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Polymorphisms in the promoter of interleukin-12β2 and interleukin-23 receptor genes influence milk production traits in Chinese Holstein cows

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SUMMARY

Interleukin-12 (*IL-12*) and interleukin-23 (*IL-23*) are proinflammatory cytokines produced by macrophages and dendritic cells in response to infection with intracellular pathogens. Given the importance of *IL-12* and *IL-23* for modulating inflammation and the host immune response, the *IL-12* and *IL-23* receptor genes may be suitable candidate genes for studying disease resistance in dairy cattle. Twenty Chinese Holstein cows were selected randomly for PCR amplification and sequencing, and used for SNP discovery in the bovine *IL-12Rβ2* and *IL-23R* promoter region. One SNP(*c.-246G > T*) in *IL-12Rβ2* gene and 2 SNPs (*c.-856A > G* and *c.-207T > C*) in *IL-23R* gene were identified. Chinese Holstein cows ($n=866$) were then genotyped using Sequenom MassARRAY (Sequenom Inc., San Diego, CA) based on the 3 identified SNPs, and the associations between SNPs or haplotype of the genes and milk production traits, SCS were analyzed by the least squares method in the GLM procedure of SAS. The *IL-23R*c.-856A > G and *IL-23R*c.-207T > C showed close linkage disequilibrium ($r^2=0.89$). No association was found with SCS, but associations were found between 3 of these SNP with milk protein content and lactose content. The software MatInspector revealed that these SNPs were located within several potential transcription factor binding sites, and may alter gene expression, but further investigation will be required to elucidate the biological and practical relevance of these SNP.

1. Introduction

Mastitis is the most frequent and important disease in dairy cattle worldwide with great economic losses to the dairy industry (Nash et al., 2003). Incidence rates for clinical mastitis in different dairy cattle populations ranged between 25% and 60% (Ruegg, 2003), and even higher incidence rates were reported for Chinese Holstein cows (Mao et al., 2011). A prerequisite for direct selection strategies on disease resistance implies installation of suitable recording schemes and recording technique. Hence, most breeding programs use the suitable indicator trait SCS being available from official milk recording schemes. (Shook, 2006). The indirect selection strategy based on SCS is effective, because of the strong positive genetic correlation with clinical mastitis (Rupp and Boichard, 1999).

The proinflammatory cytokines *IL-12* and *IL-23* are important regulators of inflammation and of the host immune response. *Interleukin-12* is a heterodimer composed of two covalently linked proteins, p35 and p40

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(Yilmaz et al., 2005). Interleukin-12 is generally produced by macrophages and dendritic cells in response to infection with intracellular bacteria (van de Vosse et al., 2003). Alluwaimi et al. (2003) investigated *IL-12* expressions in the *Staphylococcus aureus*-infected bovine mammary gland. Transcriptional activity was significantly increased 24 h after an infection. The *IL-12* receptor *(IL-12R*) is composed of two subunits, β-1 and β-2, which are each coded by different genes. *Interleukin-23* is a close relative of *IL-12,* and also plays an important role in the inflammatory response (Langrish et al., 2004). *IL-23* shares a subunit with *IL-12, IL-12/23p40*, and comprises a specific subunit, *IL-23p19*. Likewise, the *IL-12Rβ1* chain pairs with the IL-23 receptor (*IL-23R*) form the high-affinity *IL-23R*. A recent genome-wide association study (GWAS) in humans identified an association between a polymorphism in the *IL23R–IL12Rβ2* locus (rs924080) and Behcet's disease (BD) (Remmers et al., 2010). Those associations were confirmed by a subsequent study (Xavier et al., 2012). For the bovine animal, only Skelding et al. (2010) reported the polymorphism of *IL-12R* and *IL-23R* based on their study in Canadian Holsteins. They identified several SNPs in the 5′ upstream region of the *IL-12Rβ2* and *IL-23R* gene, but associations between SNPs and SCS were not significant. In contrast, pronounced associations between SNPs located in the *IL-12Rβ2* gene (c.-511A > G) and productivity (milk and protein yield) were identified. In summary, a variety of studies identified the importance of these genes with regard to the regulation of inflammatory and immune response. Furthermore, the potential impact of genetic variants within these genes on disease susceptibility for a variety of bovine inflammatory disorders, e.g. clinical mastitis and milking traits, was widely discussed. Hence, the objectives of this study were (i) to determine the occurrence of SNPs within the promoter region of bovine *IL-12β2* and *IL-23* receptor genes for Chinese Holstein cows, and subsequently (ii) to identify associations between those SNPs with SCS and milk production traits.

2. Material and methods

2.1. Animal and milk production records

The study was approved by the Animal Care Committee of Yangzhou University. Holstein cows from farms located in the district of Yanchen City were housed in a tie-stall barn. Feeding based on a total mixed ration (TMR), and cows were milked three times per day. The DC-305 software was used for dairy cow management.

The bloods of 866 cows were sampled from the tail vein by using a vacuum tube. Data included 43 sire families with 3 to 45 daughters per sire. Phenotypic data comprised 19,864 test-day records from in total 1886 lactations (lactations 1 to 3) of 866 cows. Mixed milk (morning:noon:night=4:3:3) samples were collected monthly from June 2010 to December 2012. Samples were treated with potassium bichromate (30 mg/tube) immediately after milking, and analyzed for SCC based on flow cytometry (Fossmatic 5000, Foss Electric, Denmark). Infrared technique (Milkoscan 6000, Foss Electric, Denmark) was used to determine the concentrations of fat, protein, lactose, total solids (TS) and milk urea nitrogen (MUN). Production data included test-day observations for milk yield (TDMY), fat content (FC), protein content (PC), lactose content (LC), MUN and somatic cell count (SCC). Somatic cell score (SCS) was calculated using the formula:$SCS = \log_2(SCC/100,000) + 3$(Wiggans and Shook, 1987). Data were obtained from the DHI lab of the Shanghai Dairy Cattle Breeding Center. Editing of data was performed to ensure both reliability and consistency for statistical analyses. Requirements were designed as follows: TDMY between 2 and 60 kg, FC between 2% and 7%, PC between 2% and 6%, LC between 2% and 5.5%, TS content between 9% and 18%, MUN between 5% and 40%, SCC between 1×10^3 and 9999×10^3, and SCS between 0 and 9. Only records from parities 1 to 3, and from 8 to 305 days in milk (DIM) were included. Finally, 14,157 test-day records were included in this study. The mean values and standard deviations for the analyzed traits stratified by parity are summarized in Table 1

2.2. DNA extraction

A standard phenol–chloroform procedure was used to extract DNA from blood, with a slight modification in centrifugation speed and time (Winfrey et al., 1997). An Eppendorf Biophotometer (Berlin, Germany) was used to assess the DNA concentration and DNA quality based on absorbance of UV light at 260 and 280 nm.

2.3. SNP discovery

Twenty cows were selected randomly for PCR amplification and sequencing, and then used for SNP discovery in the bovine *IL-12Rβ2* and *IL-23R* promotor region. The bovine gene sequences for *IL-12Rβ2* and *IL-23R* were accessed using the Ensembl genome browser (http://www.ensembl.org/index.html). These sequences were used to design primers with the Primer3 software (http://biotools.umassmed.edu/bioapps/primer3_www.cgi) to amplify about 1 kb of the putative promoter region of each gene.

Table 1
Descriptive statistics for milk production traits by parity (means ± SD).

Parity	Records number	TDMY (kg)	FC (%)	PC (%)	SCS	LC (%)	TS (%)	MUN (g/100 mL)
1	7077	28.91 ± 6.81	4.15 ± 0.78	3.35 ± 0.38	2.04 ± 1.46	5.01 ± 0.19	13.51 ± 1.19	10.92 ± 2.31
2	6446	32.84 ± 9.70	4.39 ± 0.79	3.39 ± 0.39	1.83 ± 1.44	4.93 ± 0.22	14.08 ± 1.30	12.51 ± 3.16
3	634	40.28 ± 10.07	4.63 ± 0.87	3.18 ± 0.33	1.94 ± 1.79	4.89 ± 0.21	14.47 ± 1.46	13.59 ± 2.00
Total	14,157	31.21 ± 8.84	4.28 ± 0.8	3.36 ± 0.38	1.94 ± 1.47	4.97 ± 0.21	13.81 ± 1.3	11.76 ± 2.85

TDMY: test-day milk yield; FC: fat content; PC: protein content; LC: lactose content; TS: total solid; MUN: milk urea nitrogen; SCS: somatic cell score.