Short communication

Association of single nucleotide polymorphisms in genes coding insulin-like growth factor 1 system and milk production traits in Montbeliarde cows

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Abstract

The insulin-like growth factor 1 system plays a central role in the growth and development of the mammary gland. Insulin-like growth factor 1 (IGF1) and insulin-like growth factor 1 receptor (IGF1R) have been proposed as candidate genes for milk production traits. This study involved a population of 163 Montbeliarde cows. Five polymorphic sites were analysed using the polymerase chain reaction: restriction fragment length polymorphism (PCR-RFLP) (TaiI and MspI restriction enzymes) and amplification-created restriction site (ACRS) (SnaBI, TasI and TaqI restriction enzymes). The frequencies of the most common alleles were 0.67 for the T allele (IGF1/SnaBI), 0.85 for the A allele (IGF1/TasI), 0.95 for the C allele (IGF1R/TaiI), 0.84 for the G allele (IGF1R/MspI) and 0.69 for the G allele (IGF1R/TaqI). In the first lactation, IGF1/TasI polymorphisms and all single nucleotide polymorphisms in the IGF1R gene were associated with differences in milk, fat and protein yields, without further confirmation in the second lactation. No differences were found in milk production traits between IGF1/SnaBI genotypes and combined genotypes. To date, this is the first association study based on polymorphisms of the primary genes encoding the IGF-1 system in a small herd of Montbeliarde cows. If specific haplotypes could be determined in large-scale studies, based on Montbeliarde and other dairy breeds, it would provide a valuable genetic tool to identify causative mutations.

Keywords: *IGF1*, *IGF1R*, milk production traits, polymorphism
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The Montbeliarde breed is a red and white breed of cattle that originated in the east of France in the nineteenth century, and is known for withstanding extreme weather conditions such as hot summers and cold winters. Milk from Montbeliarde cows is characterized by high protein levels (3.45%) and a high frequency of kappa-casein B variants (Bugeac *et al*., 2013).

The insulin-like growth factor 1 (IGF1) system plays a key role in the regulation of growth, development, metabolism and lactation in cattle (Baumrucker & Erondu, 2000). Most of IGF1 is secreted by the liver and subsequently transported to target tissues where it acts as an endocrine hormone. The main effect of this action is stimulation of the mitogenic and anabolic activity of the growth hormone in various tissues (Laron, 2001). However, an autocrine/paracrine IGF1 can be produced locally in a tissue-specific manner, including the mammary gland (Weber *et al*., 2000; Plath-Gabler *et al*., 2001), where it regulates cell differentiation. Regardless of its origin, most of the functional effects of IGF1 are modulated via binding to the cell surface receptor type 1 (IGF1R) and its activation. Subsequent downstream pathways play a crucial role in IGF system signalling (Annunziata *et al*., 2011).

Given the role played by the IGF system in mammary gland development and lactation, the aim of this study was to investigate possible associations between several polymorphic variants of the IGF1 and IGF1R genes and milk production traits in Montbeliarde dairy cows.

This study involved a population of 163 Montbeliarde cows from one herd, kept in West Pomerania Province, Poland. DNA was isolated from blood using MasterPure™ DNA Purification Kit (Epicentre Technologies), according to the manufacturer's instructions.

The genotypes were analyzed using PCR-RFLP and, if commercial enzymes were not available for the native sequence, amplification created restriction site (ACRS) (Table 1).

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Table 1 Primers used for amplification of selected fragments of insulin-like growth factor 1 and insulin-like growth factor 1 receptor genes

Gene	Localization SNP number	Primer sequence	Annealing temp. (ºC)	Length (bp)	Restriction enzyme (cut site)	Allele and digestion pattern (in base pairs)	Method
IGF1	P1 promoter rs109763947	5'-ATACAAAGCTGCCTGCCCC-3' 5'- ACCTTACCCGTATGAAAGGAATAT**A**CGT-3'*	62	249	SnaBI (TAC↓GTA)	*T*: 223 + 26 *C*: 249	ACRS[†]
	P1 promoter rs133990633	5'-TCATCCAGCTGAGAGATTTGAAT-3' 5'-TGTGTGTGTGTGTGTGTGTG**A**AT-3' *	58	146	TasI (↓AATT)	*A*: 122 + 24 *C*: 146	ACRS[†]
IGF1R	exon 7 rs41961336	5' -ACAGTGTTTGGGTCCTTAGTGG- 3' 5' -AGGTGATGATGATTCGGTTCTT- 3'	59.5	236	TaiI (MaeII) (ACGT/)	*C*: 141+45+26+24 *T*: 167+45+24	PCR-RFLP[¥]
	exon 12 rs41640706	5' -TTCTTGCCTGTTTCAATTGTTG- 3' 5' -CTCGACTTGGGATCCATATTTT- 3'	59.5	164	MspI (C/CGG)	*G*: 113 + 51 *A*: 164	PCR-RFLP[‡]
	exon 21 rs41960562	5' -GCCGGTCACCATAGGTCT**C**G- 3' * 5' -AGTGGGGGTTTTGGCAGAAT- 3'	62	163	TaqI (T/CGA)	A: 145 + 18 G: 163	ACRS[‡]

***** mismatch is underlined.
Source: [†] Szewczuk *et al.* (2013a); [‡] Szewczuk *et al.* (2013b); [¥] present work.

Data from 305-day milk production from the first and second lactations, including overall milk production, fat and protein yields and their percentages, were obtained from the official farm records. The cows were progeny of 33 sires. Statistical calculations were performed using Duncan's test and Statistica software (STATISTICA 10.0 PL software package, Statsoft Inc. 2011). The effect of the IGF1 and IGF1R genotypes and their combined genotypes on milk production traits of Montbeliarde cows were analysed using the general linear model (GLM). The following model was used:

$$Y_{ijkl} = \mu + G_i + S_j + CYS_k + b(CA_l - CA) + e_{ijkl}$$

where: Y_{ijkl} is analysed trait; μ is overall mean;
G_i is effect of IGF1 or IGF1R genotype (i = 1, 2 or 3) or combined genotypes (i = 1,…,3 ÷ 6);
S_j is random effect of a sire; CYS_k is effect of calving year/season (k = 1,…,16; assuming that each year was divided into two seasons: spring/summer and fall/winter);
b is linear regression coefficient for calving age;
CA_l is calving age of a *l*-cow;
CA is mean calving age;
e_{ijkl} is random error.

Genotype frequencies of five polymorphic sites in the IGF1 and IGF1R genes in this herd of Montbeliarde cows are shown in Tables 2 and 3.

For most of the polymorphic sites, all possible genotypes were observed. In the IGF1R/TaiI polymorphism, there were no individuals with the homozygous TT genotype. The frequencies of the most common alleles in the herd were 0.67 for the T allele (IGF1/SnaBI); 0.85 for the A allele (IGF1/TasI); 0.95 for the *C* allele (IGF1R/TaiI); 0.84 for the G allele (IGF1R/MspI); and 0.69 for the G allele (IGF1R/TaqI), without significant changes in the second lactation.

Table 2 shows the effect of the RFLP-SnaBI and -TasI polymorphisms of the IGF1 gene on milk production traits. No differences were found in milk production traits among IGF1/SnaBI genotypes. In the first lactation, the IGF1/TasI polymorphism was associated with differences in milk performance. Cows with the homozygous *CC* genotype had higher milk, fat and protein yields than those with the AA (P <0.05) and AC (P <0.01) genotypes. In the second lactation, there were no significant associations, but similar trends were observed. No statistical differences in percentage traits were found.

Table 2 Allele and genotype frequencies, means and standard errors (in parentheses) for milk production traits in Montbeliarde cows with insulin-like growth factor 1 gene variants

Polymorphism	Lactation	Allele freq.	Genotype	No. genotypes	Genotype freq.	Milk yield/ lactation (kg)	Fat		Protein	
							kg	%	kg	%
IGF1/SnaBI rs109763947	1	*T* 0.67 *C* 0.33	*CC*	15	0.0920	7915 (242.01)	304 (13.12)	3.82 (0.07)	277 (9.41)	3.51 (0.06)
			CT	77	0.4724	7685 (105.68)	305 (6.29)	3.95 (0.05)	275 (3.42)	3.58 (0.02)
			TT	71	0.4356	7689 (114.48)	300 (5.79)	3.89 (0.04)	275 (3.89)	3.58 (0.02)
	2	*T* 0.67 *C* 0.33	*CC*	15	0.0980	8063 (440.28)	301 (13.41)	3.80 (0.14)	276 (12.70)	3.46 (0.07)
			CT	70	0.4575	8099 (139.58)	309 (6.37)	3.82 (0.05)	279 (4.57)	3.45 (0.03)
			TT	68	0.4445	8436 (144.14)	319 (6.58)	3.78 (0.05)	286 (4.24)	3.41 (0.02)
IGF1/TasI rs133990633	1	*A* 0.85 *C* 0.15	*AA*	123	0.7546	7750^{a} (80.92)	303^{a} (4.25)	3.91 (0.03)	278 (2.72)	3.59 (0.02)
			AC	31	0.1902	7359^{B} (182.27)	289^{B} (11.79)	3.89 (0.09)	261^{A} (5.79)	3.53 (0.03)
			CC	9	0.0552	8337^{aB} (306.97)	335^{aB} (16.49)	4.02 (0.14)	293^{A} (11.64)	3.51 (0.04)
	2	*A* 0.84 *C* 0.16	*AA*	114	0.7451	8187 (116.38)	311 (5.00)	3.81 (0.04)	281 (3.50)	3.44 (0.02)
			AC	30	0.1961	8331 (203.91)	316 (9.76)	3.80 (0.07)	284 (6.75)	3.41 (0.04)
			CC	9	0.0588	8673 (543.33)	322 (20.24)	3.75 (0.17)	293 (16.85)	3.40 (0.07)

Means within columns of the associated polymorphism and particular lactation marked with the same superscripts differ significantly at P <0.01 (capitals), at P <0.05 (small letters).

Table 3 presents the effect of the RFLP-TaiI, -MspI and -TaqI polymorphisms of the IGF1R gene on milk production traits. Significant associations were found for all three polymorphic sites.

With regard to the IGF1R/TaiI polymorphism, Montbeliarde cows with the CC genotype showed higher milk (+ 492 kg) and fat (+ 29 kg) yields in the first lactation, compared with heterozygous cows (P <0.05). In the second lactation, there were no significant differences in the milk production traits. Furthermore, IGF1R/TaiI heterozygous cows were characterized by slightly higher yields and contents.

With respect to the IGF1R/MspI polymorphism, it was found that individuals with the GG genotype always had lower milk and fat yields than individuals with other genotypes, irrespective of lactation. However, in the first lactation, the most noticeable differences were found between the GG and AA genotypes (−639 kg for milk yield at P <0.05 and −40 kg for fat yield at P <0.01). In the second lactation, no statistical differences in percentage traits were found between the GG and AG genotypes (−612 kg for milk yield and −32 kg for fat yield, both at P <0.05).

The third polymorphic site in IGF1R, namely IGF1R/TaqI, was characterized by different average values of these traits, with low reproducibility between lactations. However, cows with the AA genotype seemed to be preferred for milk production because of their higher yields (some were significant). Briefly, fat yield was always the highest (first lactation: +30 to 32 kg at P <0.01 and + 28 kg in the second lactation), while milk and protein yields were significant only in the second lactation (P <0.05). In addition, fat content in the first lactation was highest in milk derived from cows with the AA genotype. However, protein content was highest in milk from cows with the GG genotype. In the present herd, cows that carried a heterozygous genotype of the IGF1R/TaqI polymorphism appeared to have the lowest milk performance.

Table 3 Allele and genotype frequencies, means and standard errors (in parentheses) for milk production traits in Montbeliarde cows with different insulin-like growth factor 1 receptor gene variants

Polymorphism	Lactation	Allele freq.	Genotype	No. genotypes	Genotype freq.	Milk yield/ lactation (kg)	fat		protein	
							kg	%	kg	%
IGF1R/TaiI rs41961336	1	*C* 0.95 *T* 0.05	*CC*	146	0.8957	7759^{a} (78.51)	305^{a} (4.23)	3.93 (0.03)	277 (2.61)	3.57 (0.02)
			CT	17	0.1043	7267^{a} (185.13)	276^{a} (12.57)	3.77 (0.11)	262 (7.43)	3.61 (0.04)
			TT	0	0.0000	-	-	-	-	-
	2	*C* 0.94 *T* 0.06	*CC*	136	0.8889	8217 (106.99)	311 (4.55)	3.79 (0.04)	281 (3.27)	3.27 (0.02)
			CT	17	0.1111	8457 (291.85)	326 (13.89)	3.86 (0.09)	289 (8.92)	3.44 (0.05)
			TT	0	0.0000	-	-	-	-	-
IGF1R/MspI rs41640706	1	*A* 0.16 *G* 0.84	*GG*	126	0.7730	7629^{a} (84.38)	298^{A} (4.46)	3.90 (0.03)	273 (2.79)	3.58 (0.02)
			AG	21	0.1288	7753^{b} (161.86)	302^{b} (11.71)	3.89 (0.11)	275 (7.33)	3.55 (0.05)
			AA	16	0.0982	8268^{ab} (245.92)	338^{Ab} (13.33)	4.08 (0.09)	290 (7.31)	3.51 (0.05)
	2	*A* 0.16 *G* 0.84	*GG*	117	0.7697	8114^{a} (113.70)	307^{a} (4.83)	3.79 (0.04)	278 (3.50)	3.44 (0.02)
			AG	20	0.1316	8726^{a} (229.36)	339^{a} (13.00)	3.89 (0.11)	297 (7.22)	3.42 (0.04)
			AA	15	0.0987	8614 (358.37)	323 (11.94)	3.79 (0.10)	291 (10.33)	3.41 (0.08)
IGF1R/TaqI rs41960562	1	*A* 0.31 *G* 0.69	*GG*	95	0.5828	7598 (100.90)	297^{A} (5.09)	3.90^{a} (0.04)	273 (3.46)	3.59^{a} (0.02)
			AG	36	0.2209	7768 (131.62)	295^{B} (8.68)	3.78^{B} (0.07)	273 (4.66)	3.51^{a} (0.03)
			AA	32	0.1963	7965 (166.08)	327^{AB} (9.29)	4.09^{aB} (0.07)	284 (5.00)	3.58 (0.03)
	2	*A* 0.30 *G* 0.70	*GG*	90	0.5921	8202 (135.20)	309 (5.81)	3.78 (0.05)	281 (4.10)	3.44 (0.02)
			AG	32	0.2105	8028^{a} (189.98)	304^{a} (8.36)	3.79 (0.06)	274^{a} (6.73)	3.41 (0.03)
			AA	30	0.1974	8599^{a} (223.49)	332^{a} (9.37)	3.87 (0.08)	292^{a} (5.99)	3.42 (0.05)

Means within column of the associated polymorphism and particular lactation marked with the same superscripts differ significantly at P <0.01 (capitals), at P <0.05 (small letters).

The study included an analysis of combined genotypes of IGF1/IGF1R genes in a total of eight arrangements, namely i) SnaBI/TasI; ii) SnaBI/TaiI; iii) TasI/TaiI; iv) SnaBI/MspI; v) TasI/MspI; vi) SnaBI/TaqI; vii) TasI/TaqI; and viii) TaiI/MspI/TaqI), and a number of combinations. Combinations with a low number of individuals (n <10) were omitted. For all arrangements, no differences were recorded in the analysed traits for particular combinations (data not shown owing to the considerable volume).

The five SNPs under study were silent mutations, which did not result in change in the amino acid sequence. Most of these markers were associated with production traits in other bovine breeds. Validation of previously associated polymorphisms in other cattle breeds is important in understanding the development of milk production traits.

The IGF1/SnaBI polymorphism might affect gene expression in cattle, because it alters the putative binding site for various transcription factors (Mullen *et al*., 2011). Therefore, this SNP is the most extensively investigated polymorphism in the IGF1 gene, in about 30 cattle breeds, excluding Montbeliarde (Ge *et al*.,

2001; Siadkowska *et al*., 2006; Mullen *et al*., 2011**;** Szewczuk *et al*., 2012; 2013a), but there is no conclusive evidence of association with milk traits.

As regards the IGF1/TasI polymorphism, only the Holstein-Friesian breed has been studied. In the present study, as well as in that of Szewczuk *et al*. (2011) on 658 Holstein-Friesian cows, significant differences between individuals that carried the *CC* genotype and other genotypes were found for milk, fat and protein yields. On the contrary, in the current study of a small herd of Holstein-Friesian cows with a higher frequency of the *CC* genotype and relatively few heterozygous individuals, Szewczuk *et al*. (2013a) reported that cows with the *AC* genotype were characterized by the highest milk, protein and fat yields (P <0.01); the highest yields being found in cows with the *Sna*BI^{CC}/*Tas*I^{AA} combination, compared with other combinations.

Many biological processes are strongly dependent on the mRNA secondary structure of crucial genes, which is essentially determined by SNPs within exons. So far, no articles have been published on the effects of three polymorphisms in the coding sequence of IGF1R (rs41961336-TaiI/ rs41640706-MspI/rs41960562-TaqI) and their association with milk composition and synthesis. In the present study, each polymorphism was associated with certain milk traits. According to this observation, cows carrying the TaiI^{CC}/MspI^{AA}/TaqI^{AA} combination (only n = 11 in the present work) were characterized by the highest milk production: milk yield (8420 kg in the first lactation and 8861 kg in the second), fat yield (338 and 354 kg, respectively) and protein yield (293 and 295 kg, respectively) compared with the largest group of individuals in the herd (n = 79) with the TaiI^{CC}/MspI^{GG}/TaqI^{GG} combination (7660/8133 kg; 301/305 kg; 274/278 kg; for milk, fat and protein yields in the first and second lactations, respectively), but differences were not significant (results not shown).

The current study describes the association of previously reported SNPs in IGF1 and IGF1R genes with milk yield and composition. To the authors' knowledge, this is the first such report about commercial dairy cows of the Montbeliarde breed. The results of these preliminary experiments show that IGF1R variants may affect milk production traits. However, these findings need to be confirmed on a larger population of animals.

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