

Effects of Four Gene Polymorphisms related to Fertility on Milk Production and Calving Ability in Japanese Holsteins

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ABSTRACT: We investigated the association between the gene polymorphisms on *PKP2*, *CTTNBP2NL*, *SETD6* and *CACNB2* and fertility, milk production, and calving ability of Holsteins in Japan. The genotype effects of the four genes were estimated to be significantly positive on conception rate (CR) and significantly negative on milk production. The genotype effects of *PKP2*, *CTTNBP2NL* and *CACNB2* on calving difficulty were significantly positive. The genotype effects of *CTTNBP2NL*, *SETD6* and *CACNB2* on decreasing stillbirths were significantly negative. These results suggest that selection to increase the frequencies of allele that indicate high CR for the four genes leads to an increase in calving difficulty with a decrease in milk production as well as the indirect response of decreasing stillbirths (SB).

Keywords: gene polymorphism; fertility; milk production; calving ability; Holsteins

Introduction

Milk production of Japanese Holsteins has increased steadily since the Dairy Herd Improvement Program started in 1975. In 2012, the average of 305d milk yield was 9,286 kg, which is 1.6 times the amount of 1975. On the other hand, genetic improvements that increased milk production led a decline in fertility due to the negative genetic correlations, also an increase in SB. Sugimoto et al. (2013) identified four genes related to CR of Holstein: *plakophilin 2 (PKP2)*, *cortactin-binding protein 2 N-terminal-link (CTTNBP2NL)*, *SET domain-containing 6 (SETD6)* and *voltage-dependent L-type calcium channel subunit beta 2 (CACNB2)*. They showed that the high CR genotypes on *PKP2* and *CTTNBP2NL* promoted the implantation of embryo, and improved the response action to a gonadotropin-releasing hormone on *SETD6* and *CACNB2*. The objective of this study was to investigate the effect of these four gene polymorphisms on fertility, milk production, and calving ability.

Materials and Methods

Traditional genetic evaluation. Fertility, milk production, and calving ability records were collected from Holstein cows in Hokkaido, Japan. The fertility traits consist of CR, days open (DO) and number of inseminations (NI) after first calving from January 1990 through May 2013. The milk production traits consist of test day milk, fat and protein yields for the first three lactations from January 1975 through May 2013. The calving ability traits consist of calving difficulty (CD) and SB records for the first three calvings from January 1990 through May 2013. Table 1 presents the traditional genetic evaluation procedures and models for each trait.

Genotype effects. 4,559 Holsteins were genotyped for the four genes. The data set with EBV consists of 4,378 animals (2,527 bulls and 1,851 cows) for CR, 4,476 animals (2,527 bulls and 1,949 cows) for DO and NI and 4,553 animals (2,529 bulls and 2,006 cows) for milk production, respectively. The data set with ETA of CD and SB consists of 2,512 bulls as service sires (CD-S and SB-S) and sires of daughters (CD-D and SB-D), respectively. The effects of these four gene polymorphisms on EBV or ETA of each trait were analyzed using the following model:

$$y_i = \mu + \sum_{j=1}^4 x_{ij} g_j + e_{ij},$$

where y_i is the EBV or deregressed EBV (dEBV) of the i th animal (bull or cow) for fertility and milk traits, and the ETA or deregressed ETA (dETA) of the i th bull for calving ability traits. dEBV or dETA were used because of the difference in reliabilities among EBVs and ETAs. The dEBV of i th animal was calculated using the formula: $dEBV_i = (EBV_i - base) / reliability + base$. dETA was calculated by changing EBV to ETA. μ is the general mean, x_{ij} is the genotype covariate, which was coded as 0 or 2 for the two homozygotes and 1 for heterozygotes, of j th gene of i th animal. The genotype for each gene was coded with 0, 1 or 2 according to an increase in CR. g_j is the random regression coefficient for j th gene (also known as the allele substitution effect), and e_{ij} represents the random residual for each

Table 1: Methods (models) of genetic evaluations and number of records, cows with records, bulls with daughters and animals included pedigree for fertility (CR: conception rate, DO: days open and NI: number of Inseminations), milk production (milk, fat and protein yields) and calving ability (CD: calving difficulty and SB: stillbirth)

Trait	Method (model) [§]	Records	Cows	Bulls	Pedigree
CR	ST, SL, BLUP, RP, Thr-AM	3,047,032	1,509,824	6,952	855,154
DO and NI	ST, SL, BLUP, AM	1,699,045	1,699,045	7,073	795,569
Milk and fat	ST, ML, RR, RP, TD, BLUP, AM	55,843,004	2,625,995	9,342	1,320,634
Protein	ST, ML, RR, RP, TD, BLUP, AM	47,484,734	2,259,935	7,395	1,686,694
CD and SB	ST, ML, BLUP, Thr-SMGS	3,634,118	-	7,595 [¥]	9,698

[§] ST: single-trait, RP: repeated cow records, Thr-AM: threshold animal model, SL: single lactation (first parity), ML: multiple (the first three) lactations, RR: random regression, TD: test day, Thr-SMGS: threshold sire and MGS (maternal grandsire) model

[¥] 7,595 bulls contains 7,152 Holstein bulls purebred and 443 Wagyu bulls crossbred with Holstein cows

Table 2: Genotype and allele frequencies of each gene

Gene	Genotype frequencies						Allele frequencies			
	pp=0		pq=1		qq=2		p=0		q=1	
	Genotype	Freq.	Genotype	Freq.	Genotype	Freq.	Allele	Freq.	Allele	Freq.
Bulls (2,529 records)										
<i>PKP2</i>	TA/TA	0.24	del/TA	0.49	del/del	0.27	TA	0.49	del	0.51
<i>CTTNBP2NL</i>	A/A	0.30	G/A	0.49	G/G	0.20	A	0.55	G	0.45
<i>SETD6</i>	Glu/Glu	0.56	Ala/Glu	0.38	Ala/Ala	0.06	Glu	0.75	Ala	0.25
<i>CACNB2</i>	AT/AT	0.52	del/AT	0.40	del/del	0.09	AT	0.72	del	0.28
Cows (2,030 records)										
<i>PKP2</i>	TA/TA	0.23	del/TA	0.51	del/del	0.26	TA	0.49	del	0.51
<i>CTTNBP2NL</i>	A/A	0.31	G/A	0.49	G/G	0.20	A	0.55	G	0.45
<i>SETD6</i>	Glu/Glu	0.51	Ala/Glu	0.42	Ala/Ala	0.07	Glu	0.72	Ala	0.28
<i>CACNB2</i>	AT/AT	0.46	del/AT	0.46	del/del	0.09	AT	0.68	del	0.32
Total animals (4,559 records)										
<i>PKP2</i>	TA/TA	0.24	del/TA	0.50	del/del	0.26	TA	0.49	del	0.51
<i>CTTNBP2NL</i>	A/A	0.31	G/A	0.49	G/G	0.20	A	0.55	G	0.45
<i>SETD6</i>	Glu/Glu	0.54	Ala/Glu	0.40	Ala/Ala	0.06	Glu	0.74	Ala	0.26
<i>CACNB2</i>	AT/AT	0.49	del/AT	0.42	del/del	0.09	AT	0.70	del	0.30

observation. The analysis was implemented in the MIXED procedure of SAS.

Results and Discussion

The genotype and allele frequencies in each gene had no significant difference between the genotyped bulls and cows (Table 2). The frequencies of allele TA and allele del on *PKP2* were 49% and 51%, allele A and allele G on *CTTNBP2NL* were 55% and 45%, respectively. The frequencies of allele Glu and allele Ala on *SETD6* were 74% and 26%, allele AT and allele del on *CACNB2* were 70% and 30%, respectively. The bias was large compared to *PKP2* and *CTTNBP2NL*. Table 3 shows that the fractions of residual variance for EBV and dEBV of CR were smaller (87.93% and 87.32%) than the other traits (from 90.70% to 97.71%). The variance fractions of *SETD6* and *CACNB2* of fertility traits were relatively larger than that of *PKP2* and *CTTNBP2NL*. For the milk production traits, the variance fraction of *CACNB2* was the largest in the range of 2.05% to 6.44%. The genes that have a relatively large variance on calving ability traits are *CACNB2* for CD-S, CD-D and SB-D and *CTTNBP2NL* for SB-S. The genotype effects of the four genes were estimated significantly positive ($P<0.01$) on CR and significantly negative ($P<0.01$) on DO and NI (Table 4). These results agree with Sugimoto et al. (2013)

that allele TA on *PKP2*, allele A on *CTTNBP2NL*, allele Glu on *SETD6* and allele AT on *CACNB2* improve fertility. The effects of the genotypes on the four genes on milk, fat and protein yields were estimated to be significantly negative ($P<0.01$ except $P<0.05$ of *SETD6* for fat yield). Therefore, it suggests that intensive selection for milk production has affected the allele frequencies on *SETD6* and *CACNB2* particularly and has caused a decline in fertility. The effects of genotype on *PKP2*, *CTTNBP2NL* and *CACNB2* on CD were significantly positive ($P<0.01$ or $P<0.05$). The effects of genotype on *CTTNBP2NL*, *SETD6* and *CACNB2* on SB were estimated to be significantly negative ($P<0.01$ or $P<0.05$). The improvement of the allele frequency on the four gene polymorphisms related to fertility might have lead to the increase in CD and the decline in SB.

Conclusion

The allele frequencies on *CTTNBP2NL* and *PKP2* had no bias. Meanwhile the frequencies of the conflicting two alleles on *CACNB2* and *SETD6* were biased. The selection to increase the frequencies of allele for a high CR on *CACNB2*, *SETD6* *PKP2*, *CTTNBP2NL* and *CACNB2* may have resulted in the increase in CD and the decrease in SB and milk production.

Table 3: Fractions of *PKP2*, *CTTNBP2NL*, *SETD6* and *CACNB2* genotype variances and residual variance on each total variance for EBV and deregressed EBV (dEBV) of fertility and milk production traits (305-d yields) and ETA and deregressed ETA (dETA) of calving ability traits

Trait &		Total variance	Fractions of variance (%)				
			<i>PKP2</i>	<i>CTTNBP2NL</i>	<i>SETD6</i>	<i>CACNB2</i>	Residual
CR	EBV	26.10	2.68	1.69	4.21	3.49	87.93
	dEBV	24.13	2.81	1.76	4.51	3.59	87.32
DO	EBV	236.8	0.96	0.19	2.31	4.27	92.27
	dEBV	149.7	1.29	0.31	3.27	4.43	90.70
NI × 10 ²	EBV	605.3	1.48	1.61	2.83	3.06	91.03
	dEBV	433.7	1.81	2.03	4.00	3.04	89.12
Milk	EBV	303,065	0.68	0.87	2.13	4.91	91.40
	dEBV	281,306	0.61	1.15	2.21	4.31	91.72
Fat	EBV	485.8	1.89	0.52	0.23	2.79	94.58
	dEBV	439.3	1.84	0.77	0.33	2.05	95.02
Protein	EBV	320.1	0.56	0.56	1.03	6.44	91.41
	dEBV	310.5	0.43	0.86	1.00	5.47	92.24
CD-S	ETA	3.02	0.20	0.41	0.03	1.64	97.71
	dETA	10.27	0.28	0.49	0.10	2.14	96.99
CD-D	ETA	1.02	0.37	0.61	0.03	1.11	97.88
	dETA	2.64	0.41	0.85	0.07	1.70	96.98
SB-S	ETA	1.00	0.00	2.10	1.14	0.41	96.35
	dETA	3.30	0.00	2.30	1.38	0.32	96.01
SB-D	ETA	2.31	0.04	1.47	1.76	3.95	92.78
	dETA	5.40	0.05	1.81	2.05	4.53	91.57

& See Table 1 for abbreviation of traits

Table 4: Estimates for intercept and genotype effects (regression coefficients) of *PKP2*, *CTTNBP2NL*, *SETD6* and *CACNB2* on EBV and deregressed EBV (dEBV) of fertility and milk production traits (305-d yields) and ETA and deregressed ETA (dETA) of calving ability traits

Trait #		Intercept	<i>PKP2</i>	<i>CTTNBP2NL</i>	<i>SETD6</i>	<i>CACNB2</i>
CR	EBV	41.00 **	0.83 **	0.66 **	1.04 **	0.95 **
	dEBV	41.01 **	0.82 **	0.65 **	1.04 **	0.92 **
DO	EBV	5.48 **	-1.48 **	-0.61 *	-2.31 **	-3.16 **
	dEBV	5.49 **	-1.37 **	-0.64 **	-2.20 **	-2.56 **
NI × 10 ²	EBV	11.13 **	-2.95 **	-3.09 **	-4.10 **	-4.27 **
	dEBV	10.90 **	-2.77 **	-2.95 **	-4.14 **	-3.61 **
Milk	EBV	130 **	-45 **	-50 **	-79 **	-121 **
	dEBV	106 **	-40 **	-56 **	-78 **	-110 **
Fat	EBV	3.53 **	-3.02 **	-1.53 **	-0.95 *	-3.66 **
	dEBV	2.56 **	-2.81 **	-1.80 **	-1.11 *	-2.96 **
Protein	EBV	3.31 **	-1.31 **	-1.29 **	-1.77 **	-4.52 **
	dEBV	2.52 **	-1.10 **	-1.60 **	-1.72 **	-4.10 **
CD-S	%ETA	6.98 **	n.s.	0.10 *	n.s.	0.22 **
	%dETA	6.78 **	n.s.	0.21 *	n.s.	0.46 **
CD-D	%ETA	6.54 **	0.06 *	0.07 **	n.s.	0.10 **
	%dETA	6.17 **	0.10 *	0.14 **	n.s.	0.21 **
SB-S	%ETA	6.11 **	n.s.	-0.14 **	-0.10 **	-0.06 *
	%dETA	6.16 **	n.s.	-0.27 **	-0.21 **	n.s.
SB-D	%ETA	6.64 **	n.s.	-0.18 **	-0.20 **	-0.30 **
	%dETA	7.05 **	n.s.	-0.31 **	-0.32 **	-0.49 **

P**<0.01, P*<0.05, n.s.: non significance

#See Table 1 for abbreviation of traits

Literature Cited

SAS/STAT 9.2 user's guide (2008). SAS Institute Inc., USA
 Sugimoto, M., Sasaki, S., Gotoh, Y. et al. (2013). PNAS,
 110:19495-19500.