Biotechnology in Animal Husbandry 33 (1), p 13-25 , 2017 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.291
DOI: 10.2298/BAH1701013M

GENOME-WIDE SNPs ANALYSIS OF INDIGENOUS ZEBU BREEDS IN PAKISTAN

Hamid Mustafa[1&2], Kim Eiusoo[2], Huson J. Heather[3], Adeela Ajmal[1], David Riley[4], Talat Nasser Pasha[1], Afzal Ali[1], Khalid Javed[1], Tad S. Sonstegard[2]

[1] University of Veterinary and Animal Science, Lahore-Pakistan.
[2]Bovine Functional Genomics Laboratory (BFGL), U.S.D.A, USA.
[3]Department of Animal Sciences, Cornell University, NY, USA
4Department of Animal Sciences, Texas A&M University, USA
Corresponding author: Hamid Mustafa, hamidmustafapasha@gmail.com
Original scientific paper

Abstract: Prospects of high throughput technology in animal genetics makes easy to investigate hidden genetic variation in farm animal's genetic resources. However, many SNPs technologies are currently practicing in animal genetics. In this study, we investigated genome wide SNPs variations and its distribution across the indigenous cattle population in Pakistan using Illumina Bovine HD (777K) SNPs bead chip. A total of 136 individuals from ten different breeds were genotyped and after filtration 500, 939 SNPs markers were used for further analysis. The mean minor allele frequency (MAF) was 0.23, 0.20, 0.22, 0.22, 0.20, 0.18, 0.20, 0.22, 0.21 and 0.18 observed for Achi, Bhagnari, Cholistani, Dhanni, Dajal, Kankraj, Lohani, Red sindi, Sahiwal and Tharparkar cattle, respectively. Significant difference ($P<0.001$) of MAFs were observed in selected population. A common variants minor allele frequency (≥ 0.10 and ≤ 0.5) was estimated (64%). Across all sampled populations 64% SNPs markers were observed polymorphic (MAF>0.05) within breeds and remaining 36% were considered as monomorphic markers. Average observed (H_o) and expected (H_E) heterozygosity values 0.662 and 0.640 were estimated among these breeds. In conclusion, this preliminary study results revealed that these SNPs variation level could potentially be used for genetic characterization of zebu cattle breeds and could also be used to estimate genetic potential of these cattle breeds for livestock improvement in country.

Keywords: minor allele frequency, SNPs, variation, distribution, cattle

Introduction

Bovine high density (HD) SNPs assay is a most comprehensive genotyping tool to explore genome variation with high coverage resolution across cattle breeds (*Howard et al., 2015*). This features more than 777,962 SNPs probes that are equally distributed across entire bovine genome (*Leroy, 2014*). This array was first time introduced in 2009 (*Mbole-Kariuki et al., 2014*). Applications of this array include genome wide association studies, quantitative trait loci identification, prediction of genetic merit, linkage disequilibrium and breed characterization (*Pryce at el., 2014*). The potential of this array has been proven in several studies that identified genomic regions which have strong contribution in phenotypic variation. These genomic regions that are related with feed efficiency and intake traits (*Lin at el., 2010; Edea et al., 2014*) milk production traits and meat type traits (*Howard at el., 2015; Kim at el., 2015*).

In addition, genomics values prediction in breeding programme based on genomic data have been extensively used for cattle selection (*Edea et al., 2014*). The genomic selection tools reliability is based mainly on linkage disequilibrium (LD) existence and their association between SNPs and QTL that affects the traits of interest (*Caruthers et al., 2011; Curik at el., 2014; Kim at el., 2015*). In U. S. A and other developed countries genomic information is widely used for genetic evaluation of farm animals (dairy and beef) (*Howard at el., 2015*). The Bovine HD SNP assay has also been used to identify copy number variations (CNV) that are used for QTL association with phenotypes (*Bickhart at el., 2016*). In addition, Bovine high density (HD) genotyping assay has also been used to detect genetic relationships among and within cattle breeds and also been applied to detect signature of selection in different dairy and beef breeds (*Kim at el., 2015*).

In Pakistan, all genetic improvement programmes for dairy and beef cattle breeds are based on conventional quantitative genetics methods. There is also limited availability of phenotypic and pedigree data information for estimation of breeding values in these breeds (*Mustafa at el., 2014*). Conventionally, the genetic structure of economically important traits was considered to be a black box with little information of the genes variations affecting phenotypic expression of these traits, gene interactions, and the location of these genes in the genome (*Decker et al., 2014; Hussain at el., 2016*). Meanwhile, it has been found that genetic selection has a high probability to increase genetic gain in cattle and also permits more accurate genetic predictions for traits of low heritability in farm animals than conventional phenotypic selection (*Groeneveld at el., 2010; Curik at el., 2014; Leroy, 2014; Kim et al., 2015*). Currently, indigenous cattle breeds in Pakistan still lack the opportunity for high throughput evaluation. To better understand complex evolutionary process and breeding improvement programmes. To date, no indigenous Pakistani cattle breed has been included either in training or a validation population using the Bovine HD SNP BeadChip (*Mustafa at el., 2014*).

Therefore, it is necessary to assess the usefulness of the Bovine HD SNP BeadChip in indigenous Pakistani cattle breeds. The evaluation of this high throughput technique would be help to improve the cattle farming and establish a reference population. Therefore, the aim of this analysis was to find the level of informativeness of Bovine HD SNP BeadChip by measuring loci polymorphism in indigenous cattle population in Pakistan.

Materials and Methods

Animals sampling, genomic DNA extraction and Genotyping

A 10ml Jugular blood samples were obtained from ten different breeds from potential agro-geographical area of these breeds using EDTA containing tubes (Table 1 & Figure 1-2). The gDNA extraction and quality control of data was described in a previous study (*Mustafa at el., 2014*). Genotyping of selected samples was performed at USDA platform using Illumina Bovine high density (HD) SNPs bead chip (version 2) spanning 777, 962 SNPs markers across all bovine genome. 200 ng gDNA quantities were used to genotyped these samples according to manufacture protocol.

Table 1. Animals sampling and geographical details

Population	Code	N	Agro-ecology*	Purpose	Province
Achi	AC	18	West Mountains	Milk and Meat	Khyber Phaktunpkhua
Bhagnari	BH	14	Sulaiman Piedmont	Work	Balochistan
Cholistani	CL	13	Sandy Desert	Milk and Meat	Punjab
Dhanni	DH	10	Barani Lands	Work and Milk	Punjab
Dajal	DJ	10	Sulaiman Piedmont	Work and Meat	Punjab
Kankraj	KK	12	Sandy desert	Work and Meat	Sindh
Lohanni	LH	19	Western Dry Mountains	Work and Milk	Balochistan
Red Sindhi	RH	13	Southern Irrigated	Milk	Sindh
Sahiwal	SH	14	Northen Irrigated Plains	Mlik	Punjab
Tharparkar	TH	13	Sandy desert	Mlik	Sindh

*Figure 1 showed complete agro-geographic location

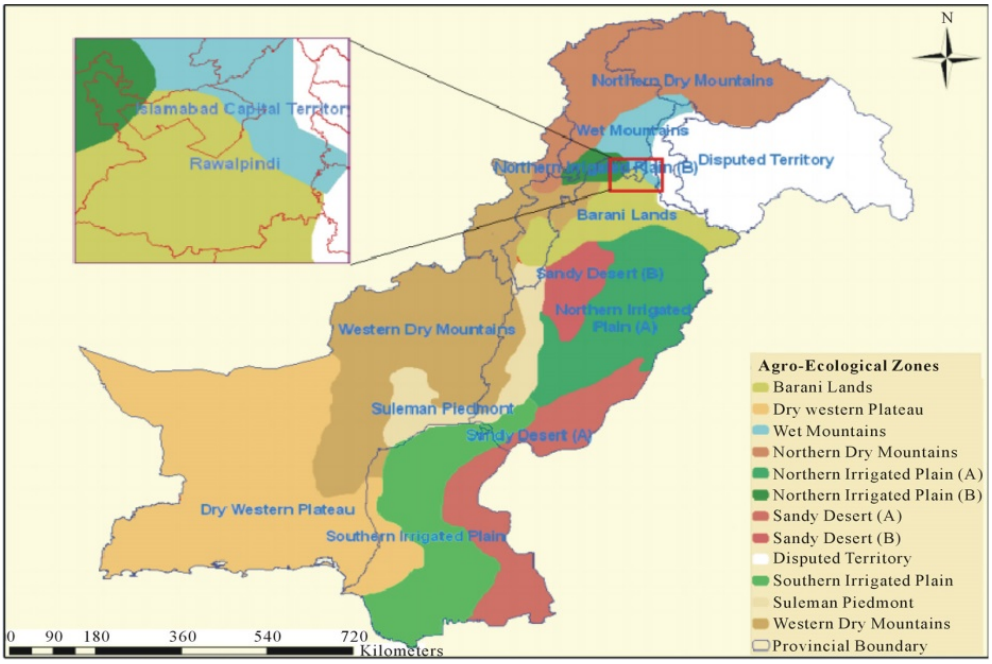

Figure 1. Agro-ecological zones of Pakistan (Kazmi & Rasul, 2012).

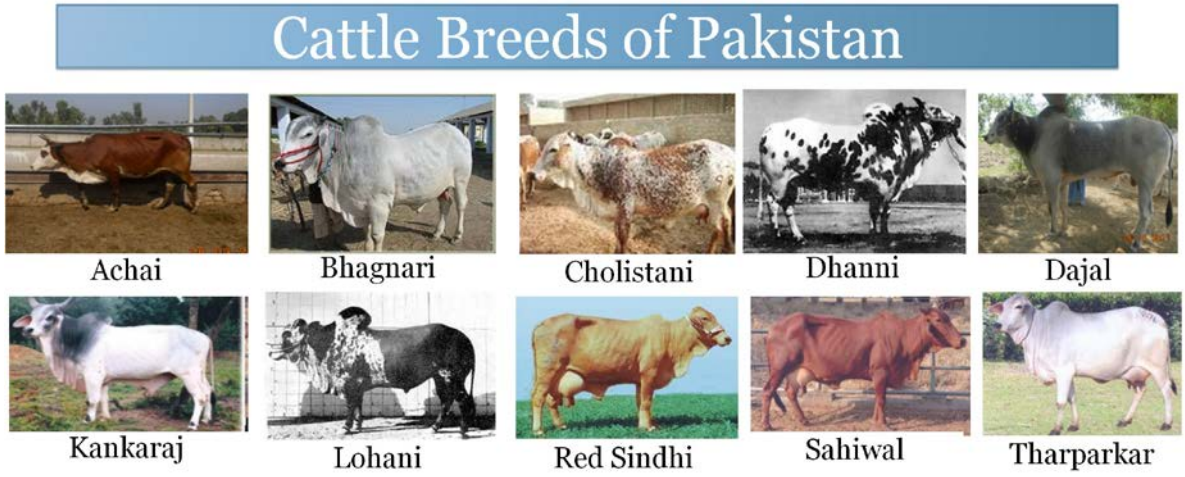

Figure 2. Cattle Breeds of Pakistan (Mustafa *et al.*, 2012)

Data analysis

Genotypic data were generated from the iScan system. The raw data analysis including genotyping calling, clustering and data normalization was performed by using genome studio version 1.9.0 software (*Edea at el., 2015*) .Pad and map. file was created for downstream analyses from the genome studio using PLINK (version 1. 9). Quality assurance module were used form SVS (version 8; Golden Helix Inc., USA) for genotypic statistics each markers were analyzed for call rate, Hardy- Weinberg equilibrium (HWE), minor allele frequency (MAF) and genotypes count. Quality control (QC) criteria for further analysis were < 95% call rate and <0.05 minor allele frequency (MAF). Hardy Weinberg equilibrium (P<0.001) was tested to help identify genotyping errors (*Kim at el., 2015*).

Results and Discussion

Minor Allele Frequency (MAF) Distribution

The minor allele frequency (MAF) was calculated and presented in Table 2 & figure 3 for each SNP from the generated data set. The analysis of 500,939 SNP markers indicate an average minor allele frequency (MAF) that is 0.23, 0.20, 0.22, 0.22, 0.20, 0.18, 0.20, 0.22, 0.21 and 0.18 for Achi, Bhagnari, Cholistani, Dhanni, Dajal, Kankraj, Lohani, Red sindi, Sahiwal and Tharparkar cattle, respectively. There was a significant difference observed among these selected breeds (p<0.001). The overall minor allele frequency (MAF) was observed in this study was higher than previous reported studies in *indicine* breeds (*McKay at el., 2008; Edea et al., 2015; Kim at el., 2015*) and lower than the average value reported for Red Chittagong that was 0.28 (*Uzzaman at el., 2014*). The lower average minor allele frequency (MAF) value is as expected than most of the Bos *taurus* cattle breeds (*Mckay et al., 2008; Mustafa at el., 2014*). The minor allele frequency (MAF) found in this study revealed that these attributes to different markers density (Illumina Bovine 8K, 10K, 50K, 80K and 700K) used in previous studies in different cattle breeds around the world and most of these breeds samples were not used before or during designing of these chips (*Chen at el., 2010; Lin at el., 2010; Melka at el., 2011; Edea at el., 2014; Uzzaman at el., 2014*).

Table 2. Minor Allele Frequency (MAF) values of indigenous cattle breeds in Pakistan.

	Breed	Minor Allele Frequency (MAF)
1	Achi	0.23
2	Bhagnari	0.20
3	Cholistani	0.22
4	Dhanni	0.22
5	Dajal	0.20
6	Kankraj	0.18
7	Red Sindhi	0.20
8	Lohani	0.22
9	Sahiwal	0.21
10	Tharparkar	0.18
		0.21

The SNP variation across all Pakistani cattle breeds was also examined. The SNPs minor allele frequency (MAFs) distribution at common variants (≥ 0.10 and ≤ 0.5) accounts is 64% (Table 3). Among these selected breeds, Dhanni cattle displayed high proportions of common variants (69%). The minor allele frequency (MAFs) variation at rare variant (>0 and <0.05) were observed 11% in overall breed samples. The higher proportion of alleles (fixed) in selected cattle populations indicate inbreeding that is due to uncontrolled breeding management in country (*Groeneveld at el., 2010; Lin at el., 2010; Leroy, 2014*). The high proportions of common variants were also reports in sheep that was 83 % (*Kijas et al., 2009*). The average minor allele frequency (MAF) distributions at ≥0.30 and ≤0.5 were displayed 32 % that is higher than previous reported polymorphism in cattle breeds (*McKay at el., 2008; Kim at el., 2015*). It is an established fact that higher proportions of minor allele frequency (MAFs) were observed in Bos *taurus* rather than Bos *indicus* using different Illumina bovine Bead chips due to limited numbers of indicus breeds were used during chip developments (*Decker at el., 2014; Mustafa at el., 2014; Bickhart at el., 2016*). The SNPs distribution at fixed level (0) was also examined and average 8% was observed among all these breeds. The highest SNPs proportion at fixed level was observed in Dhanni and Tharparkar (10%) and lower level in Bhagnari and Lohani (6%) cattle breeds, respectively.

Across all sampled populations 64% SNPs markers were observed polymorphic (MAF>0.05) within breeds and remaining 36% were considered as monomorphic markers (Figure 5). The higher proportion of polymorphism among these breeds was showed in Dhanni breed (69%). the high proportion of SNP variation in this study was higher than previous reported SNP variation in different

cattle breeds (*Curik at el., 2014; Howard at el., 2015; Kim at el., 2015*). Although, the results of SNP variations in this study revealed close similarity with the some previously reported variation in other farm animals including sheep and goat using genome wide SNP array (*Kijas et al., 2009*). The observed polymorphism in these selected breeds could explain that maximum bovine sequence data were available in the development of bead chip were from European cattle breeds (Bos *taurus)* (*Gautier at el., 2010; Melka at el., 2011; Edea at el., 2014; Decker at el., 2014*).

Genetic Diversity among Pakistani cattle breeds

The genomic variability within these cattle breeds were also examined and compare heterozygosity level between these breeds (Table 4). Across all these cattle breeds, the average observed (H_o) and expected (H_E) heterozygosity were 0.662 and 0.640, respectively. The average heterozygosity level was observed higher than the previous reported microsatellite markers analysis in some *indicine* cattle breeds (*Hussain et al., 2016*). Meanwhile, there is close agreement with previous reported values using SNPs in Brahman, Gir cattle and Nellore cattle (*Dadi at el., 2012; Leroy, 2014; Pryce at el., 2014; Decker at el., 2014; Bickhart at el., 2016*).

The F-statistics were also estimated within these selected breeds. Overall inbreeding within population (F_{IS}) value was estimated (0.073), where total inbreeding (F_{IT}) was 0.082. Genetic differentiation (F_{st}) was estimated at 0.076 (*Mbole-Kariuki at el., 2014; Edea at el., 2015; Kim at el., 2015*). Previously, in a study of Genetic characterization in Pakistani cattle population reported (inbreeding within population (F_{IS}) of 0.2819, F_{IT} (total inbreeding) of 0.3864 and F_{st} of 0.1456) using microsatellite makers (Hussain at el., 2016). The F_{st} of cattle breeds in Pakistan was observed low as reported in some previous zebu cattle studies (*Gautier at el., 2010; Groeneveld at el., 2010; Lin at el., 2010; Leroy at el., 2014*) that may be due to common origin.

The overall Hardy-Weinberg equilibrium (HWE) deviation ($p<0.05$) were significantly observed for 840 markers in these cattle breeds. Including Achi, 789; Bhagnari, 813; Cholistani, 744; Dhanni, 821; Dajal, 799; Kankraj, 811; Lohani, 787; Red sindi, 852; Sahiwal, 818; and Tharparkar, 911. Achi cattle showed lower proportion of markers deviating from Hardy-Weinberg equilibrium (HWE) similarly described in a previous study of African zebu cattle breeds (*Decker at el., 2014; Edea at el., 2015*). The proportion of SNPs variation displaying deviating from Hardy-Weinberg equilibrium (HWE) among the selected breeds could be expounded by population structure (admixture) and selection pressure.

Table 3. Distribution of minor allele frequency (MAF) of high density SNP (777, 962K) BeadChip in indigenous Pakistani cattle breeds.

		Fixed (0)		Rare (> 0 & < 0.05)		Intermediate (≥ 0.05 & ≤ 0.10)		Common (≥ 0.10 & ≤ 0.50)			≥ 0.30 & ≤ 0.5	
Breed	N	SNP	Prop.	SNP	Prop.	SNP	Prop.	SNP	Prop.		SNP	Prop.
Achi	18	54,329	0.070	84,317	0.108	98,421	0.127	431,897	0.56		215,949	0.28
Bhagnari	14	50,203	0.065	88,213	0.113	98,341	0.126	508,995	0.65		222,341	0.28
Cholistani	13	68,900	0.089	87,973	0.113	96,774	0.124	510,898	0.66		255,449	0.32
Dhanni	10	78,790	0.101	89,645	0.115	98,771	0.127	533,996	0.69		266,998	0.34
Dajal	10	69,471	0.089	89,763	0.115	98,721	0.127	498,898	0.64		256,631	0.33
Kankraj	12	55,431	0.071	89,789	0.115	97,631	0.125	499,399	0.64		243,421	0.31
Red Sindhi	13	58,991	0.076	88,976	0.114	98,984	0.127	521,999	0.67		261,000	0.34
Lohani	19	52,381	0.067	86,881	0.112	97,423	0.125	499,798	0.64		253,451	0.33
Sahiwal	14	68,360	0.088	84,953	0.109	93,946	0.121	500,968	0.64		250,484	0.32
Tharparkar	13	79,432	0.102	86,977	0.112	99,781	0.128	502,538	0.65		251,269	0.32
Overall	136	63,629	0.082	87,749	0.113	97,879	0.126	500,939	0.64		250,469	0.32

Table 4. Observed (H_o), expected (H_e) heterozygosity (F_{IT}) Total inbreeding, (F_{IS}) within population inbreeding of indigenous cattle breeds in Pakistan

Population	N	H_O	H_E	F_{IT}	F_{IS}
AC	18	0.663	0.628	0.034	0.089
BH	14	0.666	0.645	0.068	0.039
CL	13	0.657	0.645	0.039	0.017
DH	10	0.602	0.628	0.062	0.045
DJ	10	0.672	0.645	0.086	0.059
KK	12	0.628	0.619	0.028	0.040
LH	19	0.679	0.645	0.109	0.083
RH	13	0.651	0.645	0.021	0.043
SW	14	0.66	0.635	0.095	0.086
TH	13	0.701	0.645	0.179	0.164
Total	136	0.662	0.64	0.082	0.073

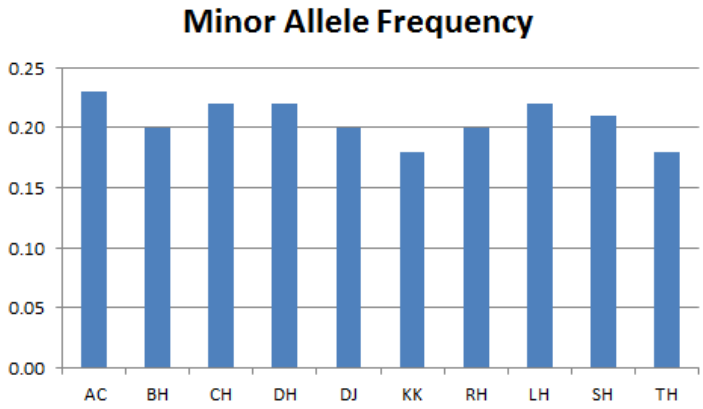

Figure 3. Minor Allele Frequency

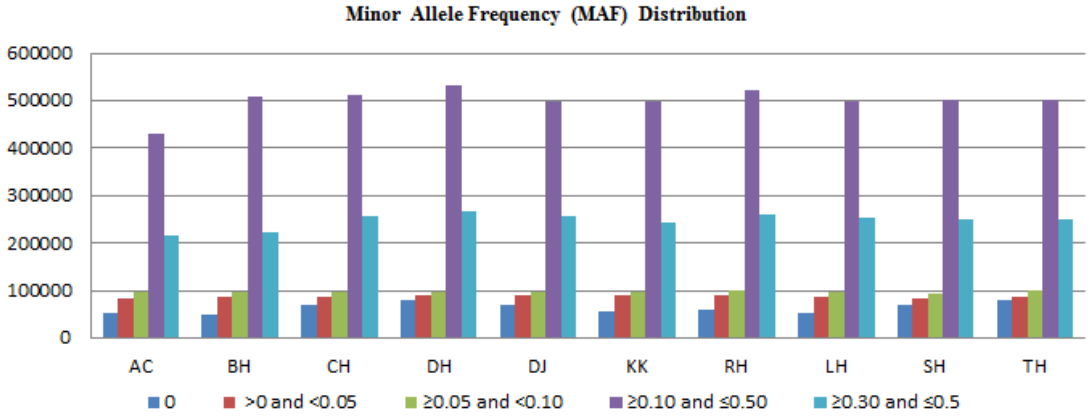

Figure 4. Minor Allele Frequency Distribution across all ten indigenous cattle Breeds in Pakistan

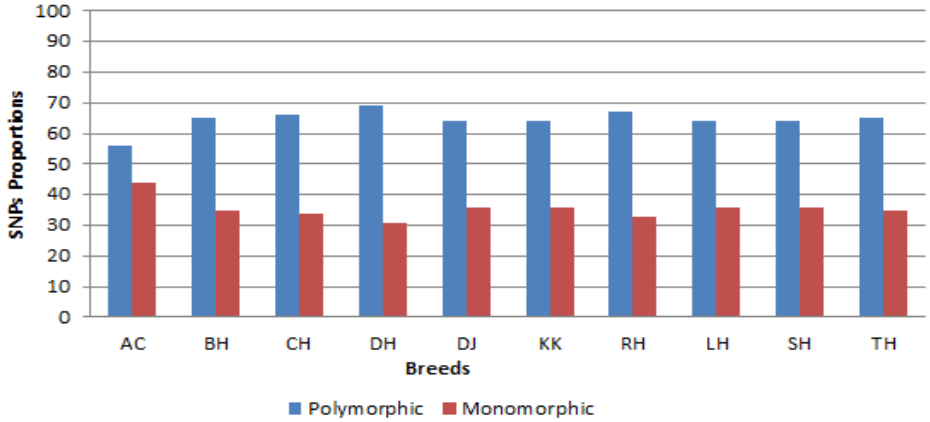

Figure 5. Polymorphic and Monomorphic SNPs distributions across all sampled breeds

Conclusion

The results of this preliminary study of Bovine high density SNPs revealed that the distribution of SNPs markers across the bovine genome of native zebu breeds in Pakistan was significantly different and identified some level of polymorphism and minor allele frequency (MAF) rate among these breeds. The levels of SNPs variation in this study encourage future use of Bovine High Density SNP assay with great extent for genetic studies in these breeds. These results could be effectively used to understand breed composition and within breed diversity, which could be an attractive opportunity to allow this important genetic resource improvements through effective population selection strategy.

SNPs analiza na nivou genoma autohtonih zebu rasa u Pakistanu

Hamid Mustafa, Kim Eiusoo, Huson J. Heather, Adeela Ajmal, David Riley, Talat Nasser Pasha, Afzal Ali, Khalid Javed, Tad S. Sonstegard

Rezime

Genetički resursi domaćih životinja (AnGR) u Pakistanu imaju jedinstven identitet širom sveta. Postoji petnaest različitih rasa goveda. Ispitali smo SNP varijacije i distribuciju među deset rasa goveda koristeći Bovine high density (777k) Bead čip. Ukupno 136 individualnih grla deset različitih rasa su genotipizirani i posle filtracije 500, 939 SNP markera je korišćeno za dalju analizu. Srednja niže frekvencija alela (MAF) je bila 0,23, 0,20, 0,22, 0,22, 0,20, 0,18, 0,20, 0,22, 0,21 i 0,18 za Achi, Bhagnari, Cholistani, Dhanni, Dajal, Kankraj, Lohani, Red Sindi, Sahival i Tharparkar goveda, respektivno. Značajna razlika ($p<0,001$) MAF je uočena u odabranoj populaciji. Zajednička varijanta niže frekvenciji alela ($\geq 0,10$ i $\leq 0,5$) je procenjena (64%). U uzorkovanoj populaciji, 64% SNP markera su polimorfni (MAF> 0,05) u okviru rasa i preostalih 36% su smatrani monomorfnim markerima. Prosečno registrovane (H_o) i očekivane (H_E) vrednosti heterozigotnosti od 0,662 i 0,640 su dobijene kod ovih rasa. Ovaj rezultat ukazuje na značajnu razliku između ovih rasa i ukazuje na to da SNP varijacije imaju potencijal koji bi mogao da se koristi u budućnosti za efikasnu selekciju i odgajivačke programe u poboljšanju stočarske proizvodnje i studijama konzervacije ovih rasa u zemlji.

Ključne reči: frekvencija minor alela, SNP, varijacija, distribucija, goveda

Acknowledgments

The authors would like to thanks USDA (BFGL) for laboratory facilities. HEC is greatly acknowledged for financial assistance for this study. All public Livestock Experiment Stations are also appreciated for providing blood samples.

References

CARRUTHERS C. R., PLANTE Y., SCHMUTZ S. M. (2011): Comparison of Angus cattle populations using gene variants and microsatellites. Can J. Anim. Sci., 91, 81-85.

CURIK I., FERENČAKOVIĆ M., SÖLKNER J. (2014): Inbreeding and runs of homozygosity: A possible solution to an old problem. Livestock Science, 26:24.

DADI H., KIM J. J., YOON D., KIM K. S. (2012): Evaluation of single nucleotide polymorphisms (SNPs) genotyped by the Illumina Bovine SNP50K in cattle focusing on Hanwoo breed. Asian-Australian Journal of Animal Science, 25, 28-32.

EUI-SOO K., TAD S. S., CURTIS P. V. T., GEORGE W., MAX F. R. (2015): The Relationship between Runs of Homozygosity and Inbreeding in Jersey Cattle under Selection. PLoS One. 10(7): e0129967.

MUSTAFA H., HUSON J. H., MATTHEW M., KIM E., AHMAD A., TAD S. S. (2012): Genome wide structure of cattle from high density SNP array on some worldwide breeds. BARC annual poster competition at USDA, ARS, Bovine Functional Genomics Laboratory, Beltsville, MD, USA. 7 April, 2012. 46.

MUSTAFA H., HUSON J. H., KIM E., NISAR A., AFZAL A., WAQAS A. K., TALAT N. P. MUHAMMAD Z. F., KHALID J., ADEELA A., TAD S. S. (2014): Comparative analysis of genome wide difference in Red Sindhi and Holstein cattle breeds using dense SNP marker. International Journal of Advanced Research, 2(4), 300-304.

HOWARD J. T., MALTECCA C., HAILE-MARIAM M., HAYES B. J., PRYCE J. E. (2015): Characterizing homozygosity across United States, New Zealand and Australian Jersey cow and bull populations. BMC Geno. 16:187 doi: 10.1186/s12864-015-1352-4.

DECKER J. E., MCKAY S. D., ROLF M. M., KIM J. W., ALCALÁ A. M., SONSTEGARD T. S., HANOTTE O., GÖTHERSTRÖM A., SEABURY C. M., PRAHARANI L., BABAR M. E., REGITANO L. C., YILDIZ M. A., HEATON P. M., LIU W. S., LEI C. Z., REECY J. M., SAIF-UR-REHMAN M., SCHNABEL

R. D., TAYLOR J. F. (2014): Worldwide patterns of ancestry, divergence, and admixture in domesticated cattle. PLoS Gene.10, e1004254.
KAZMI D., RASUL G. (2012). Agrometeorological wheat yield prediction in rainfed Potohar region of Pakistan. Agricultiral Sciences, 3, 170-177.
KIJAS J. W., DAVID T., BRIAN P. D., MICHAEL P. H., JILLIAN F. M., ANNETTE M., PETER W., ROXANN G. I., RUSSELL M., SEAN M., DAVE T., JOHN M., NOELLE C., V. H. O., FRANK W. N., HERMAN R. (2009): A genome wide survey of SNP variation reveals the genetic structure of sheep breeds. PLoS One 4:e4668.
KIM E. S., SONSTEGARD T. S., ROTHSCHILD M. F. (2015) Recent artificial selection in U.S. Jersey cattle impacts autozygosity levels of specific genomic regions. BMC Geno. 6:302 doi: 10.1186/s12864-015-1500-x.
LEROY G. (2014): Inbreeding depression in livestock species: review and meta-analysis. Animal Genetetics, 45, 618–28.
Groeneveld L. F., Lenstra J. A., Eding H., Toro M. A., Scherf B., Pilling D., Negrini R., Finlay E. K., Jianlin H., Groeneveld E., Weigend S. (2010): The GLOBALDIV Consortium 2010. Genetic diversity in farm animals – a review. Animal Genetics 41, 6–31.
LIN B. Z., SASAZAKI S., MANNEN H. (2010): Genetic diversity and structure in Bos taurus and Bos indicus populations analyzed by SNP markers. Animal Science Journal, 81, 281-289.
GAUTIER M., LALOE D., MOAZAMI-GOUDARZI K. (2010): Insights into the genetic history of French cattle from dense SNP data on 47 worldwide breeds. PLoS One 5, e13038.
MCKAY S. D., SCHNABEL R. D., MURDOCH B. M., MATUKUMALLI L. K., AERTS J., COPPIETERS W., DENNY C., EMMANUEL D. N., CLARE A. G., CHUAN G., HIDEYUKI M. Z., CURT P. V. T., JOHN L. W., JEREMY F. T., STEPHEN S. M. (2008): An assessment of population structure in eight breeds of cattle using a whole genome SNP panel. BMC Gene. 9:37.
MELKA H. D., JEON E. K., KIM S. W., HAN J. B., YOON D., KIM K. S. (2011): Identification of genomic differences between Hanwoo and Holstein breeds using the Illumina Bovine SNP50 BeadChip. Geno. Info. 9, 69-73.
MBOLE-KARIUKI M. N., Tad S. S., ORTH A., THUMBI S. M., BRONSVOORT B. D. C., KIARA H., TOYE P., CONRADIE I., JENNINGS A., COETZER K., WOOLHOUSE M., HANOTTE O., TAPIO M. (2014): Genome-wide analysis reveals the ancient and recent admixture history of East African Shorthorn Zebu from Western Kenya. Here.113 (4), 297–305.
PRYCE J. E., HAILE-MARIAM M., GODDARD M. E., HAYES B. J. (2014): Identification of genomic regions associated with inbreeding depression in Holstein and Jersey dairy cattle. Genetecs Selection Evolution, 46:71 doi: 10.1186/s12711-014-0071-7.

CHEN S., LIN B. Z., BAIG M., MITRA B., LOPES R. J., SANTOS A. M., MAGEE D. A., AZEVEDO M., TARROSO P., SASAZAKI S., OSTROWSKI S., MAHGOUB O., CHAUDHURI T. K., ZHANG Y., COSTA V., ROYO L. J., GOYACHE F., LUIKART G., BOIVIN N., FULLE D. Q., MANNEN H., BRADLEY D. G., BEJA-PEREIRA 2010. Zebu cattle are an exclusive legacy of the South Asia neolithic. Molecular Biology and Evolution, 27, 1–6.

HUSSAIN T., BABAR M. E., PETERS S. O., WAJID A., ALI A., AZAM A., AHMAD Z., MUHAMMAD W., AHMAD A., KADIR K., MARCOS D. D. IKHIDE G. I. (2016): Microsatellite Markers Based Genetic Evaluation of Pakistani Cattle Breeds. Pakistan Journal of Zoology, 48(6): 1633-164.

EDEA Z., DADI H., KIM S. W., PARK J. H., SHIN G. H., DESSIE T., KIM K. S. (2014): Linkage disequilibrium and genomic scan to detect selective loci in cattle populations adapted to different ecological conditions in Ethiopia. Journal of Animal Breeding and Genetics, 131 (5), 358–366.

BICKHART D. M., LINGYANG X., JANA L. H., JOHN B. C., DANIEL J. N., STEVEN G. S., JIUZHOU S., JOSE F. G., TAD S. S., CURTIS P. V. T., ROBERT D. S., JEREMY F. T., HARRISA L., GEORGE E. L. (2016): Diversity and population-genetic properties of copy number variations and multicopy genes in cattle. DNA Res. 1-10 (doi: 10.1093/dnares/dsw013).

EDEA Z., BHUIYAN M. S. A., DESSIE T., ROTHSCHILD M. F., DADI H., KIM K. S. (2015): Genome-wide genetic diversity, population structure and admixture analysis in African and Asian cattle breeds. Animal, 9(2):218-226.

UZZAMAN M. R., ZEWDU E., BHUIYAN M. S. A., JEREMY W., BHUIYAN A.K.F.H., KWAN –S. K. (2014): Genome-wide Single Nucleotide Polymorphism Analyses Reveal Genetic Diversity and Structure of Wild and Domestic Cattle in Bangladesh. Asian Australian Journal of Animal Science, 27 (10):1381-1386.

Received 7 November 2016; accepted for publication 22 January 2017