Validation of Somatic Cell Score-Associated SNPs from Holstein Cattle in Sudanese Butana and Butana X Holstein Crossbred Cattle

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Validation of somatic cell score-associated SNPs from Holstein cattle in Sudanese Butana and Butana x Holstein crossbred cattle

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Abstract

The Bos indicus zebu cattle Butana is the most commonly used indigenous dairy cattle breed in Sudan. In the last years, high-yielding Holstein dairy cattle were introgressed into Butana cattle to improve their milk yield and simultaneously keep their good adaption to extreme environmental conditions. With the focus on the improvement of milk production, other problems arose such as an increased susceptibility to mastitis. Thus, genetic selection for mastitis resistance should be considered to maintain healthy and productive cows. In this study, we tested 10 SNPs which had been associated with somatic cell score (SCS) in Holstein cattle for association with SCS in 37 purebred Butana and 203 Butana x Holstein crossbred cattle from Sudan. Animals were genotyped by allele-specific KASP assays and association analysis was performed using a linear mixed model. All 10 SNPs were segregating in the crossbred Butana x Holstein populations, but only 8 SNPs in Sudanese purebred Butana cattle. The SNP on chromosome 13 was suggestively associated with SCS in the Butana x Holstein crossbred population (rs109441194, 13:79,365,467, \(P_{BF} = 0.054\)) and the SNP on chromosome 19 was significantly associated with SCS in both populations (rs41257403, 19:50,027,458, \(P_{BF}=6.2 \times 10^{-16}\)). The minor allele of both SNPs showed an increasing in SCS. Therefore, selection against the disadvantageous minor allele could be used for genetic improvement of mastitis resistance in the studied populations. However, investigations in a bigger population and across the whole genome are needed to identify additional genomic loci.
Introduction

Livestock plays a significant role in the production of food and is of great socio-economic and cultural value in various societies around the world. In Sudan, the *Bos indicus* zebu cattle Butana are well adapted to the hot climate, harsh environment with low feed availability, the lack of water, and tropical diseases and parasites. But most importantly, they have a higher milk yield than reported from most African breeds [1]. Butana cattle have an average milk yield of 538.3 kg per lactation under field conditions [2], and 1,662.6 kg under improved feeding and management conditions in research stations. In order to improve milk production, Butana cattle have been crossed with high-yielding Holstein dairy cattle. The resulting Butana x Holstein crossbred cattle are as robust as Butana cattle but have higher milk yield compared to Butana cattle [3]. Thus, crossbred cows are currently used as the main source of milk in urban regions but can also be found in rural areas.

One of the main problems of dairy production in Sudan and world-wide is the high incidence of mastitis, an inflammatory udder disease causing economic losses due to reduced milk production and quality, and increased treatment costs or culling [4, 5]. In cattle breeding, the somatic cell count (SCC) in milk is used as an indirect marker to monitor mastitis infections. A SCC higher than 100,000 cells/ml corresponding to a somatic cell score (SCS) of 3 is suggestive for a subclinical mastitis infection [6].

To identify genomic loci affecting SCS or clinical mastitis directly, many association studies were performed in different dairy herds [7–9]. In this study, we tested 10 SNPs that have been previously associated with SCS in German Holstein bulls and cows [7, 8] for associations with SCS in Sudanese purebred Butana and Butana x Holstein crossbred cattle. Five of those SNPs were also found to be associated with clinical mastitis [9]. If those SNPs would be significantly associated, they could also be used as genetic markers to improve resistance against mastitis in Sudanese purebred Butana and Butana x Holstein crossbred populations. Such markers could contribute to improve the productivity of these cattle which is necessary to meet the increasing demand for milk and dairy products in Sudan.
Material and methods

Animals and phenotypic data

Data was obtained from 37 unrelated purebred Butana cows from Atbara research station in North Sudan and from 203 Butana x Holstein crossbred cows with varying levels of exotic blood belonging to five farms in Khartoum North. Milk samples were taken on multiple dates in 2017 and 2018. The somatic cell count (SCC) of milk samples was determined using the microscopic slide method [10]. SCC was transformed to somatic cell score \((SCS = \log_2 (SCC/100,000 \text{ cells/ml}) + 3)\) in order to obtain a distribution close to normal. The total dataset included 1,261 records for SCS with on average 5.25 measurements per cow. One Butana cow was removed because the age of first calving was more than 3000 days. Average SCS was calculated by first taking the mean SCS for a cow across all sample dates, and then calculating the mean SCS by breed and farm. Outliers were defined as values outside the mean \pm 3 standard deviations. After filtering, 1,222 SCS measurements were available for 34 purebred Butana cows and 201 Butana x Holstein crossbred cows.

DNA isolation and SNP genotyping

Blood samples of all cows were collected from the jugular vein in sterile tubes containing EDTA as anticoagulant. DNA was isolated using a salting out procedure [11]. Genotyping of 10 SNPs that have been identified for SCS [7, 8] was performed with allele-specific primers using KASP assays [12]. Genomic positions were referenced to the current \textit{Bos taurus} ARS_UCD2.1 genome assembly.

Association analysis

Association analysis between SCS and genotypic data was performed with a linear mixed model using lmer function implemented in R language for statistical computing. Butana and Butana x Holstein crossbred cattle were examined separately. The model for testing the additive effect for each SNP in Butana cattle was:

\[
y_{ijkl} = s_y + \text{dim}_i + g_{tk} + (1|\text{animal}_l) + e_{ijkl},
\]

(Eq. 1)

where \(y_{ijkl}\) is the SCS from multiple sample dates, \(s_y\) represents the covariate for the year of sampling followed by the covariate for days in milk \(\text{dim}_i\) and the SNP genotype \(g_{tk}\). The animal was included as a random effect \((1|\text{animal}_l)\) to compensate for repeated measurements; \(e_{ijkl}\) is the residual error.

The model for testing the additive effect for each SNP in Butana x Holstein crossbred cattle was
\[ Y_{ijklmno} = \text{sy} + \text{cs}_j + \text{fc}_k + \text{dim}_l + \text{f}_m + \text{gt}_n + \text{g}(1|\text{animal})_o + \epsilon_{ijklmno}, \]  

(Eq. 2)

where the same formula was used as in Eq. 1 extended by the covariates for calving season \(cs_j\) (dry season from November to April; wet season from May to October), age at first calving \(fc_k\), days in milk \(dim_l\) and farm \(f_m\).

The covariates year of sampling, days in milk, calving season, age at first calving, and farm were only included as fixed effects into the model, if the covariate significantly contributed to the model (Supplementary Table 1). This was tested by performing an ANOVA test between the null model \((Y = (1|\text{animal}))\) and the null model extended with one of the covariates \((Y = \text{covariate} + (1|\text{animal}))\). Bonferroni correction was used to adjust \(p\)-values for the 10 tested SNPs. \(P\)-values after Bonferroni correction \((P_{BF})\) were considered suggestive if \(P_{BF} < 0.1\) and significant if \(P_{BF} < 0.05\). SNP effect plots were done using R package ggplot2 (version 3.3.2) and \(p\)-values between genotype groups were estimated using pairwise \(t\)-tests and displayed using R package ggpubr (version 0.4.0). For the SNP effect plots, SCSs were corrected for each breed separately using significant covariates from Eq. 1 and Eq. 2 and then averaged across the animal identification numbers.

**Results**

SCS was with 4.6 lower in Butana cattle compared to on average 5.1 in Butana x Holstein crossbred cattle, whereas the mean SCS per farm ranged from 4.8 to 5.3 (Supplementary Table 2). We observed that all 10 investigated SNPs were segregating in Butana x Holstein crossbred cattle, but only eight in Butana cattle (Table 1). Two out of the 10 previously reported SNPs were associated with SCS in Butana x Holstein crossbreed cattle (Figure 1). One suggestively associated SNP was located on chromosome 13 (rs109441194, 13:79,365,467, \(P_{BF} = 0.0540\)). This SNP had a minor allele frequency (MAF) of 0.33 and its minor allele T accounted for an increase in SCS of 0.254. The other SNP located on chromosome 19 was significantly associated with SCS (rs41257403, 19:50,027,458, \(P_{BF} = 6.2 \times 10^{-16}\)). The minor allele A of the chromosome 19 SNP had a frequency of 0.24 and increased SCS by 0.726.

In the investigated Butana cattle, the SNP on chromosome 13 that was suggestive in the crossbred cattle was not segregating, but the SNP on chromosome 19 was also significantly associated with SCS in Butana.
(rs41257403, 19:50,027,458, $P_{BF} = 0.003$). The minor allele was the same as in the crossbred population and had the same direction and a similar magnitude of effect. The frequency of the unfavourable minor allele A of the chromosome 19 SNP in the crossbred population was 0.46, increasing SCS by 0.849.

**Discussion**

In the current study, we tested 10 SNPs for association with SCS in the Sudanese *Bos indicus* zebu cattle breed Butana and in Butana x Holstein crossbred cows. We confirmed suggestive and significant effects on SCS of the two SNPs rs109441194 and rs41257403 on chromosomes 13 and 19, respectively, in Butana x Holstein crossbreed cattle. In Butana cattle, the SNP on chromosome 19 was also significantly associated with SCS, while the SNP on chromosome 13 was monomorphic. Therefore, the chromosome 13 region could not be tested for potential association of the linked chromosomal region with SCS in Butana. These two confirmed SNPs were not only associated with SCS but also directly with clinical mastitis in Holstein cows [9] which makes the findings more important. In the regions of the two confirmed SNPs are the same two positional candidate genes as reported previously [7, 9]: NFATC2 (cytoplasmic nuclear factor of activated T-cells 2) and FOXK2 (forkhead box protein K2), which are both linked directly or indirectly to immune response of T-cells.

The MAFs of all tested SNP ranged from 0.00 to 0.46 in Butana and 0.10 to 0.43 in Butana x Holstein crossbred cattle (Table 1). Across all 10 SNPs, the difference of allele frequency was bigger between Holstein [7, 8] and Butana cattle (average difference of MAFs=0.17) than between Holstein and Butana x Holstein crossbred cattle (average difference of MAFs=0.10). For the two confirmed SNPs (rs109441194, rs41257403), the MAFs were similar in Holstein and in Butana x Holstein crossbred cattle (difference of MAFs=0.04). With respect to the SNP on chromosome 19, the MAF was much higher in Butana cattle, where the SNP was fixed (difference of MAF=0.26).

The directions of effects of the two confirmed SNPs were the same in both investigated breeds. In all cases, the minor allele was disadvantageous increasing SCS. Therefore, selection against the minor allele could reduce the somatic cell numeric in the milk in the population and thereby hopefully improve resistance against clinical mastitis. Comparing to previous results in Holstein bulls and cows [7, 8], the direction of effects of the
associated SNP on chromosome 13 was consistent meaning that the same region is linked to SCS or immune
response in Butana x Holstein cattle as in Holstein Friesian cattle. In contrast, the associated SNP on
chromosome 19 showed an opposite direction of effects compared to previous results in Holstein cattle [7, 8]
which could be caused by the weak linkage between the investigated SNP and the causative mutation.

In general, SCC and thus SCS were high in the investigated Butana (average SCS=4.6) and Butana x Holstein
crossbred cattle (average SCS=5.1). In contrast, SCS in different Holstein herds ranges between 2.91- 4.62
[13–15]. The SCS of milk that is allowed to be consumed varies in different countries, e.g., in USA, Canada,
and Germany, the SCS threshold is set to 5.87, 5.3, and 5, respectively [16, 17]. The high SCS in Sudanese
cattle may indicate that Sudanese farms have high rates of subclinical mastitis or even clinical mastitis. First
and foremost, better farm management, higher hygienic requirements, and proper nutrition of cattle are
necessary on Sudanese farms in order to reduce mastitis cases. However, a better understanding of the genetic
background of mastitis resistance, more accurate phenotyping, and genetic selection using mastitis markers
could improve resistance against mastitis.

The biggest pitfall of this study is the low sample size, especially of Butana cattle, where alleles might be
missed due to low MAFs. Since the sample size of Butana x Holstein crossbred cattle was larger, higher
significance was obtained for these cattle. Nonetheless, we could confirm two SNPs to be associated with SCS
in Butana x Holstein crossbred cattle and one in Butana cattle. These are SNPs which could be used for genetic
improvement of mastitis resistance in the respective breeds. In order to validate the other SNPs, higher number
of animals would be needed.

This is the first association study for SCS in Sudanese Butana and Butana x Holstein crossbred cattle. The
genetic improvement of mastitis resistance and selection for lower SCS is consistent with the goal of
maximizing genetic improvement for milk production and total economic merit and should be included in
breeding programs. We confirmed the association of two SNPs in Butana x Holstein crossbred cattle, whereof
one was also significant in purebred Butana cattle. These SNPs can be used for genomic selection to reduce
SCS in milk and thus to improve mastitis resistance and further milk quality. Follow-up associations in bigger
populations using SNP-chip or whole-genome sequencing data would allow for the identification of new loci
associated with SCS.
Declarations

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Authors’ contributions

GAB, SE and SAR designed the study. SE collected the samples and phenotypic data, and did the laboratory work. MR helped with the laboratory work. SE, PK and DA performed all statistical analysis. SE and PK drafted the manuscript. GAB and DA helped draft the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Animal owners agreed to the participation of their animals in this study, blood samples were collected based on routine procedures on these farm animals and according to the animal protection law in Sudan.

Consent for publication

Not applicable.
1. Ageeb AG, Hillers JK. Production and reproduction characteristics of Butana and Kenana cattle of the Sudan. http://www.fao.org/3/U1200T/u1200T0j.htm. Accessed 1 Apr 2021.

2. Musa MA, Peters KJ, Ahmed MKA. On farm characterization of Butana and Kenana cattle breed production systems in Sudan. Livest Res Rural Dev. 2006;18(12).

3. Musa LM-A, Bett RC, Ahmed M-KA, Peters KJ. Breeding Options for Dairy Cattle Improvement in the Sudan. Outlook Agric. 2008;37:289–95. doi:10.5367/000000008787167781.

4. Bar D, Gröhn YT, Bennett G, González RN, Hertl JA, Schulte HF, Tauer LW, Welcome FL, Schukken YH. Effects of Repeated Episodes of Generic Clinical Mastitis on Mortality and Culling in Dairy Cows. J Dairy Sci. 2008;91:2196–204. doi:10.3168/jds.2007-0460.

5. Mungube EO, Tenhagen B-A, Regassa F, Kyule MN, Shiferaw Y, Kassa T, Baumann MPO. Reduced Milk Production in Udder Quarters with Subclinical Mastitis and Associated Economic Losses in Crossbred Dairy Cows in Ethiopia. Trop Anim Health Prod. 2005;37:503–12. doi:10.1007/s11250-005-7049-y.

6. Peeler EJ, Green MJ, Fitzpatrick JL, Morgan KL, Green LE. Risk Factors Associated with Clinical Mastitis in Low Somatic Cell Count British Dairy Herds. J Dairy Sci. 2000;83:2464–72. doi:10.3168/jds.S0022-0302(00)75138-1.

7. Abdel-Shafy H, Bortfeldt RH, Reissmann M, Brockmann GA. Short communication: Validation of somatic cell score-associated loci identified in a genome-wide association study in German Holstein cattle. J Dairy Sci. 2014;97: 2481-2486. doi:10.3168/jds.2013-7149.

8. Abdel-Shafy H, Bortfeldt RH, Tetens J, Brockmann GA. Single nucleotide polymorphism and haplotype effects associated with somatic cell score in German Holstein cattle. Genet Sel Evol. 2014; 46. doi:10.1186/1297-9686-46-35.

9. Abdel-Shafy H, Bortfeldt RH, Reissmann M, Brockmann GA. Validating genome-wide associated signals for clinical mastitis in German Holstein cattle. Anim Genet. 2018:49:82-85. doi: 10.1111/age.12624.

10. Brazis AR, Jasper DE, Levowitz D, Newbould FHS, Postle DS, Schultze WD, Smith JW, Ullmann WW. Direct Microscopic Somatic Cell Count in Milk. J Milk Food Technol. 1968;31:350–4. doi:10.4315/0022-
11. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988.

12. Kreuzer S, Reissmann M, Brockmann GA. Gene test to elucidate the ETEC F4ab/F4ac receptor status in pigs. Vet Microbiol. 2013;162:293–5. doi:10.1016/j.vetmic.2012.07.049.

13. da Silva JE, Barbosa SBP, Abreu B da S, Santoro KR, da Silva EC, Batista ÂMV, Martinez RLV. Effect of somatic cell count on milk yield and milk components in Holstein cows in a semi-arid climate in Brazil. Rev Bras Saude e Prod Anim. 2018;19:391–402. doi: 10.1590/S1519-99402018000400004.

14. Toffanin V, Penasa M, McParland S, Berry DP, Cassandro M, De Marchi M. Genetic parameters for milk mineral content and acidity predicted by mid-infrared spectroscopy in Holstein–Friesian cows. Animal. 2015;9:775–80. doi:10.1017/S1751731114003255.

15. Costa A, Lopez-Villalobos N, Visentin G, De Marchi M, Cassandro M, Penasa M. Heritability and repeatability of milk lactose and its relationships with traditional milk traits, somatic cell score and freezing point in Holstein cows. Animal. 2019;13:909–16. doi:10.1017/S1751731118002094.

16. Olechnowicz J, Jaśkowski JM. Somatic cells count in cow’s bulk tank milk. Journal of Veterinary Medical Science. 2012;74:681–6. doi:10.1292/jvms.11-0506.

17. Schwarz D, Diesterbeck US, König S, Brügemann K, Schlez K, Zschöck M, Wolter W, Czerny CP. Flow cytometric differential cell counts in milk for the evaluation of inflammatory reactions in clinically healthy and subclinically infected bovine mammary glands. J Dairy Sci. 2011;94:5033–44. doi: 10.3168/jds.2011-4348.
**Figures**

Figure 1: SNP effect plots for the significantly associated SNPs on chromosomes 13 and 19 for Butana and Butana x Holstein cows. They show the corrected averaged SCS per animal for SNP genotype groups (homozygous reference allele, heterozygous, and homozygous to the alternative allele). The number of animals is visualized by grey points. P-values between genotype groups from two-sided t-tests are displayed.
Table 1: Results of the association test for SCS in Butana and Butana x Holstein crossbred cattle. The SNP-ID is listed together with the chromosome number (Chr), position (bp) with regard to the Bos taurus ARS_UCD1.2 genome assembly, the minor (A1) and the major allele (A2), the allele frequency of the minor allele (F<sub>A1</sub>), the β-estimate per minor allele change (β<sub>A1</sub>), the standard error of the β-estimate (SE(β<sub>A1</sub>)), and the Bonferroni-corrected p-value of the association test (P<sub>BF</sub>). P<sub>BF</sub><0.1 are highlighted in bold. As two SNPs in Butana were not segregating (marked as “NA”), no MAF or association analysis could be calculated on them.

| SNP ID    | Chr | Position (bp) | A1 | A2     | Butana | Butana x Holstein crossbred |
|-----------|-----|---------------|----|--------|--------|-----------------------------|
|           |     |               |    |        | F<sub>A1</sub> | β<sub>A1</sub> | SE(β<sub>A1</sub>) | P<sub>BF</sub> | F<sub>A1</sub> | β<sub>A1</sub> | SE(β<sub>A1</sub>) | P<sub>BF</sub> |
| rs41257360 | 5   | 97477421      | G  | A      | NA     | 0.190 | 0.087 | 0.313 | 1 | 0.240 | -0.091 | 0.104 | 1 |
| rs41588957 | 6   | 83803915      | C  | T      | 0.300 | 0.139 | 0.314 | 1 | 0.206 | 0.133 | 0.102 | 1 |
| rs110707460| 6   | 86373734      | G  | A      | 0.014 | -0.103 | 0.541 | 1 | 0.100 | 0.167 | 0.158 | 1 |
| rs109934030| 13  | 77914930      | T  | C      | 0.001 | -0.342 | 0.635 | 1 | 0.181 | 0.220 | 0.121 | 1 |
| rs41634110 | 13  | 79002832      | G  | A      | 0.000 | NA     | NA     | NA | 0.326 | 0.254 | 0.090 | 0.054 |
| rs41629005 | 18  | 21572729      | T  | C      | 0.140 | -0.077 | 0.373 | 1 | 0.382 | 0.009 | 0.102 | 1 |
| rs41257403 | 19  | 50027458      | A  | G      | 0.460 | 0.849 | 0.149 | 0.003 | 0.243 | 0.726 | 0.087 | 6.2x10^-16 |
| rs41636878 | 19  | 51815015      | T  | C      | 0.200 | -0.497 | 0.335 | 1 | 0.228 | 0.007 | 0.116 | 1 |

Supporting information

Additional supporting information of all tables can be found in supplementary file Supplementary Material_Butana_SCS_manuscript.docx.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterialSCSmanuscript.docx