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Characterization of A QTL Region Affecting Somatic Cell Score in Friesian Cows in Egypt

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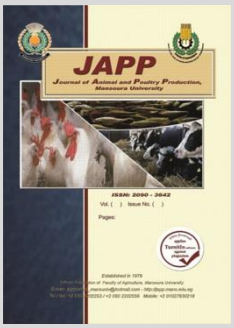

ABSTRACT

Aim of the present study was to confirm previously quantitative trait loci (QTL) affecting somatic cell score (SCS) on *Bos taurus autosomes* (BTA23) chromosomes and how can make selection by markers linked with QTL for SCS in Friesian cows in Egypt. A granddaughter design with selective genotyping was implemented that included haf-sibs from 6 sire-families cows. All sires have at least 200 daughters. Animals were genotyped for 5 microsatellites markers on BTA23. Heterozygosity on locus showed wide variation among five microsatellite markers among families. Across 6 families, the most likely QTL positions for SCS were all mapped at 48 cM, close to BM1443. Results for trait SCS, QTL with chromosome-wide significance within and across families studied identified the QTL allele substitution effects estimated for each family while fixing the QTL at the most likely position (48 cM) in 95% confidence interval (CI) QTL position. Sire families have significant QTL effect on SCS. The calculated overall QTL sire effect values across the six families (-0.154 ± 0.422), indicated decreased SCS among all families. The fact that such genotypes are found in relative high frequencies in Friesian cattle may reflect the combined breeding goal that is characterized by SCS selection to resistance mastitis. The identification of these markers raises the possibility of overcoming the unfavourable genetic correlation between milk production, SCS and mastitis traits through marker-assisted selection.

Keywords: Quantitative trait loci, somatic cell score, microsatellite, selection, Friesian cows.

INTRODUCTION

The recent development in molecular genetics has paved the way to a genomic approach of selection in livestock, as an integration of the ongoing phenotypic methodology (Dekkers, 2004). The knowledge of the association between markers and quantitative traits loci (QTL) affecting economical important traits is the basic required for the genomic approach information.

Mastitis is the inflammation of the mammary gland or udder of the cow resulting from infection or trauma. Economically, it is the most important disease in cattle, and may be caused by several reasons. The primary cause of mastitis is the invasion of the mammary gland through the teat end by microorganisms. Genetic improvement of functional traits such as resistance to mastitis may reduce the use of antibiotics and thereby the risk of residues in milk and meat products may be reduced. Therefore, improving the genetic capacity of mastitis in dairy breeds is of great importance.

Mastitis causes economic losses to the farmer and dairy industry by reducing milk production, quantitatively and qualitatively. Therefore, the determination of the genetic basis of mastitis resistance in the dairy cattle is economically of immense significance in the dairy industry, in term of milk production and in breeding management, selecting for cattle subjects with resistance to mastitis. A method of genetically selecting cows subjects with improved resistance that will yield cows less prone to mastitis would be desirable. Therefore, the objective of this investigation was to confirm the presence in this region of QTL across the cattle genome that influences SCS and resistance to mastitis in Friesian cows families in Egypt.

MATERIALS AND METHODS

The present investigation carried out at the International Livestock Management Training Center (ILMTC) Sakha, Kafrelsheikh belong to Ministry of Agriculture (MOA), Agriculture Research Center (ARC), Animal Production Research Institute (APRI).

Family structure

The experimental model was a classic daughter design (Weller *et al.*, 1990). It included six artificial insemination (AI) sire families from (ILMTC) belong to (APRI), chosen as having large variability in milk yield (MY) and somatic cell score (SCS). A total of 2289 family daughters from two farms in Egypt (Sakha, 1098 daughters and El-Karda, 1191 daughters) were used in the analysis. The average numbers of daughters per family were 381.5.

Description of phenotypic data

Milk yield (MY) and SCS consist of one to nine lactations adjusted to a 305-day lactation record. SCS is an indicator trait for resistance to mastitis, calculated as 3 + log2 (cells/100), where cells equals somatic cells by thousand per mililiter of milk (Ali and Shook 1980; Shook and Schutz 1994). Bull breeding values for milk yield MY

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and SCS were estimated on daughter records by the (ILMTC).

DNA extraction from blood

Bovine genomic DNA extracted using various techniques. DNA of blood samples collected from APRI station extracted from whole blood using the E.Z.N.A. Blood DNA Midiprep Kits following the manufacture instructions.

DNA extraction from semen

Extraction of DNA was achieved according to Masle (2007). Content of each semen straw (0.25 ml) was washed by repeated centrifugation and re-suspension of the pellet in PBS buffer to obtain clear supernatant, and then the pellet re-suspended in DNA extraction buffer (900 µl), 0.5 dithiothreitol (100 µl), and proteinase K (50 µl) were added to the suspension. The solution was incubated at 65˚ C over night. On the following day, 6M NaCl (450 µl) was added to the solution and was centrifuged for 40 minutes. The supernatant containing dissolved DNA was transported into a new tube, then mixed with ice cold 100% ethanol (3.5 ml), to stick the DNA molecules together. The DNA precipitate was "fished out" with a disposable pipet tip, re-suspended in Tris EDTA (50 µl) or molecular grade water, then incubated over night at 37° C for complete dissolution.

DNA concentration and purity

The concentration for DNA extracted was quantified using UV spectrophotometer (Eppendorf Bio-Photometer) machine at 260 and 280 nm. DNA concentration was directly calculated by the equipment in ng/µl, and DNA purity was determined as the ration of 260/280 reads. The results DNA purity was determined as the ration of 260/280 reads. The results ranged between 1.6 and 2.0, which reflects a satisfactory purity and contamination with either RNA or protein.

Microsatellite loci under study or markers tested

Five microsatellite markers (MS) were selected from the chromosome (Table 1) as the first segment of a genome-wide search for somatic cell score QTL. Selection of the markers was based on the number of alleles, map position, heterozygosity in the USDA Meat Animal Research Center (MARC) bovine map (http :// www .marc .usda.gov/genome /genome), on the basis of their informative, or their previously reported linkage with QTL, ability to co-amplify with other markers, size of the amplification product and the location of the markers on different chromosomes (Table 1). Finally 5 markers spaced at intervals of about 5–10 centi Morgan (cM) on the map of Kappes et al. (1997) on chromosome BTA 23. Bos taurus autosome (BTA), Microsatellite (MS) and all markers used are of the microsatellite type that was shown to be polymorphic in cattle (http://www.marc.usda.gov).

Polymerase chain reaction (PCR)

Studied microsatellites amplified with PCR, using primers and PCR conditions recommended by (Weimann et al. 2003).

Fragment sizing or (allele size determination)

PCR product was denatured in a mix consists of 1 µl PCR product + 2 µl loading master mix, while the loading master mix was composed of 250 µl deionized Hi-Di-Formamide (Applied Biosystems), 50 µl GENESCAN-TAMRA350 size standard (Applied Biosystems), and 50 µl loading stain (Blue dextrane, 50 mg/ml in EDT A, 25 mM, PHARMACIA). The denaturation mix was incubated at 95°C for 5 minutes and immediately chilled in ice to prevent re-annealing. A volume of 1.5 µl of such mix was loaded in polyacrylamid sequencing gel (6 M urea, Long Ranger 5%, 1X TBE, 0.05% ABS and 0.07% Temed) in the Applied Biosystems DNA sequencer 377. GeneScan v. 3.1 software (Applied Biosystems) used by the equipment for fragmentation of various alleles by comparison against the GENESCAN-TAMRA350 standard. The GENOTYPER v.3.0 software (Applied Biosystems) was used for matching and for obtaining the results of alleles.

Linkage analysis

According to Haley and Knott (1992), all genotyping data from a SCS trait were analyzed using a regression approach. A web-based version of this regression interval mapping method is available (Seaton *et al.*, 2006), and the software, GridQTL 1.4.0; (http://gridqt1.cap.ed.ac.uk), analyzes data from half-sib families to detect QTL. Data within and across the families were investigated by half-sib analysis. The software allows fitting 1-2 QTL in the model. It included tools for permutation and bootstrap analyses to compute chromosome-wise significance thresholds and 95% confidence intervals. In this experiment, about 1000 permutations were studied for each trait to calculate the chromosome-wise significance thresholds ($P<0.05$ and $P<0.01$), and the analysis of regression interval along the chromosome was determined at 1-cM. Within and across family analyses were performed, fitting one QTL in the model. Data of milk production and SCS were weighed by their respective reliabilities.

Linkage disequilibrium and linkage analysis (LDLA)

This program was used and described by (Jules *et al.*, 2009) as a module in their website (http :// cleopatra. cap.ed.ac.uk/gridsphere/gridsphere) to analyzed the genotype data and phenotypic data to detect the QTL for SCS which, is indirect measure for mastitis's resistance in dairy cattle.

Table1 Characterization of microsatellite at chromosome 23 of *Bos Taurus.*

Microsateliite	Chromosome	Heterozy.	No. allele	Allele size range	Annealing temperature (°C)
BM1815	23	56	8	140-170	56
BM1258	23	52	5	92-106	58°C
BM1818	23	56	8	274-286	56°C
BM1905	23	65	6	187-201	65°C
BM1443	23	50	8	154-170	68°C

According to Bishop, Kappes and Keele et al. (1994)

RESULTS AND DISCUSSIONS

Allele frequencies and number of allele of each five microsatellites on BTA23

Allele frequencies (Table 2) on the same locus for each family, the different ranging between 0.200 and 0.254 for locus BM1815, 0.130 and 0.0.255 for locus BM1258, 0.200 and 0.445 for BM1818, 0.253 and 0.255 for BM1905, 0.130 and 0.169 for BM1443. This indicated that the lowest allele frequency was 0.130 for loci (BM1258 and BM1443) and the highest allele frequency being 0.445 for locus BM1818. Only the 3rd family showed the overall maximum presence of allele frequency which was 0.445 on locus BM1818 and the overall minimum allele frequency being 0.130 on locus BM1258 in 4th family 4 and BM1443 in the 6th family.

The present results revealed that number of alleles per locus were different among loci, being the highest in loci BM1258 and BM1443 (9 alleles on each) and the lowest in locus BM1818 (4 alleles) as shown in table (2).The overall number of alleles on each locus, ranged between 6.2 for BM1818 and 9.8 for BM1443 with overall mean average of 7.4 for all loci.

Allele frequency in all loci for all families, ranged between 0.173 for locus BM1443, and 0.323 for locus BM1818 (Table 2). Overall number of alleles in each family was 6, 5.6, 5.6, 7, 6.2, and 6.6 for all families, respectively (Table 2). It was observed that locus BM1818 was higher frequencies than the other 4 loci in all families studied. On the other hand, locus BM1258 and BM1443 showed the lowest frequencies as compared to the other loci in all families studied. Mean of allele frequency of all loci in different families ranged between 0.183 and 0.260, being the lowest for the 4th family and the highest for the 3rd family (Table 2).

Table 2. Number of alleles (N) and allele frequency (F) for overall loci on BTA23 in 6 families.

Locus	Total no. alleles	Family 1		Family 2		Family 3		Family 4		Family 5		Family 6		Overall microsatellite	
		N	F	N	F	N	F	N	F	N	F	N	F	N	F
BM1815	6	6	0.200	5	0.254	6	0.202	6	0.200	6	0.208	6	0.210	7	0.254
BM1258	9	6	0.201	6	0.201	5	0.255	9	0.130	7	0.176	7	0.177	8	0.288
BM1818	6	5	0.255	5	0.254	4	0.445	6	0.200	5	0.253	6	0.210	6.2	0.323
BM1905	5	5	0.255	5	0.254	5	0.255	5	0.254	5	0.253	5	0.255	6	0.305
BM1443	10	8	0.145	7	0.169	8	0.145	9	0.130	8	0.144	9	0.130	9.8	0.173
Overall families	7.2	6	0.211	5.6	0.226	5.6	0.260	7	0.183	6.2	0.206	6.6	0.196	7.4	0.269

The overall mean of heterozygosity values for all loci in each family presented in table 5 and range of heterozygosity was 0.643 to 0.931, being the highest in locus BM1443 and the lowest in locus BM1258. Number of heterozygosity sires was 6 similarly in two loci BM1818 and BM1443. Also, number of heterozygosity sires was 5 similarly in two loci BM1258 and BM1905 except BM1258 was 4 (Table 3).

Table 3. Number of heterozygosity sires and marker heterozygosity of each of five microsatellites and for overall loci on BTA23 in different experimental families.

Marker	Family	No. of heterozygosity sires	Marker heterozygosity	Overall no. of heterozygosity sires	Overall marker heterozygosity
BM1815	1	1	0.8585	5	0.879
	2	0	0.8433		
	3	1	0.9600		
	4	1	0.8521		
	5	1	0.9665		
	6	1	0.7957		
BM1258	1	0	0.5023	4	0.643
	2	1	0.6983		
	3	0	0.5500		
	4	1	0.8170		
	5	1	0.5752		
	6	1	0.7243		
BM1818	1	1	0.6973	6	0.705
	2	1	0.8220		
	3	1	0.4900		
	4	1	0.7368		
	5	1	0.7391		
	6	1	0.7500		
BM1905	1	1	0.6464	5	0.646
	2	0	0.7600		
	3	1	0.5100		
	4	1	0.5539		
	5	1	0.8026		
	6	1	0.6071		
BM1443	1	1	0.9394	6	0.931
	2	1	0.9583		
	3	1	0.9500		
	4	1	0.8889		
	5	1	0.8786		
	6	1	0.9733		
Overall mean				5.2	0.712

Quantitative trait loci position for SCS on BTA23

The 3rd family showed the highest F-value (F = 4.23) for trait SCS), which appeared at 48 cM, close to marker BM1443. The test statistic for SCS within the 3rd family was lower than the 5% chromosome-wide threshold. The 2nd family ranked the second (F = 3.23). However, the profiles for 2nd, 4th, 5th and 6th family were fluctuated. Within all families for the SCS trait, the 95% confidence interval length measured from bootstrapping spanned was the same at 48 cM (Table 4).

This mean that the most likely QTL positions for SCS were observed to map at 48 cM, close to BM1443 with QTL model fitted to the analyses for all families. The F-value of SCS at the peak position was significant at a level of 5% chromosome-wide. Within family analysis, this position was also identified. The F-values for SCS was lower within the 1st family than the 5% chromosome-wide thresholds. The corresponding position was the same for all families. The F-values for SCS within the 2nd family was significant at a level of 5% chromosome-wide, and the corresponding positions were the same for all family analyses. The profiles for the 3rd, 4th, 5th and 6th family were fluctuated (Table 5). The obtained results showed differences in F-values within all families, being the highest in the 3rd family (4.23), while the lowest in the 4th family (0.67, Table 4).

However, F-value across families studied was 1.08 (Table 4).

Values of likelihood ratio were different within families studied, being the highest in the 3rd family (2.95) and the lowest in the 4th family (0.64). However, likelihood ratio across families was (6.45). This profile of likelihood results indicated that the QTL position for trait SCS located nearest the BM1443 marker.

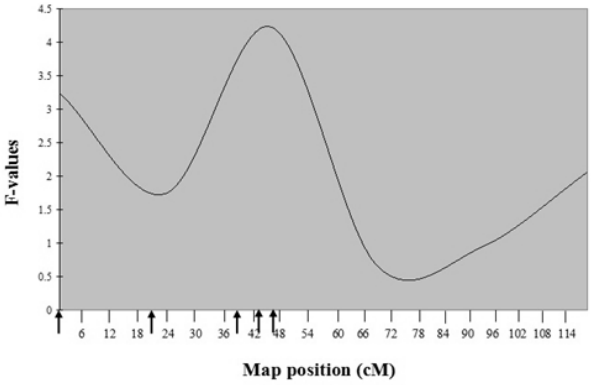

Figure 1. Test statistic profiles (F-values) for SCS trait on BTA23

The 95% confidence interval was estimated through a number of 1000 bootstrap samples. Generally, frequency distributions the samples were in association with the profiles of the statistical test, and the models were corresponded to the most likely QTL location. A putative QTL for SCS with chromosome-wide significance was identified within each family and all families. As in figure 1, the effect of a QTL allele substitution was estimated for each family, but the same QTL at the most likely position (46 cM) was in 95% CI (cM) QTL position. The effect of QTL on SCS was significant for all sire families (Table 4).

Table 4. Quantitative trait of loci position with the nearest marker (BM1443) concluded from analyses across all families in one-QTL model for SCS on BTA23.

Family	Nearest Marker	Map position, (cM)	F-value	Likelihood Ratio	F-Statistics Threshold Chrom. 5%	95% CI (cM) QTL position
1	BM1443	48	3.23	2.77	8.811	0.0 - 48.0 cM
2	BM1443	48	1.73	1.6	7.777	0.0 - 48.0 cM
3	BM1443	48	4.23	2.95	13.921	0.0 - 48.0 cM
4	BM1443	48	0.67	0.64	6.512	0.0 - 48.0 cM
5	BM1443	48	0.93	0.85	9.979	0.0 – 48.0 cM
6	BM1443	48	2.06	1.88	7.851	0.0 – 48.0 cM
Across families	BM1443	48	1.08	6.45	2. 747	0.0 – 48.0 cM

Moreover, the estimated 95% confidence interval covered very large intervals with a range of an entire chromosome (BTA23) area. This large interval of 95% confidence may be due to the relatively small size of families. The total size of sample was 29.83 daughters in total, although the sizes of families were limited (22-56 daughters per sire). Also, the small effect of QTL, the incomplete saturation of map, and a possible substantial amount of linkage disequilibrium may decrease the resolution.

Across families studied, F-values on BTA23 was higher than that found by Weimann *et al.,* (2003) who observed that F-values on the same chromosome in Holstein dairy cattle. However, F-values values on BTA23 across families studied were higher than that reported by Van Tassell *et al.,* (2004) who observed that F-values 1 in tow Holstein grandsire families on BTA27. The present values of likelihood ratio across families studied on BTA23 was less than values of likelihood ratio on BTA23 by Weimann *et al.,* (2003).

F-statistics threshold value 2.747 on BTA23 in this study was less than F-statistics 3.3 and 3 on BTA22, across six Holstein cattle families (Ashwell *et al.,* 2004). Generally, F-statistics threshold values within and across families studied were significance that indicated the position of QTL for SCS is likely in the same position in all families studied.

Sire QTL effects

The present results revealed that the absolute t value with freedom degrees equal to the informative daughters number in the family. The present t values within families were less than 2 except family 3 was 2.066 for the SCS trait (Table 5). The average of absolute was 1.431 that estimated absolute QTL substitution effect in the 6 significant families. The present results indicated that the highest QTL sire effect value within family was observed

(0.899 ± 0.435) in the 3rd family. However, the lowest value was observed (-0.790 ± 0.432) in the 1st family (Table 5).

In comparing QTL sire effect values within families would be decrease SCS by these values (-0.790 ± 0.432, -0.401 ± 0.410, -0.473 ± 0.483 and -0.598 ± 0.440) in 1st, 4th, 5th, and 6th family, respectively. On the other hand, QTL sire effect values would be increasing SCS by these values (0.439±0.333 and 0.899 ± 0.435) in 1st and 2nd family, respectively (Table 5).

Table 5. Sire QTL effects within families combined with SE and absolute t-value ABS (t) on BTA23.

Family	Sire effect		ABS(t)
	Estimate	S.E.	
1	-0.790	0.432	1.828
2	0.439	0.333	1.318
3	0.899	0.435	2.066
4	-0.401	0.410	0.978
5	-0.473	0.483	0.979
6	-0.598	0.440	1.417
Overall families	-0.154	0.422	1.431

The calculated overall QTL sire effect values across the six families (-0.154 ± 0.422), indicating decrease SCS among all families studied. The obtained sire QTL effect values from microsatellites analysis on BTA23 are a precise confirmation with the significance of absolute t value and this results indicated significance of QTL effect on trait SCS.

Interval mapping results across families confirmed the presence of the QTL affecting SCS on a chromosome-wide basis, neither of the chromosomes showed a significant effect. The present results revealed that QTL position on BTA23 found at 48 cM near of marker BM1443.

On BTA 23, the F-ratio across families studied had a value of 1.08, whereas the threshold for significance at P = 0.05 was F-statistics = 2.747. However, there was some evidence for the existence of a QTL very close to marker BM1443 (at 48 cM) (Table 4, Figure 1). The significant QTL effect at BM1818 reported by Weimann *et al.*, (2003).

CONCLUSIONS

It concluded that the usefulness of polymorphic microsatellite markers and the daughter design in the search for QTL in existing families. The present results have identified the most likely QTL to be near marker BM1443 on BTA23 which had allele 152 was associated with an increased the risk of mastitis incidence in the cows with an acute SCS from the first to the later lactations in the cows. Vice versa, alleles 154 and 156 were associated with reduced risk of high milk somatic cell score (SCS), then, reduce the risk of mastitis resistance in the cows.

The chromosome under study is more specific to for SCS and resistance to mastitis. Using these microsatellite markers under study showed a relationship between MY, SCS and resistance to mastitis. It was recommending using these microsatellite markers in genetic selection in Friesian cows. Moreover, its may be possible to use these microsatellites in the future in the marker-assisted selection in a criterion for selection for resistance to mastitis and became the cows more profitability.

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التوصيف الوراثى لمواقع الصفات الكمية التى تؤثر فى أعداد الخلايا الجسدية فى أبقار الفريزيان فى مصر

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هدفت هذه الدراسة إلى تأكيد مواقع الصفات الكمية (QTL) التي تؤثر على أعداد الخلايا الجسدية (SCS) في الأبقار الحلابة التى تقع على الكروموسوم (BTA23) وكيفية عمل الانتخاب باستخدام الواسمات الوراثية المرتبطه جينيا بمواقع الصفات الكمية (QTL) لأعداد الخلايا الجسدية (SCS) في أبقار الفريزيان في مصر. تم تصميم التجربة على أساس الإخوة أنصاف الأشقاء وذلك لعدد 6 عائلات من أبقار فريزيان. جميع الأبناء لديهم 200 بنت على الأقل. تم عمل التحليلات الجينية لكل الحيوانات وذلك باستخدام 5 واسمات وراثية تقع على الكروموسوم (BTA23). أظهرت نتائج التحليلات على المستوى الجينى اختلافات وراثية باستخدام الواسمات الوراثية المدروسة لكل العائلات المدروسة. حيث لوحظ فى الـ 6 عائلات المدروسة أن مواقع الصفات الكمية كانت أقرب إلى مسافة 48 سنتى مورجان والتى أظهرت النتائج أنها الواسمة الوراثية BM1443. حققت النتائج لصفة أعداد الخلايا الجسدية والمرتبطة بمواقع الصفات الكمية أنها كانت أكثر معنوية بين وداخل العائلات من خلال عدد الأليلات وتكراراتها فى كل عائلة من العائلات المدروسة ومدى ارتباط هذه الواسمه الوراثية بمواقع الصفات الكمية لأعداد الخلايا الجسدية والتى كانت أكثر احتمالية بالقرب من 48 سنتى مورجان بنسبة تصل إلى 95%. سجلت التاثيرات الأبوية على مستوى الصفات الكمية أن احتمالية موقع الصفات الكمية أنها أقرب إلى 48 سنتى مورجان. كانت قيم التأثيرات الأبوية (-0.15±0.422) مما كا له أثر فى إنخفاض أعداد الخلايا الجسدية على مستوى العائلات المدروسة. تجدرالإشارة إلى أن الواسمه الوراثية الأقرب للصفه الكمية لأعداد الخلايا الجسدية كانت تكرار الأليلات لها عالية نسبياً فى أبقار الفريزيان والتى بدورها تعكس الهدف المرجو منها عند عمل الانتخاب الوراثى لأعداد الخلايا الجسدية المقاومة لالتهاب الضرع فى الأبقار. وبدراسة هذه الواسمات الوراثية محل الدراسة يزيد من احتمالية التغلب على الارتباط الوراثى الغير مرغوب فيه بين كمية اللبن وأعداد الخلايا الجسدية وكذا التهاب الضرع من خلال استخدام برامج الانتخاب باستخدام الواسمات الوراثية.