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Genome wide association study of udder type traits and milk production traits in Gir cattle

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Abstract
This study investigated the association of genome wide association study of udder type traits and milk production traits in Gir cattle. Data for the current study were collected from 635 Gir cows reared by farmers in small-holder conditions, and blood samples were collected for the Genomic study. The data for the phenotypic correlation study on 305 day milk yield and udder type traits were analysed using the statistical software R (v3.4.0), and the genome wide association study was carried out using the PLINK (v1.90b4.4) software. The phenotypic correlation between udder type traits, such as central ligament, rear udder height, rear udder width, teat length, and teat thickness, and 305 day milk yield was low to moderate, but significant (p 0.05) and positive. Some traits, however, were negatively correlated, including fore udder attachment, fore teat placement, rear teat placement, and udder depth. A genome-wide association study using a customised SNP chip (INDUSCHIP1) with 45,024 SNPs distributed on 29 autosomes discovered 838 SNPs that were significantly related to nine udder type traits and 305 day milk yield. The majority of SNPs were under MAF class of 0.3-0.5 from 45,024 SNPs spread over 29 *Bos Indicus* autosomes. Thus, present study on udder type traits and 305 day milk yield and their GWAS in Gir cattle using INDUSCHIP1 a customised SNP chip has provided the base line data for incorporation of udder type traits in Genomic selection for overall genetic improvement of Gir cattle.

Keywords: GWAS, Gir, udder type traits, SNPs, milk production traits

Introduction
Milk and milk products are obviously the most widely accepted form of animal proteins in India. India has the highest livestock populations in the world, contributing 16% of the world's cattle population with its 190.9 million cattle. Among the Indian breeds of cattle, the Gir is one of the finest dairy breeds. It's significant to breed future cows with characteristics that encourage high production or extend an animal's capacity for longer productive life. Numerous type traits have variable degrees of correlation with production traits.

About a decade ago, the field of genome wide association studies (GWAS) developed as a potent method for identifying genes linked to an organism's phenotypic traits (Andreas and Christensen, 2016) [1]. The primary focus of GWASs is on the relationships between single nucleotide polymorphisms (SNPs) and various traits. Milk production in dairy animals and type traits may be correlated. In Gir cattle, the relationship between type and milk yield has not been well investigated.

In order to investigate the Genome Wide Association utilising SNP Genotypes with type features in Gir cows, the present investigation was carried out with the objectives of evaluating the measure and variability of various udder type traits and correlating the udder type traits with milk production traits.

Materials and Methods
The present study was conducted in the coastal Gujarat districts of Bhavnagar, Gir-Somnath (both of which are on Saurashtra's eastern coast), and Bidaj (Sabarmati Ashram Gaushala). Data for present study were collected from the total 208 animals for udder type traits measurements and blood samples were collected from animals for Genomic study in the villages of Bhavnagar Dist. Similarly, 427 animals were recorded for udder type traits, and blood samples were collected for a genomic study in the villages of Gir-Somnath Dist.

Milk production performance data for 529 Gir cows were obtained from performance recording done under Gir Pedigree Selection programme under NDP-I program of NDDB,

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Anand implemented by Sabarmati Ashram Gaushala, Bidaj. Udder type traits measurement of 635 Gir cows around peak milk production was scored. Scoring on the selected day was done two hours before milking. Nine traits were included as udder type traits.

The traits included in udder type traits are categorized as (i) Fore Udder Attachment (ii) Central Ligament (iii) Front Teat Placement (iv) Rear Teat Placement (v) Rear Udder Height (vi) Rear Udder Width (vii) Teat Length (viii) Udder Depth (ix) Teat thickness. Total 635 animals which calved in between 1st June 2016 to 31st March 2017 were scored for the udder type traits with proper care and accuracy in assessment. The available DNA was used for genotyping by three day procedure comprising of ten steps by Illumina laboratory. All the measured traits were scored based on scale as summarized in Table 1.

Table 1: Summary of scoring pattern of udder type traits

Traits	Scale	Range		
Fore udder attachment	1-9	1-3 (weak)	4-6 (medium)	7-9 (strong)
Central ligament	1-9	1-3 (weak)	4-6 (medium)	7-9 (strong)
Fore teat placement	1-9	1-3 (wide)	4-6 (central)	7-9 (close)
Rear teat placement	1-9	1-3 (wide)	4-6 (central)	7-9 (close)
Rear udder height	Cm	<12 (high)	12-18 (medium)	>18 (low)
Rear udder width	Cm	<7 (narrow)	7-13.5 (medium)	>13.5 (wide)
Teat length	Cm	<7.33 (short)	7.33-13 (medium)	>13 (long)
Udder depth	Cm	<6 (deep)	6-27 (medium)	>27 (shallow)
Teat thickness	1-9	1-3 (thin)	4-6 (medium)	6-9 (thick)

Analysis of Data

The data on 305 day milk yield and udder type traits for phenotypic correlation study were analyzed by a statistical software R (v3.4.0) (R Core Team, 2017) [6]. The data on genotype and phenotype (udder type traits and milk production) were used for genome wide association study (GWAS). The genome wide association study was carried out with PLINK (v1.90b4.4) software (Purcell and Chang, 2017) [5].

Before carrying out genome wide association, all the phenotype data were corrected to remove fixed effects of lactation order and stage of lactation with least squares analysis. The association of these SNPs with udder type traits and milk production was carried out in PLINK with p value <0.0001. This is basic association test with chi square values and P values based on chi square test. For analysis of data, animals were grouped by their lactation order as well as stage of lactation. The classification of data is summarized in

following Tables 2 and 3.

Table 2: Number of animals grouped by lactation order

Lactation order.	No. Animals
1	166
2	161
3	174
≥4	134
Total	635

Table 3: Number of animals grouped by stage of lactation

Lactation stage	No. of animals
1 (0-90 days or early lactation)	229
2 (91-180 days or mid lactation)	280
3 (181 days and above or late lactation)	126
Total	635

Effect of lactation order and stage of lactation on udder type traits and 305 day milk yield

The udder type traits and 305 day milk yield might be affected by lactation order and stage of lactation. The mean of different lactation order and stage of lactation were compared with statistical software IBM SPSS statistics version 24.0 software package, 2016. The differences in estimated mean and SE value in the lactation order and stage of lactation were examined for significance with further statistical analysis by Duncan's New Multiple Range Test (DNMRT).

In genotyping data total 45,024 SNPs identified with INDUSCHIP 1 prepared by Illumina. From these SNPs, 16 variants were removed due to missing genotype data (e.g. genotyping rate ≥ 90%). After association analysis P values were plotted on manhattans plot by 'qqman' package v0.1.4 (Turner, 2017) [9] in software R (v3.4.0).

Results and Discussion

The major objective of the present study was to measure the udder type traits and study the correlation of udder type traits with milk production and their genome wide association of these traits in Gir cows recorded under the Pedigree Selection programme.

Effect of lactation order and stage of lactation on mean and SE value of udder type traits and 305 day milk yield

In present study, animals of different lactation order and in different stage of lactation were measured for udder type traits and 305 day milk yield. The estimated means for udder type traits and 305 day milk yield for four different lactation order and three different stage of lactation is presented in table 4 and 5.

Table 4: Lactation order wise mean with SE for udder type traits and 305 day milk yield

Traits	1st Lactation		2nd Lactation		3rd Lactation		4th Lactation		Total	
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ±SE	N	Mean ± SE
FUA	166	5.71±0.16	161	5.35±0.15	174	5.73±0.15	134	4.92±0.17	635	5.46±0.08
CL	166	4.87±0.12	161	5.00±0.12	174	4.75±0.12	134	4.54±0.13	635	4.80±0.06
FTPL	166	4.05±0.09	161	4.13±0.11	174	4.49±0.10	134	4.34±0.11	635	4.25±0.05
RTPL	166	5.71±0.10	161	5.80±0.11	174	6.10±0.11	134	6.41±0.11	635	5.99±0.05
RUH	166	13.14±0.23	161	13.79±0.23	174	14.00±0.22	134	14.41±0.28	635	13.81±0.12
RUW	166	7.17±0.14	161	7.05±0.11	174	6.97±0.09	134	6.97±0.12	635	7.04±0.06
TL	166	7.77±0.18	161	8.63±0.20	174	8.23±0.18	134	8.48±0.22	635	8.26±0.10
UD	166	13.86±0.29	161	13.45±0.29	174	13.01±0.27	134	12.17±0.30	635	13.17±0.14
TTH	166	4.03±0.13	161	4.24±0.13	174	4.40±0.12	134	4.76±0.15	635	4.34±0.07
MILK	135	2164.22±60.98	132	2203.72±70.54	145	1891.54±55.10	117	1947.58±59.79	529	2051.42±31.38

N= number of observation, SE = standard error

Table 5: Lactation stage wise mean with SE values for udder type traits and 305 day milk yield

Traits	Stage 1		Stage 2		Stage 3		Total	
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
FUA	229	5.26±0.13	280	5.41±0.12	126	5.90±0.20	635	5.46±0.08
CL	229	5.01±0.10	280	4.67±0.08	126	4.73±0.15	635	4.80±0.06
FTPL	229	4.22±0.09	280	4.30±0.07	126	4.21±0.11	635	4.25±0.05
RTPL	229	5.77±0.09	280	6.08±0.08	126	6.19±0.14	635	5.99±0.05
RUH	229	13.48±0.19	280	13.71±0.17	126	14.63±0.30	635	13.81±0.12
RUW	229	7.34±0.11	280	6.89±0.08	126	6.81±0.11	635	7.04±0.06
TL	229	8.16±0.16	280	8.31±0.15	126	8.35±0.24	635	8.26±0.09
UD	229	13.30±0.23	280	13.16±0.21	126	12.93±0.35	635	13.16±0.14
TTH	229	4.42±0.12	280	4.43±0.09	126	3.99±0.0.15	635	4.34±0.06
MILK	229	1302.66±73.28	280	1901.33±55.05	126	2020.00±66.23	635	1708.98±40.08

N= number of observation, SE = standard error

In present study, the effect of lactation order and stage of lactation on udder type traits are found to be non-significant ($p > 0.05$). The lactation order and stage of lactation has significant ($p < 0.05$) effect on 305 day milk yield. The probable reason for non-significant differences between the lactation order and stage of lactation for these udder type traits might be due to the fact that, the means compared are from un-paired samples (animals).

The ANOVA tables for effect of lactation order and stage of lactation on udder type traits and 305 day milk yield are presented in Table 6 and 7.

Phenotypic correlation of udder type traits and 305 day milk yield & among nine udder type traits
Phenotypic correlation between nine udder type traits measured on 635 cows and correlation between each trait with milk production from 529 cows is presented in Table 8. In Table 8, phenotypic correlations for 305 day milk yield with udder type traits is summarized. Central ligament, rear udder width, teat length and teat thickness had significant and positive phenotypic correlation with 305 day milk yield, while fore udder attachment, fore and rear teat placement and udder depth had significant and negative phenotypic correlation with 305 day milk yield in this study.

Positive phenotypic correlation was reported between milk yield and udder height in Hariana cattle (0.68) by Raheja and Dalal (2007) [7], in Sahiwal cattle (0.61) by Dahiya and Rathi (1997) [2], in Guernsey cattle (0.30) by Harris and Freeman (1992) [3], in Holstein cattle (0.10) by Short and Lawlor (1992) [8] and in Australian Holstein and Jersey cattle (0.13 and 0.14 respectively). Contrary to present study, Mrode and Swanson (1994) [4] reported negative phenotypic correlation between udder height and milk yield (-0.04) in Holstein Friesian cattle.

Genome Wide Association Study (GWAS)
The 45,024 SNPs included in INDUSCHIP 1 were distributed over all 29 *Bos indicus* autosomes. For genome wide association study, each udder type trait was corrected to remove fixed effect of lactation stage and lactation order of individual cows with least squares mean as described hereafter. Before carrying out genome wide association, the phenotype scores of all type traits were corrected with least squares constants.

A total of 838 genome wise significant SNPs associated with nine udder type traits and 305 day milk yield ($p < 0.0001$) were detected with PLINK software. The identified significant SNPs associated with nine udder type traits and 305 day milk yield are described hereunder and summarized in Table 9.

Table 6: ANOVA Table for effect of lactation order on Udder type traits and 305 day milk yield

Source of variation			Sum of Squares	Df	Mean Square	F	p
FUA * Lactation	Between Groups	(Combined)	64.515	3	21.505	5.328	.001
	Within Groups		2547.044	631	4.037		
	Total		2611.559	634			
CL * Lactation	Between Groups	(Combined)	16.445	3	5.482	2.328	.073
	Within Groups		1485.948	631	2.355		
	Total		1502.394	634			
FTPL * Lactation	Between Groups	(Combined)	20.427	3	6.809	3.981	.008
	Within Groups		1079.258	631	1.710		
	Total		1099.685	634			
RTPL * Lactation	Between Groups	(Combined)	44.599	3	14.866	8.043	.000
	Within Groups		1166.324	631	1.848		
	Total		1210.923	634			
RUH * Lactation	Between Groups	(Combined)	129.378	3	43.126	4.725	.003
	Within Groups		5759.853	631	9.128		
	Total		5889.231	634			
RUW * Lactation	Between Groups	(Combined)	4.234	3	1.411	.640	.589
	Within Groups		1391.167	631	2.205		
	Total		1395.401	634			
TL *Lactation	Between Groups	(Combined)	68.010	3	22.670	3.747	.011
	Within Groups		3817.662	631	6.050		
	Total		3885.672	634			
UD * Lactation	Between Groups	(Combined)	230.280	3	76.760	5.929	.001

			Sum of Squares	Df	Mean Square	F	p
	Within Groups		8169.004	631	12.946		
	Total		8399.284	634			
TTH * Lactation	Between Groups	(Combined)	41.806	3	13.935	4.989	.002
	Within Groups		1762.398	631	2.793		
	Total		1804.205	634			
MILK * Lactation	Between Groups	(Combined)	5046222.462	3	1682074.154	1.654	.176
	Within Groups		641549973.500	631	1016719.451		
	Total		646596195.900	634			

Table 7: ANOVA Table for effect of stage of lactation on udder type traits and 305 day milk yield

			Sum of Squares	Df	Mean Square	F	p
FUA * Lac stage	Between Groups	(Combined)	34.480	2	17.240	4.228	.015
	Within Groups		2577.079	632	4.078		
	Total		2611.559	634			
CL * Lac stage	Between Groups	(Combined)	15.475	2	7.738	3.289	.038
	Within Groups		1486.919	632	2.353		
	Total		1502.394	634			
FTPL * Lac stage	Between Groups	(Combined)	1.167	2	.584	.336	.715
	Within Groups		1098.518	632	1.738		
	Total		1099.685	634			
RTPL * Lac stage	Between Groups	(Combined)	18.489	2	9.245	4.900	.008
	Within Groups		1192.434	632	1.887		
	Total		1210.923	634			
RUH * Lac stage	Between Groups	(Combined)	113.652	2	56.826	6.218	.002
	Within Groups		5775.579	632	9.139		
	Total		5889.231	634			
RUW * Lac stage	Between Groups	(Combined)	33.274	2	16.637	7.719	.000
	Within Groups		1362.126	632	2.155		
	Total		1395.401	634			
TL * Lac stage	Between Groups	(Combined)	3.734	2	1.867	.304	.738
	Within Groups		3881.938	632	6.142		
	Total		3885.672	634			
UD * Lac stage	Between Groups	(Combined)	11.352	2	5.676	.428	.652
	Within Groups		8387.932	632	13.272		
	Total		8399.284	634			
TTH * Lac stage	Between Groups	(Combined)	18.886	2	9.443	3.343	.036
	Within Groups		1785.319	632	2.825		
	Total		1804.205	634			
MILK * Lac stage	Between Groups	(Combined)	60355644.430	2	30177822.210	32.533	.000
	Within Groups		586240551.500	632	927595.809		
	Total		646596195.900	634			

Table 8: Phenotypic correlation between udder type traits and 305 day milk yield in Gir cattle

Traits	FUA	CL	FTPL	RTPL	RUH	RUW	TL	UD	TTH	MILK
FUA		-0.13***	-0.09*	-0.02	-0.14***	-0.11**	-0.33***	0.47***	-0.24***	-0.34***
CL			-0.04	-0.12*	0.05	0.10**	0.16***	-0.13***	-0.03	0.20***
FTPL				0.43***	0.02	-0.07	0.08*	-0.05	0.18***	-0.12**
RTPL					-0.05	-0.10**	-0.01	-0.01	0.15***	-0.14**
RUH						0.07	0.13**	-0.20***	-0.12**	0.08
RUW							0.21***	-0.20***	0.31***	0.35***
TL								-0.27***	0.50***	0.46***
UD									-0.27***	-0.33***
TTH										0.30***
MILK										

*** $p<0.001$, ** $p<0.01$, * $p<0.05$, $p>0.05$(non-significant)

Table 9: Chromosome wise no. of Significant SNPs per trait

Chr. No.	FUA	CL	FTPL	RTPL	RUH	RUW	TL	UD	TTH	MILK	Total
1	2	3	4	5	6	7	8	9	10	11	12
1	0	0	0	0	0	1	8	0	2	19	30
2	1	0	0	0	0	1	3	0	6	19	30
3	2	0	0	0	1	1	6	0	1	18	29
4	0	0	0	2	2	0	3	0	2	15	24
5	1	2	0	0	0	2	31	1	17	59	113
6	1	0	2	0	0	2	10	5	6	25	51
7	0	0	0	1	0	1	9	0	3	17	31
8	1	0	0	0	0	0	3	0	2	12	18
9	0	0	0	0	0	8	14	0	1	28	51
10	1	0	0	0	0	2	3	0	2	15	23
11	1	1	0	0	0	1	7	0	4	17	31
12	0	0	0	1	0	0	5	0	3	13	22
13	0	0	0	0	0	1	6	0	3	15	25
14	0	0	0	0	0	4	10	2	5	27	48
15	0	0	0	1	1	1	1	1	1	9	15
16	1	0	0	0	0	0	6	0	4	23	34
17	0	0	0	0	0	4	7	1	5	21	38
18	0	0	0	1	0	0	0	0	2	11	14
19	0	0	0	1	0	2	3	2	6	14	28
20	1	0	0	0	0	2	0	0	1	20	24
21	0	0	1	0	1	2	0	0	4	24	32
22	0	0	0	0	0	1	9	0	0	12	22
23	0	1	0	0	0	0	5	0	3	14	23
24	0	0	0	0	0	1	4	2	0	8	15
25	0	0	0	0	1	0	5	0	2	11	19
26	0	0	0	0	0	0	2	0	1	13	16
27	0	1	0	0	1	1	2	0	2	5	12
28	0	0	0	0	0	0	3	0	0	11	14
29	1	1	0	0	0	0	1	0	1	2	6
Total	11	6	3	7	7	38	166	14	89	497	838

This study has provided the base line information about the udder type traits and 305 day milk yield in Gir cows in their home tract. The study has also revealed a large no. of significant SNPs associated with udder type traits and 305 day milk yield.

The GWAS of these important traits with the SNP chip array i.e. INDUSCHIP 1 can be the footstep to embark upon the option of Genomic Selection in Gir cattle. The Genomic Selection thus incorporated into the Cattle Breeding Programme would ensure rapid genetic progress of Gir, an important indigenous dairy cattle of India.

Conclusions
The present study on udder type traits and 305 day milk yield, as well as their GWAS in Gir cattle using INDUSCHIP1, a customised SNP chip, has provided the fundamental information for implementing udder type traits in Genomic selection for overall genetic improvement of Gir cattle. The phenotypic correlation between udder type traits, such as central ligament, rear udder height, rear udder width, teat length, and teat thickness, and 305 day milk yield was low to moderate, but significant ($p0.05$) and positive. A genome-wide association study using a customised SNP chip (INDUSCHIP1) with 45,024 SNPs distributed on 29 autosomes discovered 838 SNPs that were significantly related to nine udder type traits and 305 day milk yield. The Genomic Selection approach would improve the accuracy of selection of breed able Gir animals, resulting in rapid genetic progress in India's important dairy cattle.

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