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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, Butana: $P_{BF}=0.003$, Butana x Holstein: $P_{BF}=6.2 \times 10^{-16}$). 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.

27

28 **Keywords:** Association analysis, *Bos indicus*, genotyping, mastitis, SNPs, candidate gene

29 **Introduction**

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

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

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

52 **Material and methods**

53 **Animals and phenotypic data**

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

64 **DNA isolation and SNP genotyping**

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

69 **Association analysis**

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

$$73 Y_{ijkl} = sy_i + \text{dim}_j + gt_k + (1|\text{animal}_l) + e_{ijkl}, \quad (\text{Eq. 1})$$

74 where Y_{ijkl} is the SCS from multiple sample dates, sy_i represents the covariate for the year of sampling followed
75 by the covariate for days in milk dim_j and the SNP genotype gt_k . The animal was included as a random effect
76 $(1|\text{animal}_l)$ to compensate for repeated measurements; e_{ijkl} is the residual error.

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

$$Y_{ijklmno} = sy_i + cs_j + fc_k + dim_l + f_m + gt_n + (1|animal_o) + e_{ijklmno}, \quad (\text{Eq. 2})$$

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 ($Y = (1|animal)$) 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 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 crossbred 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

103 (rs41257403, 19:50,027,458, $P_{BF}=0.003$). The minor allele was the same as in the crossbred population and
104 had the same direction and a similar magnitude of effect. The frequency of the unfavourable minor allele A of
105 the chromosome 19 SNP in the crossbred population was 0.46, increasing SCS by 0.849.

106 **Discussion**

107 In the current study, we tested 10 SNPs for association with SCS in the Sudanese *Bos indicus* zebu cattle breed
108 Butana and in Butana x Holstein crossbred cows. We confirmed suggestive and significant effects on SCS of
109 the two SNPs rs109441194 and rs41257403 on chromosomes 13 and 19, respectively, in Butana x Holstein
110 crossbred cattle. In Butana cattle, the SNP on chromosome 19 was also significantly associated with SCS,
111 while the SNP on chromosome 13 was monomorphic. Therefore, the chromosome 13 region could not be
112 tested for potential association of the linked chromosomal region with SCS in Butana. These two confirmed
113 SNPs were not only associated with SCS but also directly with clinical mastitis in Holstein cows [9] which
114 makes the findings more important. In the regions of the two confirmed SNPs are the same two positional
115 candidate genes as reported previously [7, 9]: NFATC2 (cytoplasmic nuclear factor of activated T-cells 2) and
116 FOXP2 (forkhead box protein K2), which are both linked directly or indirectly to immune response of T-cells.
117 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
118 cattle (Table 1). Across all 10 SNPs, the difference of allele frequency was bigger between Holstein [7, 8] and
119 Butana cattle (average difference of MAFs=0.17) than between Holstein and Butana x Holstein crossbred cattle
120 (average difference of MAFs=0.10). For the two confirmed SNPs (rs109441194, rs41257403), the MAFs were
121 similar in Holstein and in Butana x Holstein crossbred cattle (difference of MAFs=0.04). With respect to the
122 SNP on chromosome 19, the MAF was much higher in Butana cattle, where the SNP was fixed (difference of
123 MAF=0.26).

124 The directions of effects of the two confirmed SNPs were the same in both investigated breeds. In all cases,
125 the minor allele was disadvantageous increasing SCS. Therefore, selection against the minor allele could
126 reduce the somatic cell numeric in the milk in the population and thereby hopefully improve resistance against
127 clinical mastitis. Comparing to previous results in Holstein bulls and cows [7, 8], the direction of effects of the

128 associated SNP on chromosome 13 was consistent meaning that the same region is linked to SCS or immune
129 response in Butana x Holstein cattle as in Holstein Friesian cattle. In contrast, the associated SNP on
130 chromosome 19 showed an opposite direction of effects compared to previous results in Holstein cattle [7, 8]
131 which could be caused by the weak linkage between the investigated SNP and the causative mutation.

132 In general, SCC and thus SCS were high in the investigated Butana (average SCS=4.6) and Butana x Holstein
133 crossbred cattle (average SCS=5.1). In contrast, SCS in different Holstein herds ranges between 2.91- 4.62
134 [13–15]. The SCS of milk that is allowed to be consumed varies in different countries, e.g., in USA, Canada,
135 and Germany, the SCS threshold is set to 5.87, 5.3, and 5, respectively [16, 17]. The high SCS in Sudanese
136 cattle may indicate that Sudanese farms have high rates of subclinical mastitis or even clinical mastitis. First
137 and foremost, better farm management, higher hygienic requirements, and proper nutrition of cattle are
138 necessary on Sudanese farms in order to reduce mastitis cases. However, a better understanding of the genetic
139 background of mastitis resistance, more accurate phenotyping, and genetic selection using mastitis markers
140 could improve resistance against mastitis.

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

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

155 **Declarations**

156 **Acknowledgements**

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158 management and staff team of the Atbara Research Station. We thank Elhady A. M. Omer for the help in
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163 Scientific Research Sudan.

164 **Competing interests**

165 The authors declare that they have no competing interests.

166 **Availability of data and materials**

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

169 **Authors' contributions**

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

174 **Ethics approval and consent to participate**

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

177 **Consent for publication**

178 Not applicable.

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225

226 **Figures**

228 Figure 1: SNP effect plots for the significantly associated SNPs on chromosomes 13 and 19 for Butana and
229 Butana x Holstein cows. They show the corrected averaged SCS per animal for SNP genotype groups
230 (homozygous reference allele, heterozygous, and homozygous to the alternative allele). The number of animals
231 is visualized by grey points. P-values between genotype groups from two-sided t-tests are displayed.

233 Tables

234 Table 1: Results of the association test for SCS in Butana and Butana x Holstein crossbred cattle. The SNP-ID
 235 is listed together with the chromosome number (Chr), position (bp) with regard to the Bos taurus ARS_UCD1.2
 236 genome assembly, the minor (A1) and the major allele (A2), the allele frequency of the minor allele (F_{A1}), the
 237 β -estimate per minor allele change (β_{A1}), the standard error of the β -estimate ($SE(\beta_{A1})$), and the Bonferroni-
 238 corrected p-value of the association test (P_{BF}). $P_{BF} < 0.1$ are highlighted in bold. As two SNPs in Butana were
 239 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_{A1}	β_{A1}	$SE(\beta_{A1})$	P_{BF}	F_{A1}	β_{A1}	$SE(\beta_{A1})$	P_{BF}
rs41257360	5	97477421	G	A	NA	NA	NA	NA	0.216	-0.159	0.112	1
rs41588957	6	83803915	C	T	0.190	0.087	0.313	1	0.240	-0.091	0.104	1
rs110707460	6	86337334	G	A	0.300	0.139	0.314	1	0.206	0.133	0.102	1
rs109934030	13	77914930	T	C	0.014	-0.103	0.541	1	0.100	0.167	0.158	1
rs41634110	13	79002832	G	A	0.041	-0.342	0.635	1	0.181	0.220	0.121	1
rs109441194	13	79365467	T	C	0.000	NA	NA	NA	0.326	0.254	0.090	0.054
rs29020544	18	43157279	T	G	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	6.2×10^{-16}
rs41636878	19	51815015	T	C	0.200	-0.497	0.335	1	0.228	0.007	0.116	1
rs41629005	X	30341984	T	C	0.041	0.388	0.759	1	0.431	-0.018	0.093	1

240

241 Supporting information

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

244

245

Supplementary Files

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