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A primary screen of the bovine genome for quantitative trait loci affecting twinning rate

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Abstract. An autosomal genome scan for quantitative trait loci (QTL) affecting twinning rate was carried out in the Norwegian Cattle population. Suggestive QTL were detected on Chromosomes (Chr) 5, 7, 12, and 23. Among these, the QTL positions on both Chr 5 and Chr 23 are strongly supported by literature in the field. Our results also confirm previous mapping of a QTL for twinning to Chr 7, but definitely suggest a different location of the QTL on this chromosome. The most convincing QTL peak was observed for a region in the middle part of Chr 5 close to the insulin-like growth factor 1 (*IGF1*) gene. Since *IGF1* plays an important role in the regulation of folliculogenesis, a mutation search was performed by sequencing more than 3.5 kb of the gene in actual families. The sequencing revealed three polymorphisms in noncoding regions of the gene that will be important in fine structure mapping and characterization of the QTL.

Introduction

Higher reproduction capacity has the potential of reducing production costs of several livestock species. One way to improve fertility in cattle is to increase the proportion of the herd producing twins relative to single calves. Results from experimentation and computer simulation suggest that input costs per unit of beef output could be reduced by 20–30% in the proportion of a herd that produces twins (Guerra-Martinez et al. 1990). Twinning rate in cattle is basically a product of ovulation rate, conception rate, and embryo survival (Van Vleck and Gregory 1996). Twinning and ovulation rate in cattle are strongly genetically correlated (0.75–0.9) (Van Vleck et al. 1991; Gregory et al. 1997), which indicates that the two traits are influenced by common loci.

Most studies analyzing field data report low heritability of single-parity twinning rate on the observable scale, generally below 0.04 (Syrstad 1984; Ron et al. 1990; Karlsen et al. 2000). Despite this, it has been shown that twinning rate can be significantly increased by selection. An example is the twinning population established at the US Meat Animal Research Center (MARC), Clay Center, Nebraska, where twinning rate was increased by 25.1% (from 3.4% to 28.5%) over the years 1982 to 1993 (Van Vleck and Gregory 1996). Five of the foundation animals in this experiment were Norwegian Cattle or Swedish Friesian sires whose daughters had produced twins at a rate of about 10% (Gregory et al. 1990). A recent study by Karlsen et al. (2000), utilizing field records from the Norwegian Dairy Recording Service (NDRS), shows that twinning rate in Norwegian Cattle has increased during the time period 1978 to 1995, and that some sires in the population have extremely high or low percentages of multiple births among their daughters.

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Linkage maps in cattle (Barendse et al. 1997; Kappes et al. 1997) have sufficient marker density for genomic scans of populations for quantitative trait loci (QTL), and much of the mapping resources are now targeted towards QTL detection of economically important traits. Previous QTL reports on bovine twinning and ovulation rate are mainly from the MARC twinning population, suggesting QTL for ovulation rate on bovine Chr 5 (Kappes et al. 1998; Kirkpatrick et al. 2000), Chr 7 (Blattman et al. 1996; Kirkpatrick et al. 2000), Chr 19 (Kirkpatrick et al. 2000), and Chr 23 (Blattman et al. 1996).

Recently, a male genetic map was developed in six paternal half-sib families from the Norwegian Cattle population (Våge et al. 2000). The objective of this study was to utilize genotyping in these families together with field records from the NDRS in order to identify chromosomal regions with QTL influencing twinning rate.

Materials and methods

Norwegian cattle map (NCM). Six large paternal half-sib families with a total of 285 sons of the commercial Norwegian Cattle population were used in the construction of the map (Våge et al. 2000). The NCM covers all 29 autosomal chromosomes and summarizes to a total length of 2682 cM. The average length of marker intervals is 12.5 cM, and approximately 82% of the intervals are shorter than 20 cM. More information about the NCM, including average number of informative sons per marker, is available on http://www.nlh.no/Institutt/IHF/Genkartstorfe/.

Additional markers. In order to achieve higher marker density in the middle part of Chr 5, four additional microsatellites (BMS490, CSSM22, BM1216, and RM29) were genotyped by using primers and PCR conditions as described at USMARC Genome Database (1999). In addition, parts of the insulin-like growth factor 1 (*IGF1*) gene were sequenced in order to detect polymorphisms in addition to a low polymorphic microsatellite in the 5′ flanking region of the gene (Kirkpatrick 1992). A total of 3670 bp of the gene, including the 5′-flanking region, most of the coding sequence, and parts of introns were sequenced in the six sires by dye primer sequencing of PCR products (Amersham Pharmacia Biotech, Uppsala, Sweden). Bovine IGF1 sequences are submitted to GenBank with accession numbers AF210383, AF210384, AF210385, AF210386 and AF210387. Oligonucleotides (Table 1) for sequencing were designed from bovine sequence (Ge et al. 1997) or from homologous ovine and caprine IGF1 sequences, when bovine sequences were unavailable (Ohlsen et al. 1993; Mikawa et al. 1995).

Polymorphisms in IGF1. The sequencing of bovine IGF1 revealed three polymorphisms in non-coding regions. One of these mutations, a single nucleotide polymorphism (SNP) in the 5′-flanking end of the gene (position 1407 in AF210383), has previously been reported by Ge et al. (1997). An amplification-created restriction site (ACRS) method (Haliassos et al. 1989) was developed for efficient typing of the mutation in the actual families. Primers IGF1ACRS and IGF5′P4 were used to specifically

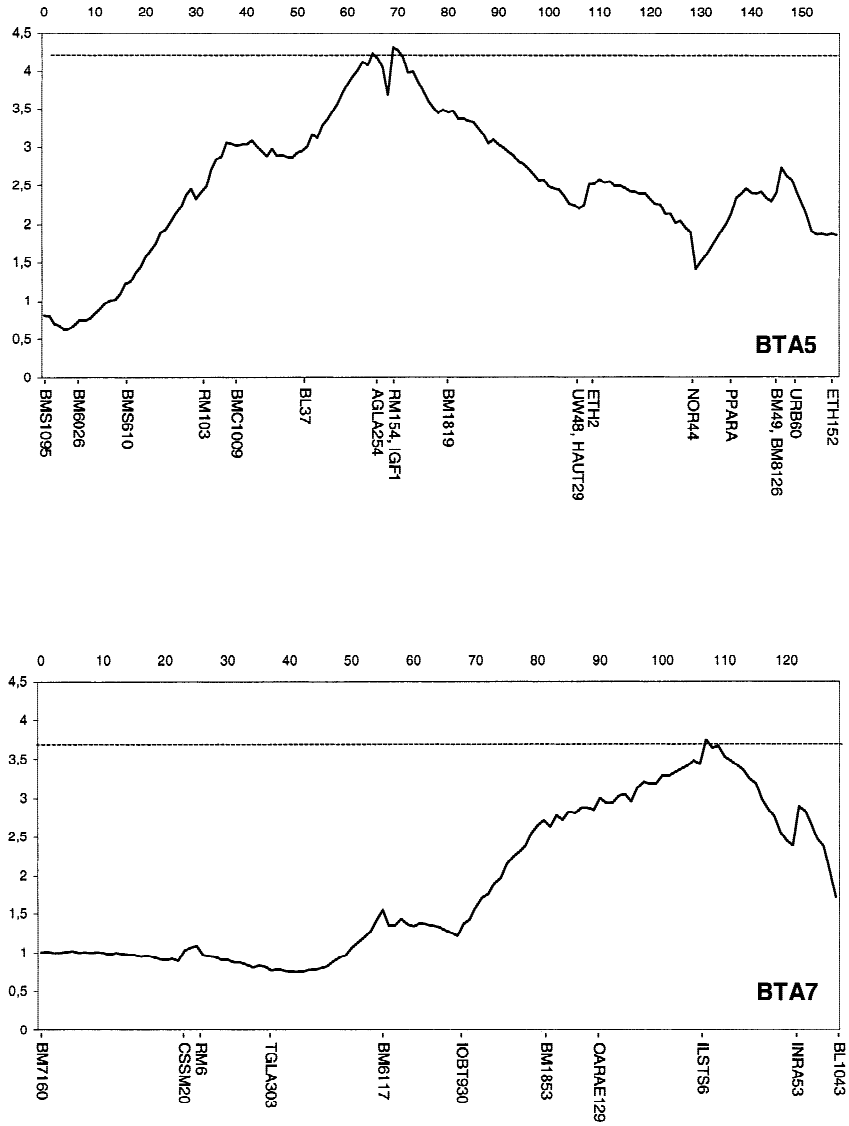

Fig. 1. Across-family QTL results for twinning rate on bovine Chrs 5, 7, 12, and 23 in the Norwegian Cattle population. Markers are pointed out on the X-axis and F-values are shown on the Y-axis. Putative QTL positions with corresponding chromosome-wise 5% (---) significance thresholds are also given for each chromosome.

Table 1. Oligonucleotides

IGFex1P1	ca/ov	5′ATTGGGTTGGAAGACTGC3′
IGF5′P2	bov	5′-21M13-TCTGCTCCTCTTGTCACTA3′
IGF5′P3	bov	5′GGCATGGTGACAAATAAC3′
IGF5′P4	bov	5′-21M13-CCAGGTTCTAGGAAATGAG3′
IGF5′P5	bov	5′TGATTTTAACCCTCAAACG3′
IGF5′P6	ca/ov	5′-21M13-GCTGAGAGATTTGAATGAC3′
IGF5′P7	ca/ov	5′CTAGCAATACCCTCTCAA3′
IGF5′P8	ca/ov	5′-21M13-AACCAATCAGGTTTTCCT3′
IGF5′P9	ca/ov	5′TGCTTGAAACTCTTGTCT3′
IGF5′P10	ca/ov	5′-21M13-GTCTTGGATTCTTCTCAA3′
IGFex2P11	ca/ov	5′AAATACTCACTGTAGGTGTAACC3′
IGFex2P12	ca/ov	5′-21M13-TACGTGGTTCTTTCAAATG3′
IGFex3P13	ca/ov	5′AAACACTAGGCTCGCATTA3′
IGFex3P14	ca/ov	5′-21M13-CTGCTCAGAGGTCACTCAC3′
IGFex4P15	ca/ov	5′CCCTATGAGCCAGAAGTCTAT3′
IGFex4P16	ca/ov	5′-21M13-CGCCTATTATCCCACTCTA3′
IGFex6P17	ca/ov	5′AAGCCTGCTGAATGAATGT3′
IGFex6P18	ca/ov	5′-21M13-GGTTGAGATCCAGTGTTAG3′
IGF1Del	bov	5′-TET-CTCCGGAAGCAGCACTCATC3′
IGF1ACRS	bov	5′TTACCCGTATGAAAGGAATATTCCT3′

bov: oligonucleotides designed from bovine IGF1 sequence (Ge et al. 1997).
ca/ov: oligonucleotides designed from homologous ovine and caprine IGF1 sequences (Ohlsen et al. 1993; Mikawa et al. 1995).
−21M13: TGTAAAACGACGGCCAGT.
TET: end labeling with the fluorescence dye TET.

Table 2. Families used in the QTL study.

Sire family	Sons	Granddaughters[a]
2005	71	67,900
2052	32	44,000
2402	54	50,700
2463	39	46,000
2946	42	17,100
3131	47	13,900
Total	285	239,600

[a]Approximate number of cows with records on twinning rate.

amplify a PCR product of 224 bp. Samples were digested with *Scr*FI, followed by separation on 3% agarose gels (fragments of 25, 42, and 157 bp). Furthermore, a 4-bp deletion (TTTG) and a point mutation were detected in intron 4 and intron 5, respectively (position 27–30 and position 270 in AF210386). The deletion in intron 4 was detected by PCR amplification by using the fluorescence-labeled primer IGF1Del together with primer IGFex4P16, followed by separation of PCR products on a DNA sequencer (ABI-373). The SNP in intron 5 was genotyped by *Dpn*II digestion of PCR products generated by primers IGFex4P15 and IGFex4P16, followed by separation on 3% agarose gels (fragments of 36, 85, and 237 bp). All polymorphisms in the *IGF1* gene were genotyped in informative

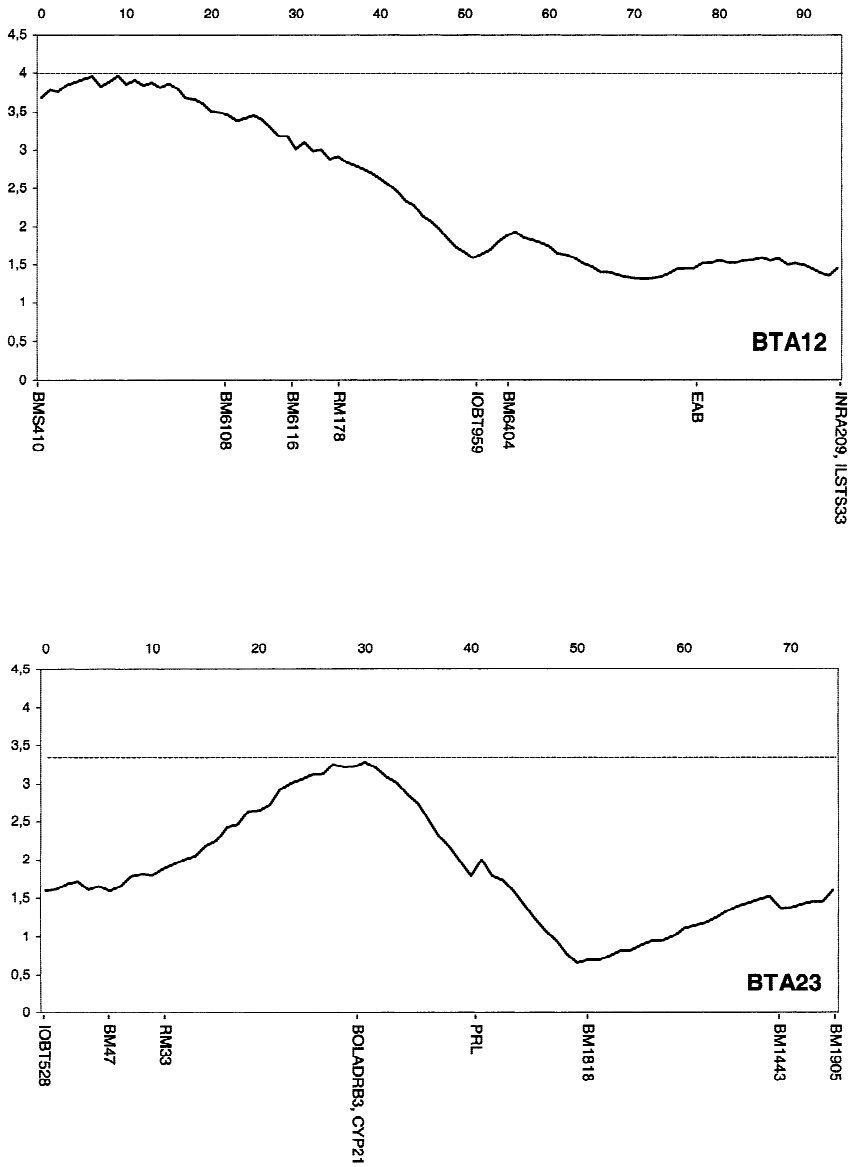

Fig. 1. *Continued*

families and included in the linkage analysis. The CHROMPIC option of CRI-MAP 2.4 (Green et al. 1990) was used to identify unlikely double crossovers, and a new linkage map for Chr 5 was constructed.

Performance data. First-parity twinning records of approximately 239,600 cows were used in the study (Table 2). All cows were daughters of 285 bulls belonging to six paternal half-sib families previously used to construct a male genetic map in Norwegian Cattle population (Våge et al. 2000). Average twinning frequency among these cows was 0.6%, whereas mean twinning rate for daughters of sons varied from 0.0 to almost 4%. The data were pre-corrected for fixed effects, such as age of calving, month of calving, and herd-year (Karlsen et al. 2000) and used in a granddaughter design (Weller et al. 1990).

Interval mapping. The statistical analysis followed the multiple marker regression method of Knott et al. (1996), but incorporating a random effect of son in the model. The method was applied both across and within all families according to the mean model:

$$y_{ijk.} = s_i + m_{ij} + a_{ijk} + e_{ijk.}$$

where

$y_{ijk.}$ = mean twinning rate of daughters of bulls corrected for fixed effects

s_i = fixed effect of the ith bull sire (i = 1 to 6)

m_{ij} = fixed effect of the jth QTL genotype (j = 1 to 2) nested within ith bull sire

a_{ijk} = random effect of the kth son nested within the jth QTL genotype of the ith bull sire

$e_{ijk.}$ = residual effect

Chromosome-wise significance threshold values were determined empirically with 10,000 permutations at each cM (Churchill and Doerge 1994). Genome-wise significance levels, taking into account testing of the whole autosomal chromosome, were calculated as: $P_{genome} = 1-(1-P_{chromosome})^{1/r}$, where (r) was obtained by dividing the length of a specific chromosome by the total length of the autosomal genome (de Koning et al. 1999).

Results

The autosomal genome scan for QTL affecting twinning rate in Norwegian Cattle revealed potential findings for Chrs 5, 7, 12, and 23 (Fig. 1). The QTL with highest significance was observed in a chromosome region close to IGF1 in the middle part of Chr 5 (Fig. 1). In order to increase the number of informative markers in the region, four additional microsatellites were genotyped in the six families. Additionally, since *IGF1* is known to play an important role in bovine folliculogenesis (Echternkamp et al. 1990), a total of

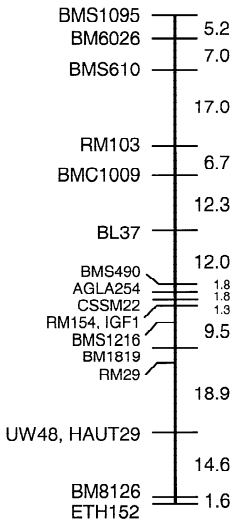

Fig. 2. A second-generation Norwegian Cattle Map for Chr 5 with a high marker density in the middle part of the chromosome.

3670 bp of the gene was sequenced in order to identify mutations in the six bull sires. Two new polymorphisms, a 4-bp deletion in intron 4 and an SNP in intron 5, were identified. These polymorphisms, as well as an SNP in the 5′end of the gene (Ge et al. 1997), were genotyped in the six families. A second-generation map, with a high marker density in the middle part of the chromosome, was constructed for Chr 5 (Fig. 2). The map was used in a new QTL search (Fig. 3a) showing the highest across-families results for marker interval IGF1-BMS1216 ($P_{chromosome} = 0.0046$). The results for three families, which are likely to segregate QTL alleles, are shown in Fig. 3b. One of the families (2463) has one rather broad peak close to the *IGF1* locus, whereas the other two families (3131 and 2005) seem to have two peaks. The largest QTL effect was found in family 3131 with a difference in twinning rate of 0.70% between sons segregating different BM1216 alleles. This corresponds to an estimated allele substitution effect of 1.57 phenotypic standard deviations.

Putative QTL affecting twinning rate were also detected on Chrs 7, 12, and 23. The F-value results from across family analyses, and chromosome- and genome-wise significance threshold levels are presented in Fig. 1 and Fig 3a. Results for Chr 7 indicate a QTL close to marker ILSTS6 located close to the telomere ($P_{chromosome} = 0.049$). Only one of the six families is segregating for a putative QTL allele. The size of the QTL effect is similar to the one detected in family 3131 on Chr 5. Also for Chr 23, the putative QTL seems to segregate in one family only. The highest peak in the across family interval mapping ($P_{chromosome} = 0.061$) was near to two loci in the BoLA region, DRB3 and CYP21. Results for Chr 12 differ from those reported for Chr 7 and Chr 23 by having two segregating families and smaller effects within each family. The highest peak for Chr 12 was detected between markers BMS410 and marker BM6108 close to the centromere ($P_{chromosome} = 0.063$).

Discussion

The granddaughter design (Weller et al. 1990), which utilizes records from breeding value estimation, is widely used for QTL mapping in cattle (Georges et al. 1995). A major limitation of this design is, however, that QTL detection is restricted to traits already recorded in the breeding scheme, which in most countries are milk production traits. Scandinavian countries have traditionally paid special attention to non-production traits in dairy cattle breeding. This is facilitated by the nation-wide recording of traits of major economic importance, such as those affecting health and fertility. As a typical Scandinavian country, Norway selects for a broad breeding goal including dairy, beef, health, and fertility traits, and progeny testing is performed on large progeny groups consisting of more than 250 daughters per son, which is about five times higher than those used in other European or American populations.

The granddaughter design in this study consisted of six paternal half-sib families. Our results show that only one or a few of these families segregate QTL affecting twinning rate, which is consistent with QTL alleles present at low frequencies in the population. The main criterion for choosing families to the QTL study in Norwegian Cattle was the number of sons per sire. The main reasons for this were that the number of sons per sire has been shown to be particularly important for the power of granddaughter designs (Weller et al. 1990) and that the families can be used in genome scans for QTL affecting milk production, health, and other fertility traits as well. An alternative strategy would be to use families with a high twinning rate and increased variation of twinning rate among sons. This is justified by our observation that most of the putative QTL detected in this study are found in such families.

Previous QTL results for twinning and ovulation rate in cattle are mainly reported in a resource population (MARC) for increased twinning rate generated at Clay Center, Nebraska (Blattman et al. 1996; Kappes et al. 1998). Interestingly, five of the foundation bulls in the MARC population were sires from Nordic countries whose daughters had produced twins at a high rate (Gregory et al. 1990). This semen export builds a link between the populations and increases the likelihood of finding identical QTL in the two studies. Both Kappes et al. (1998) and Kirkpatrick et al. (2000) have reported QTL for ovulation rate on Chr 5 in the MARC population, but at different locations on the chromosome. The most likely position reported by Kappes et al. (1998) was close to *BMC1009*, whereas Kirkpatrick et al. (2000) suggest a QTL position in the vicinity of *UW48*, approximately 60 cM apart. Our results from the across-family analysis (Fig. 3a) show the most likely QTL position close to *IGF1*, approximately in between *BMC1009* and *UW48*. When looking more closely at the results in Norwegian Cattle (Fig. 3b), two of the segregating families (2005 and 2463) seem to have two peaks; one close to marker *BMC1009* and another one close or distal to *IGF1*. Altogether, these results may indicate segregation of two QTL for twinning rate on Chr 5.

Previous studies in the MARC population also indicate putative QTL for ovulation rate on bovine Chr 7 (Blattman et al. 1996; Kirkpatrick et al. 2000) and Chr 23 (Blattman et al. 1996). Their mapping of a QTL close to the BoLA system on Chr 23 corresponds well with our results, whereas the QTL on Chr 7 are definitely positioned at different locations. Both Blattman et al. (1996) and Kirkpatrick et al. (2000) mapped the QTL to the centromere of Chr 7, whereas our results suggest a QTL positioned close to the telomere (Fig. 1).

QTL positions detected in this report can be compared with corresponding regions of the human genome in an effort to identify suitable candidate genes. This approach must be tempered with the recognition that QTL positions are only approximate and that supporting intervals are quite broad. For the QTL findings on Chr 7, Chr 12, and Chr 23, no obvious candidate genes for ovulation rate were identified on examination of human genome information. On Chr 5, however, known metabolic effect of IGF1 suggest it is a strong positional candidate gene that may underlie this QTL. IGF1 has been shown to stimulate both progesterone production and mitosis of bovine ovarian granulosa cells cultured in vitro (reviewed by Spicer and Echternkamp 1995). Echternkamp et al. (1990) showed that natural twinning is associated with increased concentrations of IGF1 in both blood and follicular fluid, which supports the hypothesis that IGF1 plays a role in the regulation of folliculogenesis and is a mediator of a genetic component of multiple ovulations in cattle. On the basis of this knowledge, we performed a search for mutations in the *IGF1* gene in families segregating the QTL. Although we did not find any mutations in

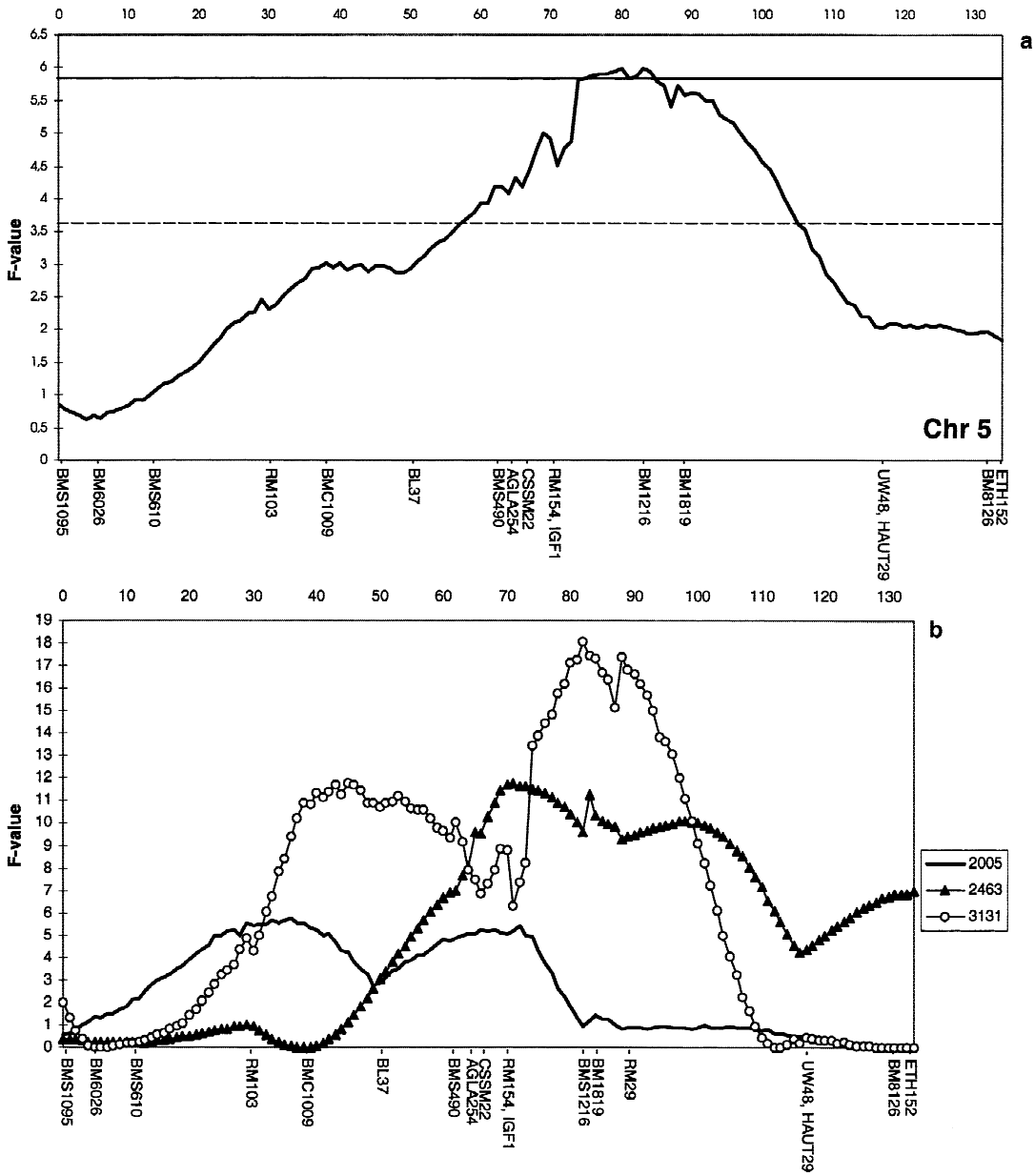

Fig. 3.a. Across-family QTL results for twinning rate on Chr 5. Markers and putative QTL positions with corresponding chromosome-wise 5% (---) and genome-wise 10 % (—) significance thresholds are indicated in the figure. **b.** Within family interval mapping results for three half-sib families of Norwegian Cattle segregating QTL for twinning rate on Chr 5.

coding or regulatory regions of *IGF1*, the detection and genotyping of several polymorphisms in noncoding regions of the gene enabled a high-resolution integration of *IGF1* with polymorphic microsatellites in the region. This may be particularly important for determining exact evolutionary breakage points between cattle Chr 5 and human Chr 12, which allow extrapolation of information from the highly developed human map to the lower-density bovine map.

Another comparative positional candidate gene for twinning rate, located in close vicinity to IGF1 on human Chr 12 (Geissler et al. 1991), is the mast cell growth factor gene (MGF). Previously, rearrangements in the mouse MGF has been shown to cause female sterility by disrupting ovarian follicle development (Bedell et al. 1995). In bovine, MGF has been associated with the roan phenotype (Charlier et al. 1996; Seitz et al. 1999), a major determinant for White Heifer Disease, and with a pleiotropic effect on fertility. However, the mapping of bovine roan locus to a region approximately 50 cM closer to the centromere than *IGF1* (Charlier et al. 1996) makes this gene less actual as candidate for the QTL findings in our study.

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