4312866	0.0214	97.8	0.445	G:A
98	BTA-04098	rs29014463	4315505	0.5101	95.8	0.466	G:T
99	BTA-75920	rs41651258	5058651	0.1527	99.2	0.074	C:T
100	bI0615A02_595	rs43702377	5077062	0.5061	99.7	0.157	C:T
101	bI0615A02_249	rs43702376	5077370	0.4385	99.9	0.172	A:G
102	bI0615A02_209	rs43702375	5077410	0.4176	100.0	0.172	G:A
103	bI0615A02_202	rs43702374	5077450	0.4601	99.7	0.171	C:T
104	bI0615A02_129	rs43702373	5077523	0.2823	99.3	0.173	T:C
105	BTA-75903	rs41595958	5156389	0.9351	97.7	0.309	T:C
106	BTA-75900	rs41651242	5190635	0.5307	96.3	0.429	G:A
107	BTA-08402	rs29021960	5354292	0.9493	97.0	0.425	T:C
108	bI0231A09_289	rs43702378	5675931	1.0	78.2	0.331	C:T
109	bI0231A09_718	rs43702379	5676362	0.9357	98.4	0.34	G:A
110	BTA-75941	rs41651273	5699747	0.5444	97.4	0.42	G:A
111	BTA-04607	rs29014966	5842254	0.0154	96.9	0.494	C:T
112	bI0557B12_532	rs43702381	5970986	0.5158	98.5	0.279	G:A
113	bI0557B12_146	rs43702380	5971371	1.0	100.0	0.02	A:G
114	BTA-121746	rs41622325	6097390	0.5353	97.0	0.188	G:A
115	BTA-75936	rs41651272	6140888	0.2963	98.4	0.371	C:T
116	BTA-11586	rs29027897	6250125	0.1455	97.9	0.342	T:C
117	BTA-24614	rs41625135	6376695	0.9858	97.3	0.481	A:G
118	BTA-107931	no hit	6489843	0.8815	96.3	0.295	G:A
119	BTA-52678	rs41644601	6549550	0.0227	99.2	0.076	G:C
120	BTA-52688	rs41644610	6639060	1.0	99.7	0.05	C:T
121	bI0558E08_383	rs43702382	6728425	0.2584	98.2	0.491	T:C
122	BTA-76003	rs41595981	6828101	0.9874	97.4	0.299	G:A
123	bI0590C12_118	rs43702383	6838744	1.0	100.0	0.014	G:A
124	bI0590C12_560	rs43702384	6839186	0.3322	98.2	0.428	T:C
125	BTA-16531	rs41578346	7244230	0.9476	98.1	0.262	G:A
126	BTA-97417	rs41665301	7375700	0.1051	99.2	0.117	T:G
127	BTA-97415	rs41665299	7501424	0.0936	96.3	0.433	C:T
128	bI1089D07_611	rs43702385	7531032	0.1842	99.9	0.088	T:A
129	BTA-06905	rs29021261	7587755	0.1042	98.1	0.498	T:C
130	BZ939648_545	rs43462273	7811999	6.5861E-9	97.0	0.188	C:T

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Table 1 (Continued). Marker names, reference single nucleotide polymorphism (rs) numbers, positions in base pairs, Hardy-Weinberg *P*-value (HWpval), percentage genotyped (%Geno), minor allele frequency (MAF), and alleles

No.	Name	rs no.	Position	HWpval	%Geno	MAF	Allele
131	BZ939648_425	rs43462272	7812119	1.0	99.0	0.434	G:A
132	BTA-76031	rs41651307	7822561	0.1162	98.2	0.219	G:T
133	PPARGC1_186	rs43702387	7925306	0.8445	99.0	0.084	C:A
134	PPARGC1_67	rs43702386	7925716	0.6479	97.7	0.34	C:G
135	BTA-76038	rs41595994	8007357	0.906	95.3	0.271	A:G
136	BTA-76048	rs41567027	8163486	0.2703	95.9	0.444	C:T
137	BTA-76033	rs41651309	8241803	0.4799	98.2	0.207	T:C
138	BTA-76051	rs41651316	8334808	0.4221	98.5	0.117	G:A
139	BTA-76050	rs41651315	8453814	0.7873	96.9	0.436	G:C
140	BTA-76049	rs41567028	8508823	0.9911	98.9	0.132	A:G
141	BTA-76111	rs41652048	8561011	0.6715	99.3	0.073	G:A
142	BTA-76107	rs41652044	8611245	0.0817	93.7	0.293	G:C
143	BTA-04998	rs29015348	8630226	0.9055	99.3	0.059	C:G
144	BTA-110806	rs41575153	8874820	0.6748	92.7	0.454	C:G
145	BTA-12005	rs29023972	9050757	0.1399	91.8	0.262	T:C
146	BTA-76106	rs41596013	9114230	0.078	92.7	0.38	T:C
147	BTA-76105	rs41652043	9140719	0.0858	93.7	0.402	T:C
148	BTA-23354	rs41578595	9568517	0.4457	99.5	0.076	T:G
149	BTA-05020	rs29019187	9592127	1.0	98.6	0.212	A:T
150	BTA-05021	rs29019188	9594819	1.0	98.4	0.211	A:C
151	BTA-76099	rs41652042	9627964	0.4537	97.8	0.129	G:A
152	IBISS4snp819	rs41257255	9676652	0.8486	96.2	0.246	A:C
153	BTA-11740	rs29017497	9734457	8.4442E-5	86.6	0.274	C:T
154	BTA-11739	rs29017496	9734515	0.7939	99.5	0.076	G:A

[1]This locus was detected and verified as a single nucleotide polymorphism (SNP), but is in fact a sequencing error because all genotyped animals turned out to be homozygous. This error was detected after the analyses were performed, and therefore this "SNP" has not been removed from the map. It does, however, affect the statistical analyses.

step was to compare the correlations in the $\mathbf{G_i}$ matrix to those in the data by using a REML analysis. The statistical model used for this analysis was

$$\mathbf{y} = \mu\mathbf{1} + \mathbf{Zh} + \mathbf{u} + \mathbf{e}, \quad [1]$$

where $\mathbf{y}$ is an n × 1 vector of records (i.e., daughter yield deviations for the trait in question); μ is the overall mean; $\mathbf{1}$ is a vector of 1's; $\mathbf{h}$ is a vector of random haplotype effects of dimension q × 1, where q is the number of different haplotypes; $\mathbf{Z}$ is an n × q incidence matrix relating observations and haplotype effects; $\mathbf{u}$ is a vector of random polygenic effects; and $\mathbf{e}$ is a vector of residuals. The variances of $\mathbf{h}$, $\mathbf{u}$, and $\mathbf{e}$ are $\mathbf{G_i}\sigma^2_h$, $\mathbf{A}\sigma^2_u$, and $\mathbf{R}\sigma^2_e$, respectively, where $\mathbf{G_i}$ is the matrix of IBD probabilities among haplotypes, $\mathbf{A}$ is the additive genetic relationship matrix, and $\mathbf{R}$ is a diagonal matrix with n_j^{-1} on the diagonals (n_j is the number of daughters of bull j).

For each marker bracket, the log-likelihood of a model containing the QTL [LogL($\mathbf{G_i}$)] was calculated as well as a model fitting only background genes [LogL(0)] by using the ASREML package (Gilmour et al., 2002). A likelihood ratio test-statistic (**LRT**) was calculated as LRT = LogL($\mathbf{G_i}$) − LogL(0). The marker bracket with the greatest LRT was expected to contain the QTL, if the LRT of that bracket was considered significant. The linkage analysis was used to test the detected QTL for its chromosome-wise significance. Approximate nominal significance levels were found by using the LRT, where 2 × LRT is approximately a chi-square distributed with 1 degree of freedom. To achieve a nominal significance level of 0.001, 2 × LRT must exceed 10.8; that is, LRT greater than 5.4 were regarded as significant. For technical reasons, the brackets were numbered from 2 to 154; that is, bracket number 2 referred to the interval between the first and second marker.

Multiple-QTL Analyses Using Linkage and Linkage Disequilibrium. A complete multiple-QTL analysis (for instance, as described by Meuwissen and Goddard, 2001) could not be performed because of convergence problems caused by the highly correlated $\mathbf{G_i}$ matrices arising from the small bracket sizes. Instead, we used the same analysis as for the single-QTL analysis, but included a random effect of a QTL in another specified marker bracket. That is, each bracket that showed a high LRT in the single-QTL analysis was included as a random effect in the QTL model in turn, and the analysis was repeated. These analyses search for additional QTL, given that the QTL in the bracket is accounted for and is similar to the fitting of cofactors (Jansen, 1993).

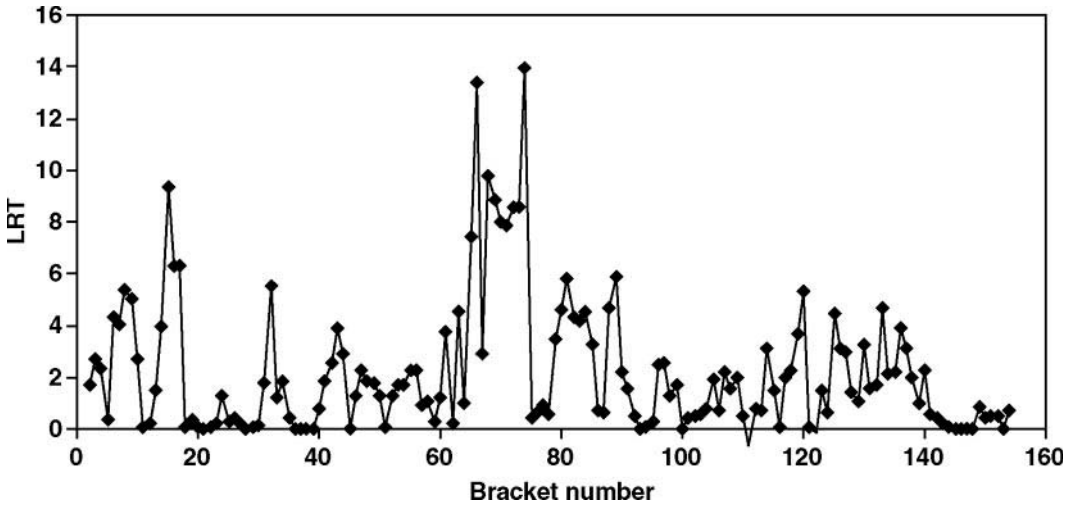

Figure 1. Single-QTL analysis for a direct effect of calving difficulty. The abscissa denotes the marker bracket number and the ordinate denotes the likelihood ratio test-statistic (LRT). Points illustrate bracket midpoints.

The above-mentioned analyses were also performed with the effect of a specific marker fitted in model 1 (instead of the bracket effect) to search for markers in high LD with the QTL. The QTL search was also repeated with the effects of both brackets 66 and 74 included in the model to investigate whether there was any evidence of more QTL segregating in other brackets.

Haplotype Analysis

Linkage phases between markers for all animals were estimated by multi-locus iterative peeling (Meuwissen, 2006). The resulting haplotypes were imported into the Haploview program (http://www.broad.mit.edu/mpg/haploview/; Barrett et al., 2005) for calculation of LD (r^2) between markers and construction of haplotype blocks.

RESULTS

Results for single-QTL analysis of CD_{dir} are shown in Figure 1. The figure reveals the presence of several peaks. The largest peak was found in bracket number 74 (i.e., the interval between markers LAP3_281 and BTA-114677), with an LRT of 13.9. A peak with similar LRT (13.4) was found in bracket 66, which is the interval between markers BTA-75776 and BTA-75780. The intervals between these 2 brackets also showed rather large test statistics because the LRT ranged from 8 to 10 for most of these brackets. A third peak was found in bracket 15 (BTA-75976 to BTA-75979), with an LRT of 9.4. All these peaks were highly significant, with nominal *P*-values of <0.0001. Several other peaks also exceeded the nominal significance level of LRT = 5.4 ($P = 0.001$). No significant results for CD_{mat} were found.

The multiple peaks for CD_{dir} could be due to either the presence of more than one QTL or the presence of one QTL with carryover effects to other regions; thus, a multiple-QTL analysis was performed. First, a QTL was fitted in bracket 74 (i.e., the interval between markers LAP3_281 and BTA-114677), and the other brackets were scanned for additional QTL. As shown in Figure 2, a sharp peak was seen in bracket 66, but the LRT was just above 4, and hence below the significance threshold. All other variation in the region was explained by the fitted QTL effect.

The result of including the effect of a QTL in bracket 66 (BTA-75776 to BTA-75780) is shown in Figure 3. The peak in bracket 74 remained, but its LRT was reduced to approximately 5. There were also a few smaller but not significant peaks, of which bracket 15 had the greatest LRT (approximately 4.5).

Figure 4 illustrates the result of fitting a QTL in bracket 15 (BTA-75976 to BTA-75979). All signals in the proximal half of the region were removed, but the peaks in brackets 66 and 74 remained. However, the LRT of these brackets were largely reduced as compared with the single QTL analysis, with the LRT of brackets 66 and 74 now being approximately 8.5 and 6, respectively.

When QTL effects in the remaining brackets were fitted, similar results as for the single-QTL analysis were found. Thus, our data do not show any evidence of further QTL in any other brackets.

The analyses yielded strong support for one or more QTL in brackets 74, 66, or both and seemed to exclude the possibility of further QTL. To verify this result, we

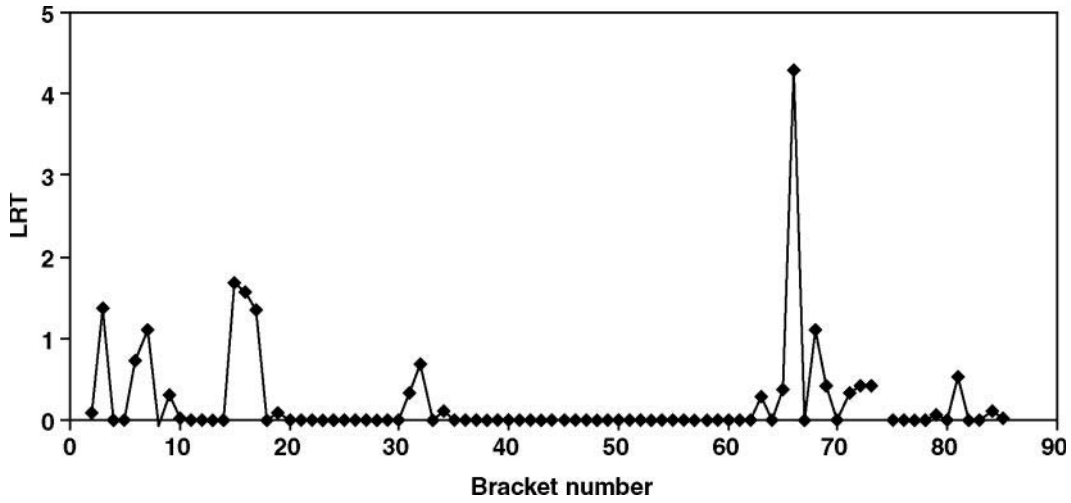

Figure 2. Multiple-QTL analysis with bracket 74 included in the model. Only the first 90 brackets are shown to improve the readability of the figure. LRT = likelihood ratio test.

also extended model [1] to include the effects of both of these brackets simultaneously. The resulting curve was completely flat (not shown), and again no evidence of further QTL in other brackets, including bracket 15, was found in our data.

Next, we aimed to identify markers in LD with the QTL by including marker effects in the QTL model. Figure 5 shows the results of including BTA-75979 (marker 15, i.e., the right boundary of bracket 15). By using this model, the bracket 15 peak was removed, whereas the LRT of brackets 66 and 74 were reduced to approximately 9 and 8, respectively. Surprisingly, such results were not found for any of the other markers; thus, only one of the 154 genotyped markers was in considerable LD with the QTL.

Finally, linkage phases of all animals were imported into the Haploview program (Barrett et al., 2005) for calculation of LD (r^2) between markers and investigation of haplotype block structure in the QTL regions. In general, levels of LD were low for the entire genotyped region, and few haplotype blocks could be constructed based on the degree of LD. None of the 6 markers surrounding brackets 15, 66, and 74 was included in a haplotype block. In addition, the LD between these markers was surprisingly low. The greatest LD was found between markers 14 and 15 (BTA-75976 and

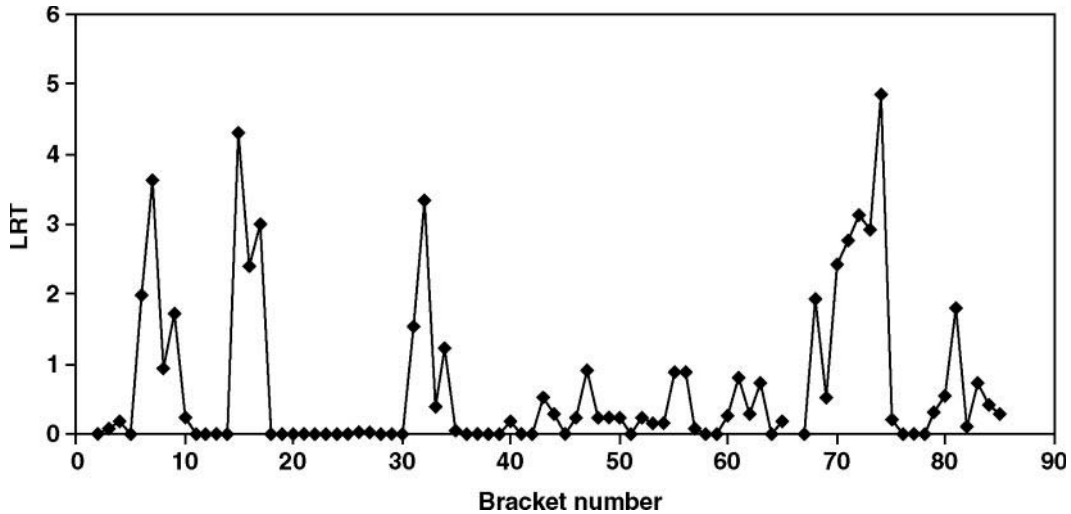

Figure 3. Multiple-QTL analysis with bracket 66 included in the model. Only the first 90 brackets are shown to improve the readability of the figure. LRT = likelihood ratio test.

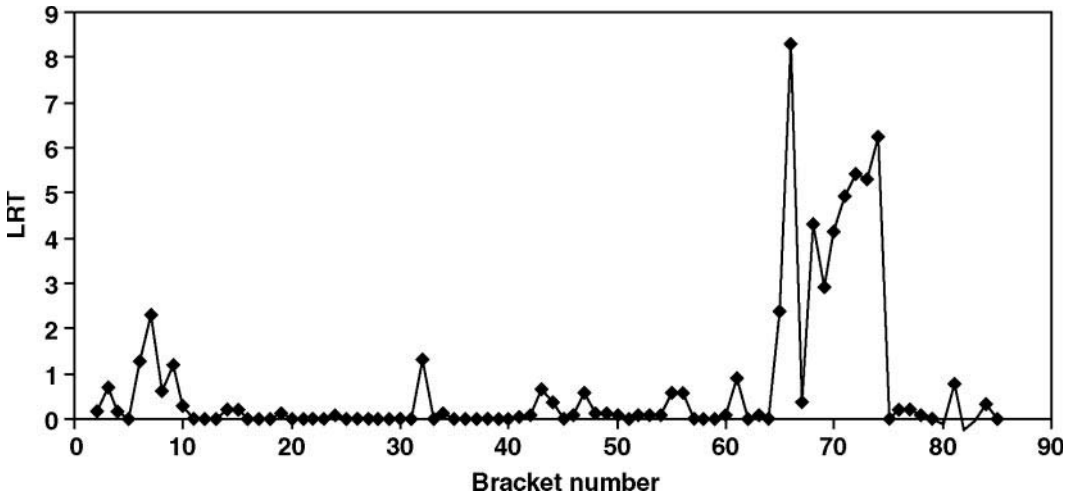

Figure 4. Multiple-QTL analysis with bracket 15 included in the model. Only the first 90 brackets are shown to improve the readability of the figure. LRT = likelihood ratio test.

BTA-75979), with an r^2 of 0.19. Marker 14 was also in some degree of LD with BTA-75780 (marker 66), with an r^2 of 0.15. For the other marker pairs, r^2 varied between 0.003 and 0.059. Figure 6 illustrates r^2 for the markers pairs between markers 65 and 74.

DISCUSSION

Preliminary analyses in Norwegian Red had indicated the presence of a QTL affecting the direct effect of calving difficulty in the middle part of BTA6 (unpublished results). The aim of this study was to refine the position of this QTL further to search for candidate genes.

The results of this study strongly confirm the presence of one or more QTL with an effect on CD_{dir} on BTA6. The single-QTL analysis yielded 3 peaks that were all significant at the nominal 0.0001 level. These peaks were situated in brackets 15, 66, and 74. However, the subsequent analyses showed that not all peaks represented true QTL. The peak in bracket 15 was reduced to below the significance threshold, both in the analysis in which bracket 74 was included and in the cases in which bracket 66 and both brackets 66 and 74 were included. Such a result could be explained by LD between the markers surrounding bracket 15 and a more distal QTL. However, the result from Haploview did not reveal high levels of LD between pairs of markers surrounding bracket 15 and markers surrounding bracket 66 or 74. Still, the fact that the peaks in brackets 66 and 74 were markedly reduced when the effect of bracket 15 was included in the model strongly

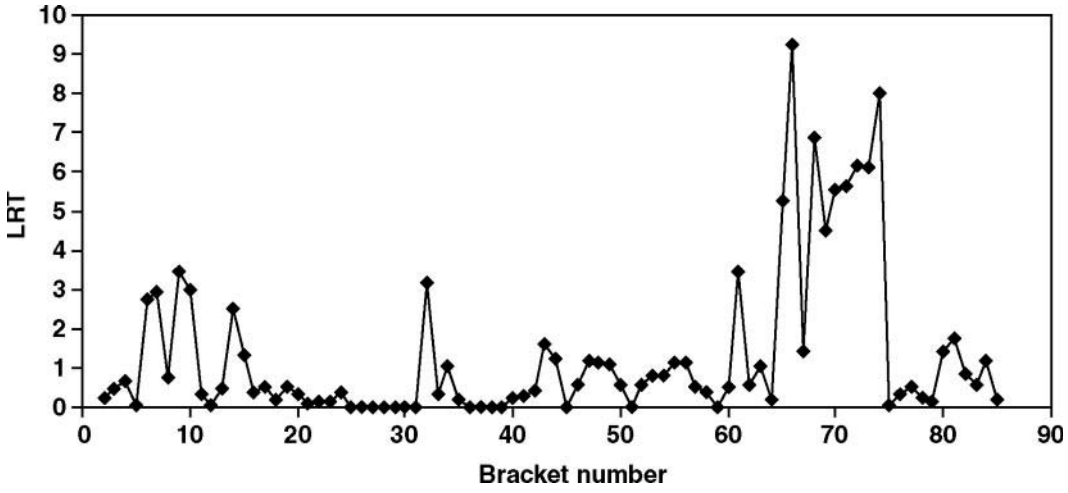

Figure 5. Multiple-QTL analysis with marker number 15 included in the model. Only the first 90 brackets are shown to improve the readability of the figure. LRT = likelihood ratio test.

indicates the existence of LD between the bracket 15 markers and combinations of alleles of several markers (i.e., haplotypes) surrounding the QTL. The same result was found when the effect of marker number 15 was fitted. Thus, we can conclude that the bracket 15 peak was merely an artifact caused by LD between the markers surrounding bracket 15 and a true QTL further downstream.

The situation for brackets 66 and 74 was somewhat less clear. The peak in bracket 74 had the greatest LRT in the single-QTL analysis, and no other brackets showed significant results when a QTL was fitted in this bracket. A reasonable explanation is then that only one QTL was segregating in our data and that this QTL was situated in bracket 74. However, the LRT of bracket 66 was reduced to only slightly below the significance threshold and was not completely removed. The fact that the 66 peak was not completely explained by a QTL in the bracket 74 QTL could indicate that bracket 66 did contain other polymorphisms with an effect on calving difficulty but that this effect was not statistically significant. Therefore, the possibility of a second QTL here cannot be completely excluded. A third possibility is that the 2 peaks could be caused by one QTL positioned somewhere between the 2 brackets. This hypothesis was supported by the high LRT in these brackets obtained by the single-QTL analysis. On the other hand, the fact that fitting a QTL in any of these brackets did not remove the QTL signals at brackets 66 and 74 contradicts this hypothesis.

Our conclusion is that the most likely explanation for the presented QTL signals is the presence of only one QTL, which was situated in bracket 74. This bracket is bordered by LAP3_281 and BTA-114677, which are separated by a physical distance of less than 150 kb. However, we cannot completely rule out the possibility of a second QTL segregating in bracket 66, or alternatively, the presence of only one QTL situated somewhere between these brackets. This explanation expands the most likely location of the QTL to a region of approximately 500 kb bounded by the SNP BTA-75776 and BTA-114677.

The reason for the difficulties in determining the correct QTL position(s) can be found from the analyses in which the effect of each marker was included in the QTL model. According to these results, marker 15 was the only one of the 154 markers whose alleles segregated in some concordance with the QTL alleles. Because the calving difficulty QTL was found in a region where much effort had been undertaken to identify a QTL for milk production (Olsen et al., 2007), the SNP density in that region was very high. Despite the high map density, the genotyped markers were not found to be causal mutations or in high LD with

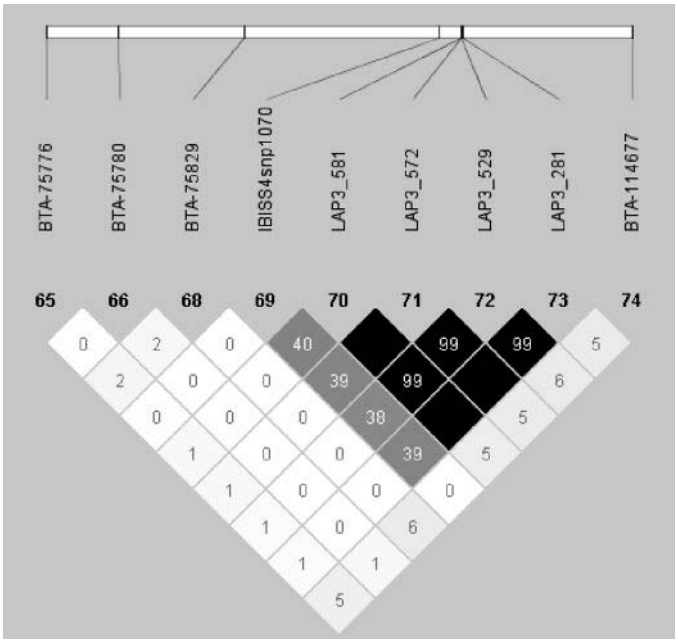

Figure 6. Linkage disequilibrium expressed as $r^2 \times 100$ between markers in the BTA-75776 (marker no. 65) to BTA-114677 (marker no. 74) region.

the real mutation. The true causal mutation could be identified by performing a systematic SNP search in the region and redoing the analyses with this new set of markers. Given the relative narrow mapping of the QTL, even resequencing the entire 500-kb region in animals carrying different QTL alleles appeared to be an affordable endeavor when using the new sequencing technology (Albert et al., 2007).

The region around brackets 66 and 74 contains several genes that can be regarded as interesting functional or positional candidates, or both. This region of BTA6 contains at least 6 known genes: osteopontin (**OPN**), extracellular matrix phosphoglycoprotein (**MEPE**), integrin-binding sialoprotein (**IBSP**), leucine aminopeptidase 3 (**LAP3**), mediator of RNA polymerase II transcription, subunit 28 homolog (*Saccharomyces cerevisiae*; **EG1**, also denoted as **MED28**), and NCAPG non-SMC condensin I complex, subunit G (**HCAP-G**). One SNP in OPN, OPN_607, was genotyped in our study and constitutes the boundary of brackets 61 and 62. The MEPE SNP BTA-02519 constitutes the boundary between brackets 67 and 68. The IBSP was not genotyped in the present study, but is mapped to the interval between MEPE and LAP3 (Cohen-Zinder et al., 2005). Of these, OPN, IBSP, and MEPE are included in a cluster of bone-tooth mineral extracellular matrix phosphoglycoproteins (Rowe et al., 2000). Although the cluster is thought to be involved in several biological processes, such as branching during

tubulogenesis of the uretic bud in the kidney (Stuart et al., 1995) and branching of the mammary epithelial ductal system (Talhouk et al., 1992), it is primarily associated with bone and cartilage morphogenesis. As an example, MEPE is thought to play an inhibitory role in bone formation, and a disruption of one of its alleles was shown to cause significantly increased bone mass in the mouse (Gowen et al., 2003). The size of the calf as compared with its mother is one of the main factors contributing to calving difficulty (e.g., Johanson and Berger, 2003); thus, this extracellular matrix cluster represents very good functional candidate genes. Several SNP of LAP3 and HCAP-G are genotyped in our study. The most likely QTL position is in bracket 74, which is the interval between the last SNP of LAP3 and the marker BTA-114677. Bracket 74 also contains the gene EG1. Very little information about the function of these genes can be found, but all are close enough to the QTL to be regarded as positional candidates.

Several studies have detected QTL for traits related to calving performance on BTA6. Holmberg and Andersson-Eklund (2006) reported a QTL for CD_{dir} close to marker BM143 and for CD_{mat} at BM1329 in Swedish dairy cattle. Based on our unpublished linkage analysis map, the distance between the bracket 74 QTL and BM143 is approximately 4 cM. Kühn et al. (2003) reported a QTL for calving difficulty and stillbirth in the proximal end of BTA6 in German Holsteins at approximately the same position where Schrooten et al. (2000) found the QTL affecting calving difficulty, size, and dairy character in Dutch Holsteins. Casas et al. (2000) reported a QTL for birth weight, which is a major cause of calving difficulty, close to BMS2508 in beef cattle. This marker is situated approximately 4 cM proximal of our QTL; thus, the results of several of these papers could reflect the presence of the same QTL segregating in different breeds.

CONCLUSIONS

Our results clearly demonstrate that at least one QTL for a direct effect of calving difficulty is segregating on BTA6 in Norwegian Red. The most likely position is in a 150-kb interval between markers LAP3_281 and BTA-114677. Some evidence was found for a second QTL between markers BTA-75776 and BTA-75780. The distance between the 2 putative QTL is less than 500 kb. Several interesting candidate genes can be found in this region, including a gene cluster affecting bone and cartilage morphogenesis.

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