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Influence of *WDR41* and *ANKRD31* gene polymorphism on udder and teat type traits and mastitis in Karan Fries cows

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ABSTRACT

In the present study, the effect of genetic polymorphism of WD-repeat containing protein 41 (*WDR41*) and Ankyrin repeat domain containing protein 31 (*ANKRD31*) gene on 17 traits related to udder and teat type and mastitis in 123 Karan Fries cows was studied. Restriction Fragment Length Polymorphism (RFLP) was used to identify the SNP (410 bp and 475 bp) in PCR amplified product of intron 4 and exon 10 in WDR41 gene. Both of them were polymorphic with Guanine to Adenine transition, and three genotypes namely AA, AG and GG were observed. In *ANKRD31* gene, RFLP was used to identify the SNP in 513 bp PCR amplified product of intron 24 and two SNPs were found. We report for the first time that intron 4 and exon 10 of WDR41 gene is significantly associated with udder depth (UD), udder balance (UB), central ligament (CL), teat circumference (TC), SFF and tear diameter (TD), rear udder width (RUW), and mastitis. SNP rs110012582 in intron 24 of ANKRD31 gene is associated significantly with UD, distance between left and right teat (DLR), SFF, and SNP rs473512406 with udder length (UL), udder width (UW), udder circumference (UC), fore teat length (FTL), rear teat length (RTL), distance between fore and rear teat (DFR), DLR and mastitis. This information can augment future studies to determine the role of *WDR41* and *ANKRD31* genes as a candidate gene marker with desired udder and teat conformation and mastitis. Thus, it is essential to work for better udder health and prevent the incidence of mastitis in the herd.

Keywords: Cows, Karan Fries, Mastitis, Udder, Teat, Trait

Molecular function of *WDR41* gene is Rab guanyl-nucleotide exchange factor activity and regulation of autophagy (Ensembl.org). This gene interacts with the C9orf72/SMCR8 heterodimer and is tightly associated with the Golgi complex. C9orf72/SMCR8/WDR41 associates with the FIP200/Ulk1 complex, which is essential for autophagy initiation (Sullivan *et al*. 2016). Non-catalytic component of the C9orf72-SMCR8 complex, a complex that has guanine nucleotide exchange factor (GEF) activity and regulates autophagy (statement unclear). The C9orf72-SMCR8 complex promotes the exchange of GDP to GTP, converting inactive GDP-bound RAB8A and RAB39B into their active GTP-bound form, thereby promoting autophagosome maturation. *ANKRD31* gene is modified by both biotinlylation of lysines and sulfoxidation of methionines. Mastitis is one of the most devastating diseases of dairy animals hampering the growth of the dairy sector (Karahan *et al*. 2011, Khan *et al*. 2013) Udder conformation traits are moderately heritable (Jaayid *et al*. 2011, Bradford *et al*. 2015, Khan and Khan 2016). Udder traits are affected by BTAX, BTA2, BTA10, BTA11, BTA20, BTA16, BTA13, BTA22 and BTA25; teat traits are affected by BTA6, BTA7, BTA9, BTA16, BTA11, BTA26 and BTA17 (Cole *et al.* 2011). Heritability for clinical mastitis is very low (Negussie *et al*. 2008). Data on nucleotide sequence polymorphism in the bovine for *WDR41* and *ANKRD31* genes are limited. In India, NDDB started recording of udder and teat traits in INAPH project. Realizing the importance of these functional traits and as a research priority, we designed this study for future analysis by taking large sample size. By giving weightage to these traits, we may probably increase the disease resistance. The present study aimed to explore the genetic polymorphism in *WDR41* gene and *ANKRD31* gene in Karan Fries cows and to find out the association of genetic variants with udder and teat type traits and mastitis.

MATERIALS AND METHODS

Experimental animals and data recording: The present study was conducted on 123 Karan Fries by generating udder and teat type traits data within period of research work (2 years) from Livestock Research Centre (LRC) of

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ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana. The traits studied included udder balance (UB), udder depth (UD), udder length (UL), udder width (UW), udder circumference (UC), central ligament (CL), teat circumference (TC), fore teat length (FTL), rear teat length (RTL), distance between fore and rear teat (DFR), distance between left and right teat (DLR), shortest distance of floor from fore teat (SDF), shortest distance of floor from rear teat (SDR) and teat diameter (TD) and mastitis. Blood samples (123) (approximately 8–10 ml) were collected from lactating Karan Fries cows. Incidence of mastitis among the lactating Karan Fries cows was recorded by considering the animals which were affected by mastitis at least once in their life time, from Mastitis Register for a period of 20 years (2000–2019) recorded with date of onset of symptoms, treatment history, date of cure/recovery maintained in the Animal Health Complex of ICAR-National Dairy Research Institute, Karnal.

Geographical and climatic description: Karnal, a city of Haryana State in North of India, is situated between 29.68°N latitude and 76.98°E longitude with altitude ranging from 235 to 252 meters above mean sea level. The climate of Karnal is sub-tropical with temperature in summer months, i.e. April to June, ranging between 24°C–46°C. Karnal experiences moderate rainfall in the months of July and lasts till September with annual rainfall of 744 mm. The winter months (December to March) are extremely cold with the temperature ranging from 4°C to 32°C.

DNA isolation and primers: Genomic DNA was extracted from aseptically collected venous blood using the standard phenol chloroform extraction method (Sambrook and Russel 2001). The quality check of isolated DNA was done using 0.8% agarose gel electrophoresis and quantification was done by using Nanodrop. Forward primer (PF 5′-CATGTATGTGCCAGGCATTG-3′) and reverse primer (PR 5′-ACACCTTTGGGCTTACTCCA-3′) and Forward primer (PF 5′-GCAGTTGTGTCCTTC-GACCT-3′) and reverse primer (PR 5′-ACAGCCATA-GGGTAGTCTCCA-3′) respectively, both having TM of 61.1°C were used to amplify intron 4 and exon 10 region of *WDR41* gene. Forward and reverse primers (PF 5′-ACTGGAGTGGGTTGCCATAG-3′ and PR 5′-CCTTA-GAGCCAGAGCA CCAC-3′) with TM of 63.9°C and 63.9°C respectively, were designed using Primer 3 plus software to amplify intron 24 region of *ANKRD31* gene from published NCBI (National Center of Bioinformatics, USA) sequences. The optimization of PCR was done to get the best possible amplification of the product, at an annealing temperature of 64°C for 1 min. The genotyping was carried out using PCR-RFLP (Polymerase Chain Reaction–Restriction Fragment Length Polymorphism) technique with *Hinf1* and *Hha1* restriction enzyme at 37°C for 6 to 8 h. The PCR products of different genotype were sequenced using the 1st base sequencing INT (Malaysia) for purification and custom sequencing from both ends (5′ and 3′ ends).

PCR amplification and genotyping conditions: Amplification of the intron 4 and exon 10 region of *WDR41* gene was optimized to get the best possible amplification of the 410 and 475 bp product. Amplification of the intron 24 region of *ANKRD31* gene was optimized to get the best possible amplification of the 513 bp product. Each PCR reaction cocktail was prepared using PCR Master Mix (2×) (Fermentas) and PCR was carried out in 25 μl reaction volume consisting of 0.5 μl forward primer, 0.5 μl reverse primer, 13 μl PCR Master Mix (2×), 9 μl Milli Q Water and 2.0 μl Template DNA.

Amplification was performed using using Thermal cycler (BioRad T100) with an initial denaturation at 95°C for 4 min followed by 30 cycles of 94°C for 60 sec, annealing temperature 61°C for 60 sec and 72°C for 60 sec, with a final extension for 10 min at 72°C. All the Karan Fries cows were screened for the presence of *WDR41* gene polymorphism using PCR-RFLP technique. Genotyping for *WDR41* gene was carried out using *Stu1 and Hha1* restriction enzyme at 37°C for 6 to 8 h. Genotyping was carried out using *Hinf1 and Hha1* restriction enzyme at 37°C for 6 to 8 h for *ANKRD31* gene. Genotyping was evaluated by running a small aliquot of PCR-RFLP product on 2.5% agarose gel. Genotype and allele frequencies were calculated by gene computing method (Falconer and Mackay 1996).

Data and statistical analysis: Data on udder and teat type traits were genertated and adjusted for non-genetic factors, viz. season of calving, stage of lactation and parity. Least-squares analysis of fitting constants (Harvey 1990) was used to overcome the non-orthogonality of effects due to unequal and disproportionate sub-class frequencies, model considered was as follows:

$$Y_{ijkl} = \mu + L_i + P_j + S_k + e_{ijkl}$$

where Y_{ijkl}, lth observation of cow in ith stage of lactation, jth parity and kth season of calving; μ, overall mean of the traits; L_i, fixed effect of ith stage of lactation (0–90 days), (90–180 days), (180–270 days) and (>270 days); P_j, fixed effect of jth parity (1–5 or >5); S_k, Effect of kth season of calving (Summer (April to June), Rainy (July to August), Autumn (September to November), Winter (December to March), and e_{ijkl}, random error which is normally and independently distributed with zero mean and unit variance [NID (0, σ^2_e)]. The effects of non-genetic factors, viz. season, stage and parity on udder and teat type traits were adjusted through least squares analysis using significant effects as shown by analysis of variance.

To study the effect of WDR41 and ANKRD31 genotypes on adjusted udder and teat type traits, fixed linear model with SNP genotypes as fixed effects was used. The model considered was

$$Y_{ij} = \mu + G_i + e_{ij}$$

where Y_{ij}, adjusted value of udder and teat type traits of jth animal of ith genotype; μ, overall mean; G_i, effect of ith genotypes; e_{ij}, residual error NID (0, σ^2_e). The differences

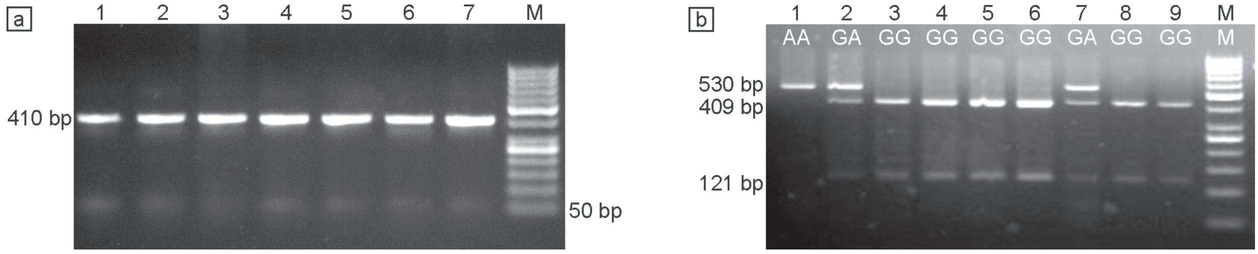

Fig. 1. **a**. PCR product of primer 1 of *WDR41* gene in Karan Fries cows. Lane 1–7: PCR product (410 bp) of Karan Fries cow samples; Lane M, 50 bp DNA ladder. **b**. PCR-RFLP of SNP locus G8598212A (rs133276561) in *WDR41* gene in Karan Fries cows using *StuI* restriction enzyme. Lane 3, 4, 5, 6, 8, 9 : GG genotype 409, 121 bp; Lane 2, 7: GA genotype 530, 409, 121, bp; Lane 1: AA genotype 530, 121 bp; Lane M: 50 bp DNA ladder.

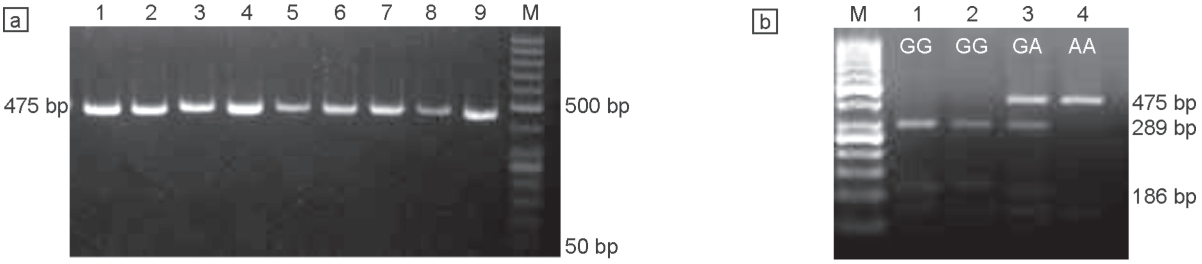

Fig. 2. **a**. PCR product of primer 5 of *WDR41* gene in Karan Fries cows. Lane 1–9: PCR product (475 bp) of Sahiwal cow samples; Lane M: 50 bp DNA ladder. **b**. PCR-RFLP of SNP locus G8600884A (rs211664388) in *WDR41* gene in Karan Fries cows using *HhaI* restriction enzyme. Lane 1, 2: GG genotype 289, 186 bp; Lane 3: GA genotype 475, 289, 186 bp; Lane 4: AA genotype 475, 186 bp; Lane M: 50 bp DNA ladder.

of least square means of udder and teat type traits between subclasses of WDR41 genotype were tested for significance using Duncan's Multiple Range Test (DMRT) as modified by Kramer (1957).

Association of genotypes with incidence of clinical mastitis: Association of genotypes with incidence of mastitis in animals was calculated using χ^2 test (Snedecor and Cochran 1994) and logistic regression.

Table 1. Udder and teat type traits (measurements) in Karan Fries cattle

Trait name	Mean±SE	CV (%)
Fore udder attachment (degree)	123.00±1.27	11.47
Rear udder width (cm)	9.13±0.23	28.47
Rear udder height (cm)	25.70±0.35	15.4
Udder balance (cm)	–0.63±0.46	–8.122
Udder depth (cm)	36.56±0.79	24.09
Udder length (cm)	59.26±0.88	16.55
Udder width (cm)	68.64±1.02	16.5
Udder circumference (cm)	128.33±1.72	14.9
Central ligament (cm)	4.30±0.16	42.7
Teat circumference (cm)	7.44±0.09	13.7
Rear teat length(cm)	4.70±0.11	27.2
Fore teat length (cm)	5.29±0.12	26.46
DFR (cm)	7.78±0.25	35.60
DLR (cm)	7.17±0.31	48.9
SDF (cm)	49.5±0.67	15.17
SDR (cm)	49.66±0.70	15.6
Teat diameter (cm)	1.47±0.04	33.3

RESULTS AND DISCUSSION

A 410 and 475 bp fragment of intron 4 and exon 10 of WDR41 gene was successfully amplified in Karan Fries cows (Figs 1a, 2a). RFLP test using Stu*1 and Hha 1* restriction enzyme indicated that intron 4 and exon 10 region of WDR41 gene was highly polymorphic in Karan Fries cows, with the presence of three genotypes namely, AA, AG and GG as shown in Figs 1 and 2. Homozygous GG was the most frequent with highest genotype frequency of 0.64 and 0.82, and AA allele was found to be rare with least frequency of 0.04 and 0.03 in Karan Fries cows. Least squares technique was used to adjust the non-orthogonality of the udder and teat type traits data. Means, standard errors and coefficients of variation (CV) unadjusted data of udder and teat type traits of Karan Fries cows is presented in Table 1. The coefficient of variation varied from –8.12% (UB) to 48.9% (DFR); however, majority of the udder and teat type traits showed moderate to high CV which could be due to the selection for these traits not operating in the herd. Genotypes of intron 4 and exon 10 of WDR41 gene significantly influenced UD, UB, CL, TC and SDF, and RUW and teat diameter respectively ($p<0.05$) and others traits were unaffected by effect of genotype in studied population of Karan Fries cows. Gonzalez *et al.* (2017) also performed genome-wide association studies of body conformation traits in Mexican Holstein cattle and found association of SNP rs109191831 of *WDR41* gene with udder depth trait. There were no earlier reports available to compare or contrast the present findings with regard to association of genetic variants of *WDR41* gene with clinical mastitis in cattle. Su *et al.* (2020) reported that *WDR41* is a beta-propeller protein that binds to SMCR8. C9ORF72 and

SMCR8 both contain longin and DENN (differentially expressed in normal and neoplastic cells) domains. Contacts between *WDR41* and the DENN domain of SMCR8 drive the lysosomal localization of the complex in conditions of amino acid starvation. *WDR41* gene supports lysosomal response to changes in amino acid availability (Amick *et al*. 2018) and regulation of autophagy. Autophagy removes aggregates of misfolded proteins and/or defective organelles, provides energy, and recycles cellular components and is essential for cellular homeostasis (Mizushima and Komatsu 2011), *Streptococcus* spp. and *Staphylococcus spp*. are most common etiological agent of mastitis in cattle and buffaloes, *S. aureus* by inhibiting autophagy flux and damaging lysosomal function after invading bovine mammary epithelial cells (BMECs) could escape autophagic degradation (Geng *et al*. 2020). *Escherichia coli* invade primary bovine mammary cells *in vitro* and the process did not involve in the rearrangement of the actin cytoskeleton. Intracellular bacteria were observed within membrane-bound compartments that labelled with the early endosomal marker phosphatidylinositol 3-phosphate [PtdIns(3)P] and also within late endosome-like compartments labelled with the small GTPase Rab7, indicating an endocytic mechanism of bacterial internalization. Endocytic pathway serve as survival niche for the internalized bacteria, allowing them to evade host immune responses and establish an infection reservoir that could later re-emerge as a recurrent clinical mastitis episode (Passey *et al*. 2008).

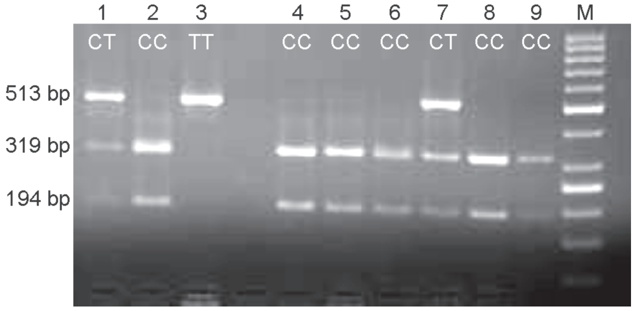

Fig. 4. PCR-RFLP of SNP locus C6476252T (rs110012582) in *ANKRD31* gene in Karan Fries cows using *HinfI* restriction enzyme. Lane 2, 4, 5, 6, 8, 9: CC genotype 319, 194 bp; Lane 1, 7: CT genotype 513, 319 and 194 bp; Lane 3: TT genotype 513 bp; Lane M: 50 bp DNA ladder.

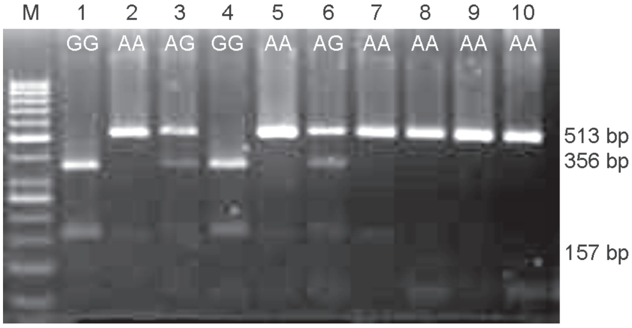

Fig. 5. PCR-RFLP of SNP locus A6547390G (rs473512406) in *ANKRD31* gene in Karan Fries cows using *HhaI* restriction enzyme. Lane 1, 4: CG genotype 356, 157 bp; Lane 3, 6: AG genotype 513, 356 and 157 bp; Lane 2, 5, 7, 8, 9, 10: AA genotype 513 bp; Lane M: 50 bp DNA ladder.

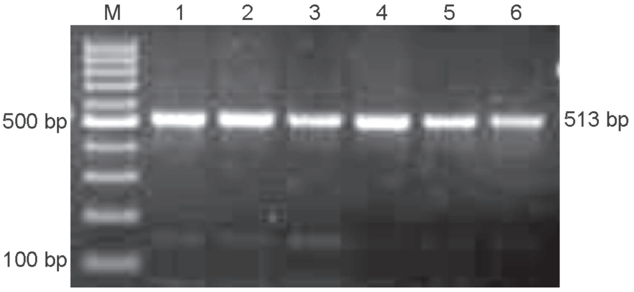

Fig. 3. Resolution of PCR product of primer 1 of *ANKRD31* gene in Karan Fries cows. Lane 1–6: PCR product (513 bp) of Karan Fries cow samples. Lane M: 100 bp DNA ladder.

A 513 bp fragment of intron 24 of ANKRD31 gene was successfully amplified in Karan Fries cows (Fig. 3). The PCR product of 513 bp in intron 24 of *ANKRD31* gene revealed SNP (rs110012582) at locus C6476252T in KF cows, which was digested with *Hinf I* enzyme and resulted in three genotypes in Karan Fries cows namely CC (319 and 194 bp), CT (513, 319 and 194 bp) and TT (513) (Fig. 4). RFLP test using *Hha1* restriction enzyme indicated that intron 24 region of *ANKRD31* gene is highly polymorphic in Karan Fries cows, with the presence of three genotypes namely, GG (356 and 157 bp), AG (513, 356 and 157 bp) and AA (513) as shown in Fig. 5. Genotypes of SNP rs110012582 of intron 24 of *ANKRD31* gene significantly influenced UD, DLR and SFF at P≤0.05 and others traits are unaffected by effect of genotype in studied population of Karan Fries cows. In KF cattle, SNP rs110012582 genotypes were found to be significantly (P≤0.05) associated with RUW, UD, DLR and SFF. Gonzalez *et al.* (2017) reported this SNP (rs110012582) of *ANKRD31* gene to be associated with udder depth in Mexican Holstein cattle by doing genome-wide association analysis of body conformation traits using a mix of sampled and imputed SNP genotypes. Genotypes of SNP rs473512406 significantly (P≤0.05) influenced UL, UW, UC, FTL, RTL, DFR, DLR and mastitis in Karan Fries cows. Lysine biotinylation and methionine oxidation in the heat shock protein HSP60 synergize in the elimination of reactive oxygen species. Biotinylated proteins play important role in defense against reactive oxygen species and thereby decreasing oxidative stress (Li *et al*. 2014). Biotin helps in improving milk production through either an increased ruminal fibre digestion or hepatic gluconeogenesis in dairy cows (Weiss and Zimmerly 2000). Supplementation of biotin had an influence on hepatic lipidosis (fatty liver) in dairy cows around calving (Rosendo *et al*. 2004). There are no earlier reports available to compare or contrast the present findings with regards to association of SNP 473512406 of *ANKRD31* gene with udder and teat type, milk production and mastitis in Karan Fries cows. Several workers reported the associations of genes with udder conformation and mastitis (Wu *et al*. 2015, Pausch *et al*. 2016, Kumari *et al.* 2019) but did not find any on BTA 10. Vallée *et al*. (2016) opined associations of SNP at 70.3 and 98.7 Mb on BTA 5 were detected in Charolais cattle for teat length. Jardim *et al.* (2017) opined that 10 and 8 chromosomes harbouring markers that were significantly associated with udder index and milking speed, respectively in Nordic Holstein cattle. Kurz *et al*. (2019) reported SNP associations with mastitis resistance and teat length of Holstein dairy cattle in a GWAS by using a high density SNP array.

Cows with SNP rs211664388 of *WDR41* gene and SNP rs110012582 of *ANKRD31* gene was found to be significantly (P≤0.05) associated with incidence of clinical mastitis in Karan Fries cattle (Tables 2 and 3). This is the first report of SNP rs110012582 of *ANKRD31* gene and its association with udder and teat type traits and mastitis. In the present study, non-significant (P≤0.05) effect of seasons and parity on mastitis was found as depicted in Tables 2 and 3. On the contrary, prevalence of udder quarters affected with clinical mastitis tended to increase with the increase in parity (Breen *et al*. 2009, Heikkila *et al.* 2012, Nakov and Trajcev 2012, Boujenane *et al*. 2015, Kumari *et al*. 2019). Abebe *et al*. (2016) reported that cows with four or more parities had pendulous udder and prone for mastitis. Nakov *et al*. (2014) found in a logistic regression study that the relative risk of clinical mastitis was lower for primiparous cows which increased with further parity. Increasing parity number was also one of the predictors that was found to associate with the presence of mastitis (Katsande *et al.* 2013, Abrahmsen *et al*. 2014, Mureithi *et al*. 2016). They reported that likelihood of mastitis as 24.8 times higher in multiparous cows having four or more calvings compared with primiparous cows. Similar findings were reported by Kumari *et al.* (2019) that season of calving did not show any significant effect on mastitis in Karan Fries cattle. Rainy season had 2.99 times greater odds of clinical mastitis event as those with winter in KF cows. Sinha *et al.* (2019) also reported higher incidence of clinical mastitis in rainy season in Sahiwal and Karan Fries cows.

Table 2. Association of identified genetic variants of *WDR41* gene with incidence of clinical mastitis in Karan Fries cows by logistic regression model

Variable	Clinical Mastitis		P value
	Odds ratio	95% confidence interval	
Season			
Winter	Reference level		
Summer	0.62	0.13–2.81	0.54
Rainy	1.99	0.33–11.95	0.44
Autumn	2.09	0.56–7.73	0.26
Stage of lactation			
4	Reference Level		
1	0.96	0.23–3.99	0.95
2	0.70	0.18–2.73	0.61
3	0.15	0.03–0.66	0.012
Parity			
5	Reference level		
1	2.47	0.48–12.73	0.27
2	4.30	0.82–22.30	0.08
3	1.39	0.20–9.61	0.73
4	2.86	0.46–17.73	0.25
WDR41 (*rs1333276561*)			
GG	Reference level		
AA	2.07	0.28–15.14	0.47
GA	0.95	0.34–2.60	0.92
WDR41 (*rs211664388*)			
GG	Reference level		
AA	30.57	1.82–511.1	0.017
GA	1.65	0.49–5.49	0.415

Table 3. Association of identified genetic variants of *ANKRD31* gene with incidence of clinical mastitis in Karan Fries cows by logistic regression model

Variable	Clinical Mastitis		P value
	Odds ratio	95% confidence interval	
Season			
Winter	Reference level		
Summer	0.60	0.13–2.78	0.51
Rainy	2.06	0.35–11.89	0.41
Autumn	1.63	0.44–6.07	0.46
Stage of lactation			
4	Reference Level		
1	1.07	0.26–4.38	0.91
2	0.86	0.23–3.26	0.83
3	0.20	0.05–0.80	0.02
Parity			
5	Reference level		
1	2.57	0.48–13.70	0.26
2	3.01	0.57–15.82	0.19
3	1.08	0.15–7.62	0.93
4	3.09	0.51–18.70	0.21
ANKRD31 (*rs110012582*)			
TT	Reference level		
CC	0.96	0.17–5.42	0.97
CT	0.74	0.12–4.60	0.76
ANKRD31 (*rs473512406*)			
GG	Reference level		
AA	0.17	0.04–0.79	0.02
GA	0.49	0.10–2.27	0.36

It is possible to select cows for their strength, mastitis resistance, production and reproductive efficiency and look for morphometric markers in type traits that can define the overall genetic merit of the cow. Udder and teat conformation and udder hygiene score predict the incidence of mastitis. Results suggest that intron 4 and exon 10 of *WDR41* gene is highly polymorphic in Karan Fries (KF) cows. GA genotype of SNP rs211664388 of *WDR41* gene and AA genotype of SNPrs110012582 of *ANKRD31* gene in Karan Fries cattle, respectively may be used as an aid to selection with desired udder conformation and lesser susceptibility to mastitis after validation in larger population. This A to G transition can be used further as SNP markers, which could be helpful to breeders for carrying out future association studies and selecting superior Karan Fries cows for longer herd life. This study may help to select the animals based on morphometric markers as it is difficult to select animals based on their production performance in field conditions.

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REFERENCES

Abebe R, Hatiya H, Abera M, Megersa B and Asmare K. 2016. Bovine mastitis: Prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Veterinary Research* **12**: 270.

Abrahmsén M, Persson Y, Kanyima B M and Båge R. 2014. Prevalence of subclinical mastitis in dairy farms in urban and peri-urban areas of Kampala, Uganda. *Tropical Animal Health and Production* **46**: 99–105.

Amick J, Tharkeshwar A K, Amaya C and Ferguson S M. 2018. WDR41 supports lysosomal response to changes in amino acid availability. *Molecular Biology of the Cell* **29**: 2213–27.

Boujenane I and El Aimani J. 2015. Incidence and occurrence time of clinical mastitis in Holstein cows. *Turkish Journal of Veterinary and Animal Sciences* **39**: 42–49.

Bradford H L, Moser D W, Minick Bormann J and Weaber R L. 2015. Estimation of genetic parameters for udder traits in Hereford cattle. *Journal of Animal Science* **93**: 2663–68.

Breen J E, Bradley A J and Green M J. 2009. Quarter and cow risk factors associated with a somatic cell count greater than 199,000 cells per milliliter in United Kingdom dairy cows. *Journal of Dairy Science* **92**: 3106–15.

Cole J B, Wiggans G R, Ma L, Sonstegard T S, Lawlor T J, Crooker B A, Van Tassell C P, Yang J, Wang S, Matukumalli L K and Da Y. 2011. Genome-wide association analysis of thirty-one production, health, reproduction and body conformation traits in contemporary US Holstein cows. *BMC Genomics* **12**(1): 408.

Falconer D S and Mackay. 1996. *Introduction to Quantitative Genetics*. 4th edn. Addison Wesley Longman Ltd. Essex, England.

Geng N, Wang X, Yu X, Wang R, Zhu Y, Zhang M, Liu J and Liu Y. 2020. *Staphylococcus aureus* avoids autophagy clearance of bovine mammary epithelial cells by impairing lysosomal function. *Frontiers in Immunology* **11**: 746.

Gonzalez M E, Gonzalez V M, Montaño M F, Medina G E, Mahadevan P, Villa C and Villa R. 2017. Genome-wide association analysis of body conformation traits in Mexican Holstein cattle using a mix of sampled and imputed SNP genotypes. *Genetics and Molecular Research* **16**(2).

Harvey W R. 1990. User's Guide for LSMLMW and MIXMDL, Mixed Model Least-Squares and Maximum Likehood Computer Program. PC-2 version. The Ohio State University, Columbus, USA.

Heikkilä A M, Nousiainen J I and Pyörälä S. 2012. Costs of clinical mastitis with special reference to premature culling. *Journal of Dairy Science* **95**: 139–50.

Jaayid T A, Yousief M Y, Hamed F H and Owaid J M. 2011. Body and udder measurements and heritability and their relationship to the production of milk in the Iraqi Buffalo. *International Journal of Biotechnology and Biochemistry* **7**: 553.

Jardim J G, Guldbrandtsen B, Lund M S and Sahana G. 2018. Association analysis for udder index and milking speed with imputed whole-genome sequence variants in Nordic Holstein cattle. *Journal of Dairy Science* **101**(3): 2199–212.

Karahan M, Acik M N and Cetinkaya B. 2011. Investigation of virulence genes by PCR in *Stapylococcus aureus* isolates originated from subclinical bovine mastitis in Turkey. *Pakistan Veterinary Journal* **31**: 249–53.

Katsande S, Matope G, Ndengu M and Pfukenyi D M. 2013. Prevalence of mastitis in dairy cows from smallholder farms in Zimbabwe. Onderstepoort. *Journal of Veterinary Research* **80**(1): 253.

Khan M A and Khan M S. 2016. Genetic parameters of udder traits and their relationship with milk yield in Sahiwal cows of Pakistan. *Journal of Animal and Plant Sciences* **26**: 880–86.

Khan A, Hussain R, Javed M T and Mahmood F. 2013. Molecular analysis of virulent genes (coa and spa) of *Staphylococcus aureus* involved in natural cases of bovine mastitis. *Pakistan Journal of Agricultural Sciences* **50**: 739–43.

Kramer C Y. 1957. Extension of multiple range tests to group means with unequal number of replications. *Biometrics* **12**: 307–10.

Kumari R. 2019. 'Genetic variants in FAM19A1, KCNB1 and *EDN3* gene and their association with udder type traits, clinical mastitis and milk production in Karan Fries and Sahiwal cattle.' PhD Thesis, ICAR-National Dairy Research Institute, Karnal, India.

Kurz J P, Yang Z, Weiss R B, Wilson D J, Rood K A, Liu G E and Wang Z. 2019. A genome-wide association study for mastitis resistance in phenotypically well-characterized Holstein dairy cattle using a selective genotyping approach. *Immunogenetics* **71**(1): 35–47.

Li Y, Malkaram S A, Zhou J and Zempleni J. 2014. Lysine biotinylation and methionine oxidation in the heat shock protein HSP60 synergize in the elimination of reactive oxygen species in human cell cultures. *Journal of Nutritional Biochemistry* **25**: 475–82.

Mizushima N and Komatsu M. 2011. Autophagy: renovation of cells and tissues. *Cell* **147**: 728–41.

Mureithi D K and Njuguna M N. 2016. Prevalence of subclinical mastitis and associated risk factors in dairy farms in urban and peri-urban areas of Thika Sub County, Kenya. *Livestock Research Rural Development* **28.**

Nakov D, Hristov S, Andonov S and Trajchev M. 2014. Udder-related risk factors for clinical mastitis in dairy cows. *Veterinarski Arhiv* **84**: 111–27.

Nakov D and Trajcev M. 2012. Udder quarter risk factors associated with prevalence of bovine clinical mastitis. *Macdonian Veterinary Review* **35**: 55–64.

Negussie E, Strandén I and Mäntysaari E A. 2008. Genetic association of clinical mastitis with test-day somatic cell score and milk yield during first lactation of Finnish Ayrshire cows. *Journal of Dairy Science* **91**: 1189–97.

Passey S, Bradley A and Mellor H. 2008. *Escherichia coli* isolated from bovine mastitis invade mammary cells by a modified endocytic pathway. *Veterinary Microbiology* **130**: 151–64.

Pausch H, Emmerling R, Schwarzenbacher H and Fries R. 2016. A multi-trait meta-analysis with imputed sequence variants reveals twelve QTL for mammary gland morphology in Fleckvieh cattle. *Genetics Selection Evolution* **48**: 14.

Rosendo O, Staples C R, McDowell L R, McMahon R, Badinga L, Martin F G, Shearer J F, Seymour W M and Wilkinson N S. 2004. Effects of biotin supplementation on peripartum performance and metabolites of Holstein cows. *Journal of Dairy Science* **87**: 2535–45.

Sambrook J and Russell D. 2001. *Molecular Cloning: A Laboratory Manual*. 3rd edn. Cold Spring Harbor Laboratory Press, New York.

Sinha R, Sinha B, Kumari R, MR V, Verma A and Gupta I D. 2019. Effect of season, stage of lactation, parity and level of milk production on incidence of clinical mastitis in Karan Fries and Sahiwal cows. *Biological Rhythm Research* **22**: 1–0.

Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 7th edn. Iowa State University, Press, Iowa.

Su M Y, Fromm S A, Zoncu R and Hurley J H. 2020. Structure of the C9orf72 ARF GAP complex that is haploinsufficient in ALS and FTD. *Nature* **585**: 251–55.

Sullivan P M, Zhou X, Robins A M, Paushter D H, Kim D, Smolka M B and Hu F. 2016. The ALS/FTLD associated protein C9orf72 associates with SMCR8 and WDR41 to regulate the autophagy-lysosome pathway. *Acta Neuropathologica Communications* **4**: 51.

Vallée A, Daures J, van Arendonk J A and Bovenhuis H. 2016. Genome-wide association study for behavior, type traits, and muscular development in Charolais beef cattle. *Journal of Animal Science* **94**(6): 2307–16.

Weiss W P and Zimmerly C A. 2000. Effects of biotin on metabolism and milk yield of dairy cows. *Proceedings of Cornell Nutrition Conference*. Rochester NY.

Wu X, Lund M S, Sahan G, Guldbrandtsen B, Sun D, Zhang Q and Su G. 2015. Association analysis for udder health based on SNP-panel and sequence data in Danish Holsteins. *Genetics Selection Evolution* **47**: 50.