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

Salma Elzaki1,2 · Paula Korkuc1 · Danny Arends1 · Monika Reissmann1 · Siham A. Rahmatalla1,3 · Gudrun A. Brockmann1

Received: 20 September 2021 / Accepted: 4 January 2022 / Published online: 12 January 2022
© The Author(s) 2022

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 single nucleotide polymorphisms (SNPs) which had been associated with somatic cell score (SCS) in Holstein cattle for association with SCS in 37 purebred Butana and 203 Butana × Holstein crossbred cattle from Sudan. Animals were genotyped by competitive allele-specific PCR assays and association analysis was performed using a linear mixed model. All 10 SNPs were segregating in the crossbred Butana × Holstein populations, but only 8 SNPs in Sudanese purebred Butana cattle. The SNP on chromosome 13 was suggestively associated with SCS in the Butana × Holstein crossbred population (rs109441194, 13:79,365,467, PBF = 0.054) and the SNP on chromosome 19 was significantly associated with SCS in both populations (rs41257403, 19:50,027,458, Butana: PBF = 0.003, Butana × Holstein: PBF = 6.2 × 10^{-16}). The minor allele of both SNPs showed an increase 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.

Keywords Association analysis · Bos indicus · Genotyping · Mastitis · SNPs

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 (Ageeb and Hillers 2021). Butana cattle have an average milk yield of 538.3 kg per lactation under field conditions (Musa et al. 2005; 2006), and 1,662.6 kg under improved feeding and management conditions in a research station (Musa et al. 2005; 2006). The age at first calving ranges from 1168 to 1588 days and the lactation length was reported from 190 to 268 days (Ageeb and Hillers 2021; Musa et al. 2005; 2006). In order to improve milk production and reproduction traits, Butana cattle have been crossed with high-yielding Holstein dairy cattle. The resulting Butana × Holstein crossbred cattle are as robust as Butana cattle but have higher milk yield compared to purebred Butana with the milk yield per lactation being on average 3988.2 kg (Elnazeir et al. 2019). The lactation length is on average 356 days, and the age at first calving 1040 days (Eshraga 2011). Thus, crossbred Butana × Holstein cattle are currently used as the main source of milk in urban regions, but they can also be found in rural areas.
One of the main problems of dairy production in Sudan and worldwide 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 (Bar et al. 2008; Mungube et al. 2005). 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 (Peeler et al. 2000). Because of the low heritability of clinical mastitis and SCS, traditional methods of selection have limited success (Hinrichs et al. 2011). Thus, selection using genomic markers has potential to improve mastitis resistance (Meuwissen et al. 2001).

Many studies in different Holstein populations have been performed, which identified genomic loci associated with SCC or clinical mastitis on chromosomes 1, 5, 6, 8, 13, 16, 18, 19, and 20 (Abdel-Shafy et al. 2014, 2018; Viale et al. 2017; Jiang et al. 2019). In this study, we tested 10 single nucleotide polymorphisms (SNPs) that have been previously associated with SCC in German Holstein bulls and cows (Abdel-Shafy et al. 2014) for associations with SCC in Sudanese purebred Butana and Butana × Holstein crossbred cattle. Five of those SNPs were also found to be associated with clinical mastitis (Abdel-Shafy et al. 2018). 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 × 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 × Holstein crossbred cows with varying levels of exotic blood belonging to five farms in Khartoum North. The cows were in lactation 1 to 8 with lactations of 5 and above being set to 5 + for statistical analyses. In this study, the average lactation length of Butana cattle was 243 days and of Butana × Holstein crossbred cattle 309 days. The age at first calving of Butana cattle was on average 1896 days and of Butana × Holstein crossbred cattle 998 days. One Butana cow was removed because the age at first calving was 3000 days and thereby more than 50% higher than the average of the population.

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 which is a technique for the estimation of the number of nucleated somatic cells in unprocessed milk (Brazis et al. 1968). SCC was transformed to somatic cell score (SCS = log2 (SCC/100,000 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. 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 ±3 standard deviations. After filtering, 1,222 SCS measurements were available for 34 purebred Butana cows from Atbara research station (134 test-day records) and for 201 Butana × Holstein crossbred cows from five farms (1,088 test-day records).

**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 salt out procedure (Miller et al. 1988). Genotyping of 10 SNPs that have been identified for SCS (Abdel-Shafy et al. 2014) was performed with allele-specific primers using competitive allele-specific PCR assays (KASP) (Kreuzer et al. 2013). Primer information for SNPs genotyped in this study is fully provided in Abdel-Shafy et al. (Abdel-Shafy et al. 2014). Genomic positions were referenced to the current *Bos taurus* ARS_UCD2.1 genome assembly (Rosen et al. 2018).

**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 × Holstein crossbred cattle were examined separately. The model for testing the additive effect for each SNP in Butana cattle was:

$$Y = SY + DIM + GT + (1|\text{animal}) + e$$  

(1)

where $Y$ is the SCS from multiple sample dates and $SY$ represents the covariate for the year of sampling followed by the covariate for days in milk DIM and the SNP genotype GT. The animal was included as a random effect ($1|\text{animal}$) to compensate for repeated measurements; $e$ is the residual error.

The model for testing the additive effect for each SNP in Butana × Holstein crossbred cattle was

$$Y = FA + SY + CS + FC + DIM + GT + (1|\text{animal}) + e$$  

(2)

where the same formula was used as in Eq. 1 plus the covariates for farm FA, calving season CS (dry season from...
November to April; wet season from May to October), and age at first calving in days FC.

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|animal)) and the null model extended with one of the covariates (Y = covariate + (1|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 ggtabin (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×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×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 cattle (Table 1). Two out of the 10 previously reported SNPs were associated with SCS in Butana×Holstein crossbred cattle (Fig. 1). One suggestively associated SNP was located on chromosome 13 (rs109441194, 13:79,365,467, P<BF = 0.054). 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 × 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 purebred Butana population was 0.46, increasing SCS by 0.849.

Discussion

In the current study, we confirmed suggestive and significant effects on SCS of the two SNPs rs109441194 and rs41257403 on chromosomes 13 and 19, respectively, in Butana×Holstein crossbred 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 (Abdel-Shafy et al. 2018) which makes the findings more important. In the regions of the two confirmed SNPs are the same two positional candidate genes as reported previously (Abdel-Shafy et al. 2014, 2018):

Table 1 Association results for SCS in Butana and Butana×Holstein crossbred cattle. Each tested SNP is listed with its SNP ID, chromosome number (Chr), genomic positions in bp with regard to the Bos taurus ARS_UCD1.2 genome assembly, the minor allele (A1), the major allele (A2), the minor allele frequency (F_A1), the β-estimate per minor allele change (β_A1), the standard error of the β-estimate (SE(β_A1)), and the Bonferroni-corrected P-value of the association test (P<BF). P<BF < 0.1 are highlighted in bold. Two SNPs were not segregating in Butana. Thus, no association analysis could be calculated with them.

| SNP ID     | Chr | Position     | A1 | A2 | Butana | Butana×Holstein crossbred |
|------------|-----|--------------|----|----|--------|---------------------------|
| rs41257360 | 5   | 97,477,421   | G  | A  | 0.000  | -                         |
| rs41588957 | 6   | 83,803,915   | C  | T  | 0.190  | 0.087                     |
| rs110707460| 6   | 86,337,334   | G  | A  | 0.300  | 0.139                     |
| rs109934030| 13  | 77,914,930   | T  | C  | 0.014  | −0.103                    |
| rs41634110 | 13  | 79,002,832   | G  | A  | 0.041  | −0.342                    |
| rs109441194| 13  | 79,365,467   | T  | C  | 0.000  | -                         |
| rs290020544| 18  | 43,157,279   | T  | G  | 0.140  | −0.077                    |
| rs41257403 | 19  | 50,027,458   | A  | G  | 0.460  | 0.849                     |
| rs41636878 | 19  | 51,815,015   | T  | C  | 0.200  | −0.497                    |
| rs41629005 | X   | 30,341,984   | T  | C  | 0.041  | 0.388                     |

\( P_{BF} = 6.2 \times 10^{-16} \)
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 × Holstein crossbred cattle (Table 1). Across all 10 SNPs, the difference of allele frequency was bigger between Holstein (Abdel-Shafy et al. 2014) and Butana cattle (average difference of MAFs = 0.17) than between Holstein and Butana × 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 × 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 (Abdel-Shafy et al. 2014), 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 × 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 (Abdel-Shafy et al. 2014) 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 × Holstein crossbred cattle (average SCS = 5.1). In contrast, SCS in different Holstein herds ranges between 2.91 and 4.62 (da Silva et al. 2018; Toffanin et al. 2015; Costa et al. 2019). The SCS of milk that is allowed to be consumed varies in different countries, e.g., in the USA, Canada, and Germany, and the SCS threshold is set to 5.9, 5.3, and 5.0, respectively (Olechnowicz and Jaśkowski 2012; Schwarz et al. 2011). 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 × 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 × 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 × 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 × 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.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11250-022-03048-3.

Acknowledgements We gratefully thank the cattle owners in Sudan for providing the samples and phenotypes, as well as the management and staff team of the Atbara Research Station. We thank Elhady A. M. Omer for the help in sample collection.

Author contribution 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.

Funding Open Access funding enabled and organized by Projekt DEAL. SE was supported by the Sudanese Ministry of Higher Education and Scientific research and the Fiat panis foundation. The collection of data and samples was supported by the Ministry of Higher Education and Scientific Research Sudan.

Data availability All genotypes and phenotypes analysed are included in Supplementary Table 3.

Declarations

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.

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

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(4): 2481-2486. https://doi.org/10.3168/jds.2013-7149.

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(35). https://doi.org/10.1186/1297-9686-46-35.

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(1):82-85. https://doi.org/10.1111/age.12624.

Ageeb AG, Hillers JK. Production and reproduction characteristics of Butana and Kenana cattle of the Sudan. World Anim Rev. 1991;2:49–56. http://www.fao.org/3/Ui1200U/Ui1200T0j.htm. Accessed 1 Apr 2021.

Bar D, Gruhn 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(6):2196–204. https://doi.org/10.3168/jds.2007-0460.

Brazis AR, Jasper DE, Levowitz D, Newbould FHS, Postle DS, Schulze WD, Smith JW, Ullmann WW. Direct Microscopic Somatic Cell Count in Milk. J Milk Food Technol. 1968;31(11):350–4. https://doi.org/10.4315/0022-2747-31.11.350.

Costa A, Lopez-Villalobos N, Visentin G, De Marchi C, Casalvino 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(5):909–16. https://doi.org/10.1017/S1751731118002094.

da Silva JE, Barbosa SRP, 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(4):391–402. https://doi.org/10.1590/1519-99402018000400004.

Elzair H, Mohammed A., Elkhidir O, Atta M. Effects of parity order, age at first calving and season of calving on productive performance of Friesian crossbred cattle in Sudan. J Vet Med Anim Prod. 2019;8(2). https://adabjournal.uofk.edu/index.php/vet/ article/view/2540. Accessed 1 Oct 2021.

Eshraga Ahamed E. Assessment of Productive and Reproductive Traits and Management Practices in Dairy Farms in Khartoum State. MSC thesis, University of Khartoum, Sudan. 2011. http://khartoumspace.uofk.edu/bitstream/handle/123456789/8701/ASSES SMENTOFPRODUCTIVEAND.pdf?sequence=1&isAllowed=y. Accessed 1 Oct 2021.

Hinrichs D, Bennewitz J, Stamer E, Junge W, Kalm E, Thaller G. Genetic analysis of mastitis data with different models. J Dairy Sci. 2011;94(1):471–8. https://doi.org/10.3168/jds.2010-3374.

Jiang J, Ma L, Prakapenka D, VanRaden PM, Cole JB, Da Y. A Large-Scale Genome-Wide Association Study in U.S. Holstein Cattle. Front Genet. 2019;10:412. https://doi.org/10.3389/fgene.2019.00412.
Kruezer S, Reissmann M, Brockmann GA. Gene test to elucidate the ETEC F4ab/F4ac receptor status in pigs. Vet Microbiol. 2013;162(1):293–5. https://doi.org/10.1016/j.vetmic.2012.07.049.

Meuwissen TH, Hayes BJ, Goddard ME. Prediction of total genetic value using genome-wide dense marker maps. Genetics. 2001;157(4):1819–29. https://doi.org/10.1093/genetics/157.4.1819.

Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16(3):1215–1215. https://doi.org/10.1093/nar/16.3.1215.

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(6):503–12. https://doi.org/10.1007/s11250-005-7049-y.

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). http://lrrd.cipav.org.co/lrrd18/12/musa18177.htm. Accessed 1 Oct 2021.

Musa LMA, Ahmed M-KA, Peters KJ, Zumbach B, Gubartalla KEA. The reproductive and milk performance merit of Butana cattle in Sudan. Arch Anim Breed. 2005;48:445–59. https://doi.org/10.5194/aab-48-445-2005.

Olechnowicz J, Jaśkowski JM. Somatic cells count in cow’s bulk tank milk. J J Vet Med Sci. 2012;74(6):681–6. https://doi.org/10.1292/jvms.11-0506.

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(11):2464–72. https://doi.org/10.3168/jds.S0022-0302(00)75138-1.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.