

Original study

Association between lactoferrin single nucleotide polymorphisms and milk production traits in Polish Holstein cattle

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Abstract

Bovine lactoferrin exhibits strong potential for further applications as a mastitis resistance marker. Since selection for mastitis resistance should not interfere with dairy performance, we investigated the association between bovine lactoferrin gene polymorphism and production traits in Polish Holsteins. The associations between four SNPs, localized in the 5'-flanking region and in exons 4 and 9 of the lactoferrin gene, and dairy performance were examined. SNPs were associated with almost all test-day milk performance traits. Significant associations were found between lactoferrin genotypes and the estimated breeding values for those traits. To find out whether the discrepancies between the lactoferrin gene SNP's influence on phenotype (test-day milk performance) and on estimated breeding values originate from the impact of other factors, we explored the genotype by environment interaction. Substantial impacts of SCC, lactation stage and parity were found. This paper suggests that the genotype by environment interaction may significantly change associations between genes and traits. It is important to include similar analyses to the studies on disease markers before using them in the selection.

Keywords: mastitis marker; lactoferrin gene; estimated breeding values; milk performance traits

Abbreviations: DIM: days in milk; EBV: estimated breeding value; GLM: general linear model; HWE: Hardy-Weinberg equilibrium; LF: lactoferrin; LS: least squares; SCC: somatic cell count; SCS: somatic cell score; SNP: single nucleotide polymorphism

Archiv Tierzucht 57 (2014) 27, 1-12
doi: 10.7482/0003-9438-57-027

Received: 21 February 2014
Accepted: 15 Juli 2014
Online: 5+ October 2014

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Introduction

Genetic variance in dairy performance traits in cattle has been explored for more than 20 years now, giving not much more than a few QTLs strongly associated with the traits of interest. *ABCG2*, *DGAT1*, *CASB*, *IGF2* and *GHR* genes are among these exceptions, but not without inconsistency in the results (Komisarek *et al.*, 2004, Bagnicka *et al.*, 2010, Olsen *et al.*, 2007, Olenski *et al.*, 2012, 2010). It has been shown that genes, connected originally to health and development traits, like *IL8*, *IL8R*, *CCL2*, *CCR2*, can be associated with dairy performance. Polymorphisms found in the above-mentioned genes are connected to at least one of the five main performance traits: milk, protein and fat yield, protein and fat percentage (Leyva-Baca *et al.* 2007, Chen *et al.* 2010, Sanz *et al.* 2010). Lactoferrin (LF), an iron-chelating protein, is present in many mammalian biological fluids, including milk. Lactoferrin is a potential genetic marker for mastitis resistance, due to the following reasons: this protein plays a crucial role in immune response during mastitis (e.g. Rainard & Riollet 2005); the magnitude of its expression is connected to the udder health state (e.g. Chen & Mao 2004); and, importantly, the LF gene and its promoter are highly polymorphic. SNPs localized within the LF gene and its 5' regulatory regions have been investigated with reference to gene expression and disease susceptibility (see review by Pawlik *et al.* 2009). It was found that LF SNPs are connected to the somatic cell count (SCC) or score (SCS) (O'Halloran *et al.* 2010, Sender *et al.* 2010), and SNPs in the 5'-regulatory region are connected to LF expression (unpublished data). Since LF exhibits potential for further application as a mastitis resistance/susceptibility marker and selection for mastitis resistance cannot interfere with selection for dairy performance (which would diminish milk production profitability) there is a need to evaluate whether LF is associated with dairy performance traits. The unfavourable effect of LF on milk traits may possibly make it an inferior mastitis marker.

Only a few papers have so far included bovine chromosome 22 (in which the LF gene is localized) among the regions of importance for milk traits (Ashwell *et al.* 2004, Kolbehdari *et al.* 2009). There are, however, reports that the LF genotype influences dairy performance, apart from its impact on SCC (Kamiński *et al.* 2006, O'Halloran *et al.* 2010).

It is known that genetic merit for traits of animal health and reproduction is in opposition to merit for production traits. Thus, it is of great importance to discover associations between genes related with animal health and performance simultaneously. Other genes, like *IGF1* and LF »ancestor gene« – TF, were proved to influence milk performance, despite their primary role in health, development or reproduction (Sanz *et al.* 2010, Szewczuk *et al.* 2012). This suggests the pleiotropic effect, or the existence of a linkage between genes coding for the non-productive traits and dairy performance traits.

Due to the above-mentioned facts, the aim of this paper was to investigate the associations between four SNPs, localized within the LF gene and its 5'-flanking region, and dairy performance traits. To achieve this, LF genotype impact on the phenotypic values of milk traits was evaluated. To carry out an in-depth analysis of LF genotype impact on the traits of interest, genotype \times environment interactions analysis was performed. Finally, the influence of the genotype on estimated breeding values (EBV) for those traits was investigated.

Material and methods

Animals

Lactoferrin gene genotypes were determined for 480 Polish Holsteins cows – daughters of 165 sires from one herd. Performance data were scored in an official milk recording system. Milk samples were collected between 1998 and 2011. Test day milk yield, milk protein and fat yields, as well as protein and fat percentages were taken into account. Animals were in their 1st to 8th lactation. The number of animals was lowered with the subsequent parities. Samples were taken from 473 animals in the 1st lactation, 374 in the 2nd, 216 in the 3rd, 104 in the 4th and 36 in the 5th to 8th lactations.

Genotyping

The LF gene SNPs used in this study were previously described by Seyfert *et al.* (1994) and Li *et al.* (2004). Two SNPs were located in the 5'-flanking region of the LF gene: G/A at position -926 (in this paper: »LF-926«) and G/C at the +32 position (the 5'-UTR region, here: »LF+32«). SNP at the +32 position was previously called +33 by some authors (Li *et al.* 2004). Two other SNPs were placed in the LF gene exons: non-synonymous SNP A/G (ILE/VAL) in exon 4 (rs109741625, here: »LFex4«) and synonymous SNP C/T in exon 9 (rs43765461, in this paper: »LFex9«).

The DNA was extracted from blood by the method according to Kanai *et al.* (1994). Primers used in the PCR reactions for the amplification of the three SNPs: LF-926, LFex4 and LFex9 were taken from the paper by Li *et al.* (2004). Primers for the amplification of the fragment containing LF+32 SNP were designed with the use of Primer3 software (Untergasser *et al.* 2012; <http://bioinfo.ut.ee/primer3>) and were (5'-3'): TTT CTC GCT CCC TCG GTC T (fwd) and CCC CGC CCC CAC TCA TAC (rev). Webcutter 2.0 software (<http://rna.lundberg.gu.se/cutter2/>) was used for restriction enzyme selection. RFLP reaction was done with the use of Fermentas nucleases (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's recommendations. The restriction enzymes used in the study were as follows: LF-926: *FaqI*, LF+32: *Eco0109I*, LFex4: *MboI*, LFex9: *Eco81I*. Agarose gel electrophoresis bands, obtained for each allele, measured: LF-926: A: 229bp, G: 168 and 61bp; LF+32: C: 264, 166 and 56bp, G: 430 and 56bp; LFex4: A: 117 and 78bp, G: 195 bp; LFex9: C: 159bp, T: 103 and 56bp.

Statistical analysis

Association between the LF genotype and milk performance traits

The analysis of variance for the estimation of the LF gene polymorphism effect upon dairy performance traits was performed with 14045 test day observations. Traits chosen for the analysis were: test-day milk yield, protein and fat yields, and protein and fat percentages. The classification used for the analysis of the association between LF SNP polymorphism and dairy performance included the fixed effects of the LF genotype, animal nested in LF genotype, year×season of calving interaction (55 levels – starting from the 1st quarter of 1998), parity (5 levels – 6th and subsequent lactations were included in the 5th) and linear regression on days in milk (DIM). Differences between animals carrying a particular LF genotype were tested for significance with Tukey range test in the GLM procedure (SAS Software). The exact test (ALLELE procedure in SAS Software v 9.1, SAS Institute Inc., Cary, NC, USA) tested deviation from the Hardy-Weinberg equilibrium (HWE).

Genotype by environment interaction

An effect of the genotype×environment interaction was added to the above described model. If genotype×environment interaction was significant, performances observed for different genotypes were categorized and the differences between them were analysed. The significance of LF genotype impact on milk performance was investigated for subsequent parities: 1st, 2nd and 3rd (later lactations were included in the 3rd parity) (respectively 473, 374 and 216 animals), different lactation stages (DIM: 5-30, 31-250, >250, respectively 467, 477 and 434 animals) and SCC> or <200 000 per ml (respectively 470 and 461 animals). For example, if genotype×lactation stage interaction significantly influenced milk yield, the differences in milk yield between the individuals carrying particular LF genotypes were tested for significance with Tukey range test in the GLM procedure in different lactation stages.

Association between LF genotype and the animal EBV for milk traits

Animal EBVs for milk traits were estimated by the authors for the 480 cows described earlier. The pedigree information spanned two generations. The variance components of test-day milk recordings were estimated by the REML (restricted maximum likelihood) method using VCE 6.02 software (<http://vce.tzv.fal.de>) and EBV were estimated by BLUP (best linear unbiased prediction) using PEST software (Groeneveld *et al.* 2002) by the mixed animal model:

$$y = Xb + Z_1a + Z_2pe + e \quad (1)$$

where y is the vector of observations containing the test day results for each of the milk performance traits of each cow (14 045 records), vector b represents the fixed effects, including the effects of interaction year×season of calving, lactation number, linear regression on DIM, vector pe represents the permanent environmental effects and vector a denotes the random additive genetic effects. Vector e considers the residual effects. The known incidence matrices X , Z_1 , Z_2 relate to observations of the corresponding fixed and random effects.

For 252 dairy cows from the above-mentioned 480, official (according to the Polish Ministry of Agriculture Decree 5/1999) animal life EBV data, with the accuracy range of 0.71, were available. Official EBVs and the EBVs estimated by the authors were used separately to analyse the LF genotype influence on animal EBVs for performance traits. The differences between LF genotypes were tested for significance with Tukey range test in the GLM procedure.

Additive and dominance effects of each SNP on animal EBV for milk performance traits were estimated with the use of the orthogonal contrasts. The dominance effect was assumed to be the difference between a solution for heterozygotes and mean solution for the homozygotes.

Results and discussion

Table 1 shows genotype frequencies for the analysed SNPs. No deviation from HWE was observed for the chosen SNPs, although minor allele frequencies, especially regarding the LF-926 A allele, were very low.

Table 1
Genotype frequencies for LF gene SNPs in the studied population

SNP	Genotype	No of animals	Frequency
LF-926	AA	3	0.01
	AG	56	0.12
	GG	410	0.87
LF+32	CC	19	0.04
	GC	130	0.27
	GG	326	0.69
LFex4	AA	284	0.60
	AG	175	0.37
	GG	16	0.03
LFex9	CC	22	0.04
	CT	151	0.32
	TT	306	0.64

Test day LS means for milk performance traits are presented in Table 2. Each SNP was connected to at least one of the traits. All four SNPs influenced fat yield, but only one (LF+32) was associated with fat percentage. The most favourable genotypes in the context of the dairy performance were: LFex9 TT, LF+32 GG and LFex4 AA.

Table 2
Test day LS means (and SE) for milk performance traits in cows carrying different LF genotypes (G)

SNP	G	Milk, kg	SE	Protein, kg	SE	Protein, %	SE	Fat, kg	SE	Fat, %	SE
LF-926	AA	26.02	2.82	0.90	0.10	3.93 ^{c*}	0.38	0.7 ^{AB}	0.12	3.34	0.48
	AG	25.98	0.96	0.81	0.03	3.40	0.13	0.93 ^A	0.04	3.60	0.16
	GG	26.47	1.19	0.84	0.04	3.44 ^c	0.16	0.93 ^B	0.05	3.57	0.20
LF+32	CC	24.38 ^{GH}	1.43	0.79 ^{jk}	0.05	3.44	0.19	0.80 ^U	0.06	3.32 ^X	0.24
	GC	26.42 ^G	1.18	0.84 ^j	0.04	3.44	0.16	0.91 ^{lb}	0.05	3.53	0.20
	GG	26.65 ^H	1.19	0.84 ^k	0.04	3.45	0.16	0.93 ^{lb}	0.05	3.60 ^X	0.20
LFex4	AA	27.01 ^F	1.21	0.86 ^R	0.04	3.47 ^H	0.16	0.94 ^{hg}	0.05	3.57	0.20
	AG	25.94 ^F	1.19	0.82 ^R	0.04	3.43 ^k	0.16	0.91 ^{Dh}	0.05	3.57	0.20
	GG	22.16 ^F	1.12	0.72 ^R	0.04	3.30 ^{Hk}	0.15	0.82 ^{GD}	0.05	3.60	0.19
LFex9	CC	23.22 ^B	1.30	0.76 ^M	0.04	3.37	0.18	0.80 ^{VZ}	0.06	3.48	0.22
	CT	25.75 ^B	1.08	0.81 ^M	0.04	3.40	0.15	0.91 ^{Vz}	0.05	3.58	0.18
	TT	27.03 ^B	1.23	0.86 ^M	0.04	3.48	0.17	0.94 ^{Zz}	0.05	3.57	0.21

Means marked in bold and by the same upper-case letter differ at $P < 0.01$; marked with the same lower-case letter differ at $P < 0.05$

The significance of the genotype by environment interactions are shown in the Tables 3, 4 and 5. Lactoferrin gene variants' influence on milk performance traits was significantly altered by the lactation stage (Table 3). The associations between: LF-926 and LFex9 and milk yield, LFex9 and protein yield, LFex4 and LFex9 and protein and fat percentages, as well as fat yield depended on the stage of lactation. Figure 1 presents an example of a significant interaction: LFex4 genotypes influence fat percentage in different ways, according to the lactation stage. The highest fat percentage was observed in the milk of cows carrying the

GG genotype in early lactation, but not in full or late lactation. The SCC class was the factor that modified the association between the L_Fex9 genotype and milk and protein yield, as well as the associations between L_Fex4 and L_Fex9 genotype and protein and fat percentage (Table 4). An example chart is shown in Figure 2: the L_Fex9 genotype is associated with the fat percentage with the influence of the SCC. The highest fat percentage was observed in healthy cows ($SCC < 200\,000 \text{ ml}^{-1}$) with the L_Fex9 TT genotype, but in cows with subclinical mastitis ($SCC > 200\,000 \text{ ml}^{-1}$) that carried the CC genotype. Parity also influenced the L_F gene variants' impact on the performance traits (Table 5). The correlations between all four SNPs and protein and fat yield were modified by the age of the cow (parity). Also, the associations between L_F gene variants and milk yield were altered by the parity regarding three mutations (excluding L_Fex4 SNP). Despite the differences in numbers of the observation in each class of the environmental factors (e.g. majority of the cows were in their first lactation, which could have affected the estimation), interactions between L_F genotype and environment were significant for most of the performance traits.

Table 3

Significance (*P*-value) of an interaction between L_F genotype and lactation stage (SNP×Stage) effects on milk performance traits

Interaction	Milk, kg	Protein, kg	Protein, %	Fat, kg	Fat, %
LF-926×Stage	0.0387	0.1285	0.4386	0.2196	0.7072
LF+32×Stage	0.7984	0.1154	0.2529	0.7058	0.7058
LFex4×Stage	0.0853	0.1237	0.0482	0.0109	0.0080
LFex9×Stage	0.0002	0.0041	0.0367	0.0001	0.0111

Table 4

Significance (*p*-value) of an interaction between L_F genotype and SCC class (SNP×SCC) effects on milk performance traits

Interaction	Milk, kg	Protein, kg	Protein, %	Fat, kg	Fat, %
LF-926×SCC	0.1738	0.2284	0.2317	0.2072	0.0514
LF+32×SCC	0.2064	0.2047	0.7089	0.8760	0.8073
LFex4×SCC	0.1205	0.0702	0.0014	0.5093	0.0333
LFex9×SCC	0.0421	0.0126	0.0042	0.0523	0.0043

Table 5

Significance (*P*-value) of an interaction between L_F genotype and parity (SNP×parity) effects on milk performance traits

Interaction	Milk, kg	Protein, kg	Protein, %	Fat, kg	Fat, %
LF-926×parity	0.0001	0.0205	0.0629	0.0019	0.0944
LF+32×parity	0.0006	0.0099	0.1128	0.0002	0.1982
LFex4×parity	0.0574	0.0389	0.2069	0.0066	0.0960
LFex9×parity	0.0003	0.0168	0.3013	0.0028	0.3159

Animal EBVs for the milk performance traits in cows carrying different L_F genotypes are shown in Table 6. Results obtained with phenotypic values were compared with the animal EBVs for the corresponding traits. It was observed that the favourable effects of the L_Fex9 genotype TT on animal EBV correspond with phenotypic values for all three milk performance traits (the highest: milk yield, protein and fat yield). The effects of the L_Fex4 AA genotype were

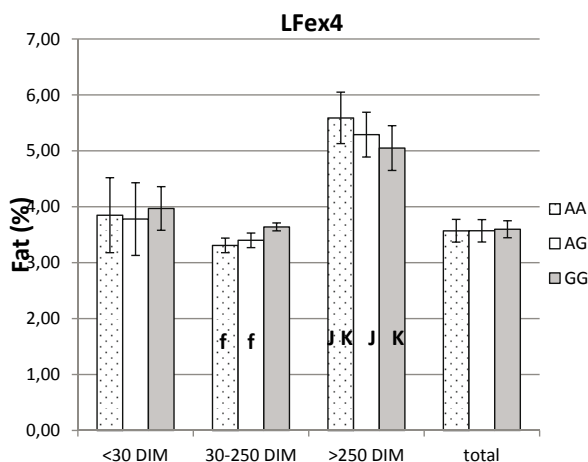


Figure 1

An example of genotype by environment interaction: the influence of LFex4 SNP on fat percentage in different stages of lactation and in whole lactation (total) (Means marked by the same upper-case letter differ at $P < 0.01$; marked with the same lower-case letter differ at $P < 0.05$)

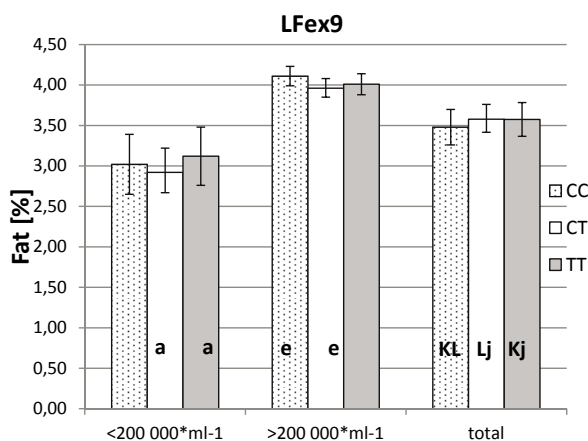


Figure 2

An example of genotype by environment interaction: the influence of LFex9 SNP on fat percentage with regard to SCC (threshold value: 200 000 cells per ml) (Means marked by the same upper-case letter differ at $P < 0.01$; marked with the same lower-case letter differ at $P < 0.05$)

retained for milk and protein yields but not for the fat yield (although a significant difference in milk fat percentage between LFex4 AA and GG genotypes was observed). The effects of the genotypes' influence on milk yield, protein and fat yields are in accordance with the well-known genetic and phenotypic correlations between those traits (Van Vleck & Dong 1988). Therefore, when SNP is positively associated with milk yield it is also positively associated with protein yield. LF-926 and LF+32 genotypes were associated only with animal EBV for fat yield, whereas LFex4 and LFex9 genotypes were associated with milk yield, protein yield and either fat yield (LFex9) or fat percentage (LFex4).

The official EBVs for the milk performance traits carrying different LF genotypes are shown in Table 7. The favourable effect of three genotypes: AG (LF-926) as well as GG (LF+32) on fat yield, and AA (LFex4) on milk yield were confirmed by the analysis based on official EBVs, despite the small number of animals used in the variance analysis (due to the limited availability of official data).

It has been shown that the interrelationships between animal EBV and LF genotype reflect, but only to a limited extent, the associations between the analysed SNPs and milk performance phenotypic value. This can be caused either by the sample size, by the low

Table 6
Animal EBV (LSmeans and SE) for milk performance traits of cows carrying different LF genotypes

SNP		Milk, kg	SE	Protein, kg	SE	Protein, %	SE	Fat (kg)	SE	Fat, %	SE
LF-926	AA	-1.849	1.590	0.062	0.051	0.033	0.080	-0.084 ^{xy}	0.048	-0.031	0.202
	AG	0.212	0.368	0.011	0.012	0.008	0.019	0.011 ^y	0.012	0.008	0.047
	GG	0.037	0.136	0.004	0.004	0.008	0.007	0.008 ^x	0.005	0.022	0.017
LF+32	CC	-0.530	0.633	-0.001	0.020	0.006	0.032	-0.030 ^A	0.019	-0.070	0.080
	CG	0.018	0.242	0.003	0.008	0.026	0.012	-0.001	0.007	-0.014	0.031
	GG	0.088	0.153	0.005	0.005	0.006	0.008	0.013 ^A	0.005	0.037	0.019
LFex4	AA	0.282 ^A	0.162	0.011 ^A	0.005	0.005	0.008	0.010	0.005	0.001 ^a	0.021
	AG	-0.153 ^B	0.206	-0.001 ^B	0.007	0.008	0.0104	0.007	0.006	0.032	0.026
	GG	-2.070 ^{AB}	0.681	-0.061 ^{AB}	0.022	0.071	0.034	-0.029	0.021	0.185 ^a	0.087
LFex9	CC	-1.446 ^{ab}	0.583	-0.039 ^A	0.019	0.050	0.029	-0.027 ^a	0.017	0.116	0.074
	CT	-0.223 ^a	0.223	-0.007 ^b	0.007	0.002	0.011	-0.001	0.067	0.016	0.028
	TT	0.247 ^B	0.156	0.012 ^{ab}	0.005	0.009	0.008	0.012 ^a	0.005	0.013	0.020

Means marked by the same upper-case letter differ at $p < 0.01$; marked with the same lower-case letter differ at $p < 0.05$

Table 7
Official EBV for milk performance traits of cows carrying different LF genotypes

SNP	G	N	Milk, kg	SE	Protein, kg	SE	Protein, %	SE	Fat, kg	SE	Fat, %	SE
LF-926	AG	32	364.757	104.146	10.739	2.523	-0.015	0.024	17.294 ^A	3.321	0.023	0.022
	GG	220	259.945	75.005	8.822	1.817	0.011	0.018	7.571 ^A	2.392	-0.04	0.045
LF+32	CC	8	203.482	163.913	8.221	3.971	0.029	0.038	3.386 ^a	5.227	-0.082	0.098
	GC	64	437.586	76.811	13.059 ^b	1.861	-0.012	0.018	15.807	2.449	-0.027	0.046
Lfex4	GG	180	295.984	93.906	8.062 ^b	2.275	-0.024	0.022	18.104 ^a	2.994	0.084	0.056
	AA	151	430.931 ^c	98.02	11.236	2.375	-0.049 ^e	0.023	11.786	3.125	-0.106	0.059
	AG	92	227.012 ^c	91.252	8.626	2.211	0.021 ^e	0.021	9.979	2.91	0.014	0.055
	GG	9	279.109	172.039	9.48	4.168	0.021	0.04	15.531	5.486	0.067	0.103
Lfex9	CC	14	337.48	133.415	10.907	3.232	-0.004	0.031	16.493	4.254	0.036	0.08
	CT	84	349.013	108.499	8.722	2.629	-0.042 ^d	0.025	12.35	3.46	-0.05	0.065
	TT	154	250.559	103.461	9.713	2.507	0.039 ^d	0.024	8.454	3.299	-0.011	0.062

Means marked by the same upper-case letter differ at $p < 0.01$; marked with the same lower-case letter differ at $p < 0.05$

accuracy of the animal EBV prediction, or by the environmental impact on the animal genome expression. We speculate that the basis of the observed divergence in our population lies in the latter, as the analysis of environmental factors has shown that they significantly influence the relationship between LF SNPs and dairy performance.

We found that the »re-ranging« of LF genotype's influence on milk production traits occurs when cows are suffering from subclinical *mastitis* (SCC > 200 000 per ml). Moreover, it shows up in successive parities, and even within the particular lactation (effects of the genotypes on milk traits change with DIM). It is not common to use factors like those used in our study (lactation stage, parity, SCC class) to evaluate genotype × environment interaction's effect on milk performance. The main environmental factors analysed so far included: feeding system (Kearney *et al.* 2004), reproduction-related factors (Haile-Mariam *et al.* 2008) or the geographic environment (Hammami *et al.* 2009). To our knowledge, an exclusive analysis, where the parity was included as a factor that influenced associations between LF genotype and health, was made by Wojdak-Maksymiec *et al.* (2013). The above-mentioned authors found that parity remarkably influences LF genotype impact on mastitis susceptibility.

The additive and dominance effects in each of four LF loci were estimated. No dominance effect was observed for any of the SNPs and additive effects were significant only considering milk and protein yields for LFex4 and LFex9 SNPs, fat yield for LF-926, LF+32 and LFex9 SNPs and fat percentage for LFex4 SNP. Summing up the results, we found that the most favourable lactoferrin genotypes in the context of dairy performance are: LF-926 GG, LF+32 GG and GC, LFex4 AA, LFex9 TT.

The relationship between LF SNPs and animal EBV is cleared from the environmental impact, therefore we suspect that LF-926, LF+32 and LFex4 SNPs are the most promising SNPs regarding their associations with dairy performance.

The result which is worth highlighting is LFex4 polymorphism's influence on the milk traits. The association between genotype and milk yield is neither violated by the lactation stage, nor by the other two environmental factors analysed in this study. Moreover, the favourable effect of LFex4 AA genotype was retained with the official EBV data used for the estimation.

The pleiotropic effect of LF gene variants on functional and performance traits was studied previously by other groups (Zabolewicz *et al.* 2012, O'Halloran *et al.* 2010, Kaminski *et al.* 2006). Zabolewicz *et al.* (2012) analysed SNP, located in the LF gene TATA box (in position: 5'–28), but found no statistically significant influence of lactoferrin genotype on milk performance in Polish Holsteins. The same polymorphism, along with two others, localized in the promoter region (in positions: –586, and –190) was investigated by O'Halloran *et al.* (2010). None of the three polymorphisms were associated with the Daughter Yield Deviations (DYD) for any of the dairy performance traits (milk yield, fat yield, protein yield). O'Halloran *et al.* (2010) carried out an additional analysis, in which they included genotype as a class effect and found a significant association of LF-28 SNP with milk yield and protein yield. On the other hand, significant associations of bovine LF gene with milk traits were also found and were described in the two following papers (Kaminski *et al.* 2006 and Sender *et al.* 2007). The results of Kaminski *et al.* (2006) regarding LF+32 SNP were contrary to those presented in this paper (they found LF+32 genotype CC to be the most favourable), but the authors based their deduction on the phenotypic values of milk performance traits only. Such analysis can display a fake picture of the animal genetic effect. A study conducted by Sender *et al.* (2007) showed

a significant interrelationship between one of the intronic variants of the LF gene and the milk yield of 516 Polish Holsteins (phenotypic value). Discrepancies in the results obtained by different groups may come from different statistical models used for the evaluations (e.g. underestimation of other effects, like genotype by environment interaction), or the usage of phenotypic data without the animal EBV information. They may also be a result of the linkage phase between the actual QTLs for performance traits and analysed SNPs in different populations. Moreover, lactoferrin is involved in many physiological processes and possesses multiple activities (eg. Adlerova *et al.*, 2008); the results of experiments, conducted on dairy cows during their lactation may also be influenced by the biological functions of the protein, especially regarding to the analyse of the phenotype

In conclusion, it must be highlighted that the pleiotropic effects of genes coding for functional traits should be taken into account during the analysis of their impact on health (or fitness)-related traits, like mastitis susceptibility. It was shown that each of four genetic variants of LF is associated with at least one of the main dairy performance traits and animal EBV for those traits. The strongest association was observed for LF+32 and LFex4 SNPs, for which associations between genotype and performance traits were observed for both self-estimated and official EBVs.

We would like to highlight the genotype by environment interaction which was described in this study. The results of this study suggest that analyses similar to ours can be helpful for the assessment of genetic markers' usefulness for further selection. This paper asserts a validation of the obtained results regarding age (parity), health (SCC) and lactation stage, as genotype by environment interaction significantly changes associations between genes and corresponding traits, which was hereby shown.

Acknowledgements

This work was supported by the Polish National Science Centre, Project Nos. N N311311535, and 2011/01/N/NZ9/00567.

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