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Short Communication

The bovine CXCR1 gene is highly polymorphic

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ABSTRACT

Several single nucleotide polymorphisms (SNP) in the bovine CXCR1 gene have been implicated in resistance to mastitis and milk somatic cell counts in several sample populations of Holstein dairy cows. As such, a more thorough understanding of SNP present in and near the bovine CXCR1 gene is needed. This study identified 36 SNP in the coding region and surrounding sequences of CXCR1 in 88 Holstein dairy cows. Four SNP induced amino acid changes and 1 SNP an early stop codon. Two amino acid changes occur in the third intracellular loop and C-terminus in locations tied to intracellular signaling. The 36 SNP could be subdivided into 4 separate linkage groups. Using representative or 'tag' SNP from each linkage group, haplotypes or the combination of SNP found on a single allele were generated to increase the specificity of an animal's genetic background. Four haplotypes were identified that represented 99% of the sample population. The haplotypes generated using tag SNP agreed with haplotypes generated from SNP causing amino acid changes. In conclusion, the CXCR1 gene is highly polymorphic and has potential implications towards genetic selection and understanding host factors that increase the risk of infection.

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1. Introduction

The bovine CXCR1 gene is a high affinity receptor for the chemokine interleukin (IL)-8 or CXCL8 (Lahouassa et al., 2008). The CXCR1 ligand, IL-8 is released during infection by epithelial cells, macrophages, endothelial cells, fibroblasts and other cells in response to invading pathogens or stressors such as low oxygen, oxidative stress, and low glutamine (DeForge et al., 1993; Desbaillets et al., 1997; Shuster et al., 1997; Rambeaud et al., 2003; Bannerman et al., 2004; Bobrovnikova-Marjon et al., 2004). Subsequent binding of IL-8 to CXCR1 or the more promiscuous CXCR2 induces migration, regulates cell survival, modifies cytokine production, and increases phagocytosis, and reactive oxygen species generation (Kettritz et al., 1998; Mitchell et al., 2003; Lahouassa et al., 2008). The primary population influenced by IL-8/CXCR1 binding has traditionally been attributed to neutrophils that have the greatest expression of CXCR1 (Grob et al., 1990). However, recent studies have implicated this ligand–receptor combination in migration and survival of metastatic breast cancer, melanoma, and prostate cancer cells (Charafe-Jauffret et al., 2009; Gabellini et al., 2009; Shamaladevi et al., 2009). CXCR1 also has been observed on NK, CD4+ T, CD8+ T, dendritic, epithelial, and endothelial cells and presumably would also influence cellular migration and survival (Sallusto et al., 1998; Murdoch et al., 1999; Takata et al., 2004; Berahovich et al., 2006; Gasser et al., 2006). With the significant rise in IL-8 typically observed during periods of inflammation and stress, the presence of CXCR1 on multiple cell types that influence immunity, and the ability to modify cellular migration and survival, suggests the IL-8/CXCR1 axis could have a significant impact on disease resistance.

Recent studies have demonstrated the bovine CXCR1 gene contains at least 8 single nucleotide polymorphisms (SNP) (Youngerman et al., 2004b; Leyva-Baca et al., 2008). A SNP at position CXCR1+777 (G>C) relative to

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the mRNA sequence (U19947) leads to a Q245H amino acid replacement that has been significantly associated with alterations in bovine neutrophil migration, apoptosis, adhesion molecule expression, and reactive oxygen species generation (Rambeaud and Pighetti, 2005, 2007; Rambeaud et al., 2006). In a sample Holstein population, cows with a CC genotype experienced a higher incidence of chronic intramammary infections (37%) versus cows with either a GG (22%) or GC (22%) genetic background (Youngerman et al., 2004a). Later studies examining the relationship of this SNP to a common indicator of intramammary infection, somatic cell count (SCC) or score (SCS) have provided mixed results. Two large-scale studies that examined SCS in daughters or granddaughters of bulls, revealed no significant association with SCS (Leyva-Baca et al., 2008; Goertz et al., 2009; Beecher et al., 2010). However, an on-farm study with approximately 250 cows across 5 breeds revealed an association between CXCR1+777 SNP and SCS ($P < 0.10$) (Beecher et al., 2010). In contrast to CXCR1+777, the SNP CXCR1−1768 relative to NM_001557.2 was significantly associated with SCS (Leyva-Baca et al., 2008). The association of at least two SNP with bovine neutrophil function and measures of mastitis susceptibility suggests this gene is critical to disease resistance. Thus a better understanding of the genomic structure and polymorphisms present in the bovine CXCR1 gene, along with a common numbering system are needed.

2. Materials and methods

Total RNA was isolated from bovine neutrophils (TriZol, Invitrogen, Carlsbad, CA). The 5′ and 3′ ends were identified by rapid amplification (GeneRacer; Invitrogen), cloning into pGEMTeasy vectors, and sequencing by the University of Tennessee core facility. The 5′ and 3′ cDNA ends were assembled using Invitrogen Vector NTI (v 10.0) to generate the full length cDNA sequence. The full length cDNA sequence was then aligned with the Bovine UCSC browser (Bos taurus, build 4.0) to identify exon and intron boundaries and submitted to Genbank (accession HM367082).

To identify polymorphisms in CXCR1, genomic DNA was isolated from 88 cows located at the Middle Tennessee Research and Education Center (Springfield, TN) that had completed at least one full lactation. Genomic DNA spanning the coding region and sequences 5′ and 3′ to the coding region were amplified by PCR, sequenced by the University of Tennessee core facility, and polymorphisms detected using Sequencher 4.2 software (Gene Codes Corp., Ann Arbor, MI). Selected areas of the CXCR1 gene were amplified as follows: 40 ng of bovine genomic DNA was used as template in a 20 μl reaction containing specific primers and Eppendorf HotMaster Mix (Eppendorf North America; Westbury, NY) according to manufacturer's guidelines. The conditions for amplification were as follows: an initial hot-start denaturation occurred at 94 °C for 2 min, followed by 37 cycles of 94 °C denaturation for 30 s, 58–61 °C annealing for 30 s, and 68 °C extension for 45 s. After the last cycle, a 10 min final extension step at 68 °C was added before reactions were chilled to 4 °C. Amplified products were purified to remove primer and excess nucleotides by treatment with exonuclease-shrimp alkaline phosphatase (Exosap-it, USB Corporation; Cleveland, OH) as per the manufacturer's guidelines. Haplotypes were generated using PHASE (Stephens et al., 2001; Stephens and Scheet, 2005). Using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/), Bovine CXCR1 (HM367082/ADK47157.1) also was compared against human (NM_000634.2/NP_000625.1), mouse (NM_178241.4/NP_839972.1), rat (NM_019310.1/NP_062183.1), rabbit (NM_001171082.1/NP_001164553.1), and swine (XM_003133655.2/XP_003133703.2) CXCR1 protein sequences to determine amino acid conservation and similarity among species (Table 1 and Fig. 1). Swine currently does not have a reference sequence for CXCR1 but the sequence chosen for comparison (XM_003133655.2) was based on chromosomal position on Sscrofa9.2 (UCSC genome browser).

Table 1
Similarity scores among CXCR1 protein sequences evaluated through ClustalW2.

	Bovine	Human	Swine	Mouse	Rat	Rabbit
Bovine	100	72	86	62	61	76
Human		100	75	64	66	83
Swine			100	63	63	76
Mouse				100	89	66
Rat					100	67
Rabbit						100

3. Results and discussion

The current full length cDNA sequences available for bovine CXCR1 in Genbank indicate a gene with a single exon (NM_001105038.1, U19947.1, NM_174360.2). However, in humans CXCR1 contains two exons (Sprenger et al., 1994), suggesting the full length bovine mRNA sequence has not yet been determined. Therefore, in order to accurately reflect the nature of polymorphisms identified, as well as conform to naming standards, it was necessary to clone the full length cDNA sequence. An 1800 bp cDNA sequence was generated from bovine neutrophils (Genbank HM367082) which shares 99% identity with the bovine CXCR1 reference sequence (NM_001105038.1). Subsequent alignment to genomic DNA (AC150887.4) using BLAST (http://blast.ncbi.nlm.nih.gov) revealed CXCR1 has two exons and 1 intron, which corresponds to the genomic structure of human CXCR1 (NG_011814.1) (Sprenger et al., 1994). Exon 1 is 87 bp (149,023–149,109 bp of AC150887.4), exon 2 is 1713 bp (151,327–153,041 bp of AC150887.4) with an open reading frame of 1081 bp, and the intervening intron is 2219 bp, which is consistent with predicted bovine CXCR1 (Pighetti and Rambeaud, 2006).

In CXCR1, 36 nucleotide polymorphisms were identified (Table 2). Eleven were located in the coding region: 4 introduced amino acid changes, 1 introduced a stop codon, and 6 were synonymous. The remaining 25 polymorphisms were located in the first intron, 3′ untranslated region, and 3′ to the gene. Naming of the polymorphisms is based on the Human Genome Variation Society guidelines (http://www.hgvs.org/mutnomen/recs.html), with the A of the ATG translation start site serving as position 1. Six of

```
Bovine          ------MTIILKDLSNSSYLWEGFEDEFENYSGTPPTEDYDYSPCEISTETLNKYAVVVI 54
Swine           MNMAGDSDRIMTAVFDDKDLLELFGDDFENFTGTPPTDEHYYSPCRIDTESLNKYAVVVI 60
Human           -------------MSNITDPQMWDFDDLN-FTGMPPADE-DYSPCMLETETLNKYVVIIA 45
Rabbit          ---------MEVNVWNMTDLWTWFEDEFANATGMPPVEK-DYSPCLVVTQTLNKYVVVVI 50
Mouse           --------MAEAEYFIWTNPEGDFEKEFGNITGMLPTGD-YFIPCKRVPIT-NRQALVVF 50
Rat             --------MAEAEYFIWIAPEGDFEEEFGNITRMLPTGE-YFSPCKRVPMT-NRQAVVVF 50
                                          .::   :   *. .  : **   . : *: .:::

Bovine          YALVFLLSLLGNSLVMLVILYSRIGRSVTDVYLLNLAMADLLFAMTLPIWAASKAKGWIF 114
Swine           YALVFLLSLLGNSLVMLVILYSRVGRSVTDVYLLNLAMADLLFALTLPIWATSKAKGWIF 120
Human           YALVFLLSLLGNSLVMLVILYSRVGRSVTDVYLLNLALADLLFALTLPIWAASKVNGWIF 105
Rabbit          YALVFLLSLLGNSLVMLVILYSRSNRSVTDVYLLNLAMADLLFALTMPIWAVSKEKGWIF 110
Mouse           YALVSLLSLLGNSLVMLVILYRRRTRSVMDVYVLNLAIADLLFSLTLPFLAVSKLKGWIF 110
Rat             YALVFLLSLLGNSLVMLVILYRRRTRSVTDVYVLNLAIADLLFSLTLPFLAVSKWKGWIF 110
                **** *************** *  *** ***:****:*****::*:*: *.** :****
                       V122A
Bovine          GTPLCKVASLLKEVNFYSGILLLACISMDRYLAIVHATRTLTQKRHWVKFICLGIWALSV 174
Swine           GTPLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRSLTQKRHWVKFICTGIWALSL 180
Human           GTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRHLVKFVCLGCWGLSM 165
Rabbit          GTPLCKVVSLVKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRHLVKFICLGIWALSL 170
Mouse           GTPLCKMVSLLKEFNFFSGILLLACISVDRYLAIVHATRTLARKRYLVKFVCVGIWGLSL 170
Rat             GTPLCKMVSLLKEVNFFSGILLLACISVDRYLAIVHATRTLTRKRYLVKFVCMGTWGLSL 170
                ** ***:.**:**.**:**********:***********:*::**: ***:* * *.**:
                                                 W207X
Bovine          ILALPIFIFREAYQPPYSDLVCYEDLGANTTKWRMIMRVLPQTFGFLLPLLVMLFCYGFT 234
Swine           VLALPIFIFREAYHPPYSSPVCYEDLGANTTKWRMVMRVLPQTFGFLLPLLVMLFCYGLT 240
Human           NLSLPFFLFRQAYHPNNSSPVCYEVLGNDTAKWRMVLRILPHTFGFIVPLFVMLFCYGFT 225
Rabbit          ILSLPFFLFRQVFSPNNSSPVCYEDLGHNTAKWRMVLRILPHTFGFILPLLVMLFCYGFT 230
Mouse           ILSLPFAIFRQAYKPFRSGTVCYEVLGEATTDFRMTLRGLSHIFGFLLPLLTMLVCYGLT 230
Rat             VLSLPFAIFRQAYKPYRSGTVCYEVLGEATADLRITLRGLSHIFGFLLPLFIMLVCYGLT 230
                 *:**: :**:.: *  *. **** **  *:. *: :* *.: ***::**: **.***:*
                           Q245H
Bovine          LRTLFSAQMGQKHRAMRVIFAVVLVFLLCWLPYNLVLIADTLMRAHVIAETCQRRNDIGR 294
Swine           LRTLFAAHMGQKHRAMRVIFAVVLVFLLCWLPYNLVLVADTLMRARVIAETCERRNDIGR 300
Human           LRTLFKAHMGQKHRAMRVIFAVVLIFLLCWLPYNLVLLADTLMRTQVIQESCERRNNIGR 285
Rabbit          LRTLFQAHMGQKHRAMRVIFAVVLIFLLCWLPYNLVLLADTLMRTHVIQETCQRRNDIDR 290
Mouse           LRMLFKTHMRQKHRAMGVIFAVVLVFLLCCLPYNLVLLSDTLLGAHLIEDTCERRNDIDQ 290
Rat             LRTLFKAHMRQKRRAMWVIFAVVLVFLLCCLPYNLVLLSDTLLGAHLIQDTCERRNNIDQ 290
                ** ** ::* **:*** *******:**** *******::***: ::* ::*:***:*.:
                                           K327R H332R
Bovine          ALDATEILGFLHSCLNPLIYVFIGQKFRHGLIRIMAIRGLISKEFLAKDGRPSFVGSSSG 354
Swine           ALDATEILGFLHSCLNPLIYVFIGQKFRHGLIKIMAIHGLISKEYLVKDGRPSFVGSSSG 360
Human           ALDATEILGFLHSCLNPIIYAFIGQNFRHGFIKILAMHGLVSKEFLARHRVTSYT-SSSV 344
Rabbit          ALDATEILGFLHSCLNPIIYAFIGQNFRNGFIKMLAARGLISKEFLTRHRVTSYT-SSST 349
Mouse           ALYITEILGFSHSCLNPIIYAFVGQNFRHEFIKILANHGLVRKEVLT-HRRVAFHTSLTA 349
Rat             ALYITEILGFSHSCLNPVIYAFVGQSFRHEFIKILAN--LVHKEVLT-HHSASFRTSLTT 347
                **  ****** ******:**.*:**.**: :*::*    *: ** *. .   ::  * :

Bovine          NTSTTL 360
Swine           NTSTTL 366
Human           NVSSNL 350
Rabbit          NVPSNL 355
Mouse           IY---- 351
Rat             IY---- 349
```

Fig. 1. CXCR1 protein sequences of bovine, swine, human, rabbit, mouse and rat. Bovine CXCR1 SNP that cause amino acid changes are indicated and marked with a red box. Conserved residues are indicated by an asterisk (*), residues with strongly similar properties and a score >0.5 in the Gonnet PAM 250 matrix by a semicolon (:), and residues with weakly similar properties and a score <0.05 in the Gonnet PAM 250 matrix with a period (.). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

the polymorphisms presented in Table 2 have been identified previously, but the naming is slightly different based on numbering conventions used within each paper and is noted within the table.

Because CXCR1 is highly polymorphic, identifying polymorphisms in linkage would provide 'tag' SNP that could be used to represent each group, provide insight to gene evolution, and offer greater specificity for genetic selection and disease association studies. Four linkage groups were identified using LDselect (Carlson et al., 2004) with a minimum allele frequency of 10% and a minimum correlation of 95% (Table 2). The first linkage group contains the most polymorphisms, including CXCR1c.+365 and CXCR1+735 which induce amino acid changes. The

Table 2
Identification of bovine CXCR1 SNP and division into linkage groups.

CXCR1 SNP position[a]	Nucleotide change	Amino acid change	Location	Position	Linkage group	SNP equivilant in prior publications
c.−20-1648	A>G			Intron 1		−1830 in Leyva-Baca et al. (2008)
c.−20-1586	T>A			Intron 1		−1768 in Leyva-Baca et al. (2008)
c.−20-461	T>C			Intron 1	2	
c.−20-445	T>A			Intron 1	2	
c.−20-357	T>A			Intron 1	1	
c.−20-185	C>T			Intron 1	4	
c.−20-162	T>C			Intron 1	2	−344 in Leyva-Baca et al. (2008)
c.+291	C>T		CDS	Exon 2	3	
c.+365	T>C	V122A	CDS	Exon 2	1	
c.+570	G>A		CDS	Exon 2	1	
c.+621	G>A	W207X	CDS	Exon 2	4	
c.+735	C>G	H245Q	CDS	Exon 2	1	+777 in Youngerman et al. (2004a,b)
c.+816	C>A		CDS	Exon 2	3	+858 in Youngerman et al. (2004a,b)
c.+819	G>A		CDS	Exon 2	1	+861 in Youngerman et al. (2004a,b)
+980	A>G	K327R	CDS	Exon 2	2	
+995	A>G	H332R	CDS	Exon 2	2	
+1008	C>T		CDS	Exon 2	3	
+1068	G>A		CDS	Exon 2	1	
*6	C>A		3′ UTR	Exon 2	1	
*7	A>C		3′ UTR	Exon 2	1	
*10	C>T		3′ UTR	Exon 2	1	
*15	C>T		3′ UTR	Exon 2	1	
*97	G>A		3′ UTR	Exon 2	2	
*199	G>A		3′ UTR	Exon 2	1	
*275	T>A		3′ UTR	Exon 2	1	
*278	G>A		3′ UTR	Exon 2	1	
*290	C>A		3′ UTR	Exon 2	1	
*295	T>C		3′ UTR	Exon 2	1	
*298	T>C		3′ UTR	Exon 2	1	
*301	A>T		3′ UTR	Exon 2	1	
*316	G>A		3′ UTR	Exon 2	1	
*428	A>G		3′ UTR	Exon 2	1	
*792	G>A			3′ to gene	3	
*854	A>G			3′ to gene	1	
*866	G>C			3′ to gene	1	
*1067	T>C			3′ to gene	1	
*1079	C>T			3′ to gene	3	
*1100	G>A			3′ to gene	1	

[a] Naming conventions are based on Human Genome Variation Society Guidelines, with the c. denoting a cDNA sequence and position relative to the transcription start site and genomic DNA sequence.
The 'A' of the ATG translation initiation sequence is 1. The position in the intron is given as the start of exon 2 (−20 in this case) minus the number of nucleotides 5′ to the beginning of exon 2. The * indicates a position 3′ to the stop translation signal.

second linkage group is smaller, containing 6 polymorphisms, of which two induce amino acid changes: CXCR1c.+980 and CXCR1c.+995. The third linkage group contains 5 synonymous polymorphisms, while the fourth linkage group contains two polymorphisms, of which an early stop codon is introduced by CXCR1c.+621. The tag SNP chosen were: +621, +735, +816, and +980 which were identified in prior publications and/or induced an amino acid change (Youngerman et al., 2004b). The combination of polymorphisms found in an allele (e.g. haplotype) then was determined using PHASE which resolved greater than 95% of the haplotypes (Table 3). Of the seven haplotypes identified, four represented approximately 99% of the sample population: GCCA (31.8%), AGCG (29.5%), GCAG (19.3%), GGCG (18.2%), GCCG (0.6%), GCAA (0.6%), and ACCA (0.03%).

With four amino acid changes and early termination being possible within bovine CXCR1, the combination of SNP in a single allele could provide insight into potential changes in receptor function. To address this, an alternative set of haplotypes based on amino acid changes were generated that reflected SNP combinations occurring at nucleotide positions +365, +621, +735, +980, and +995 (Table 3). Of the five haplotypes identified, four represented >99% of the population: TGCAA (32.4%), CAGGG (29.5%), TGCGG (19.9%), CGGGG (18.2%), and TACAA (0.01%) which led to amino acid combinations of VWHKH, AXQRR, VWHRR, AWQRR, and VXHKH at positions 122, 207, 245, 327, and 332, respectively. The frequencies and composition of the dominant amino acid haplotypes were consistent with those identified using CXCR1 tag SNP (Table 3). This finding was not unexpected as the amino acid haplotypes generated contained 3 of the 4 linkage groups.

Based upon the amino-acid generated haplotypes, SNP at locations CXCR1c.+365 and CXCR1c.+735 generate receptors expressing either $V_{122}H_{245}$ (~52%) or $A_{122}Q_{245}$ (~47%). The SNP CXCR1c.+365T>C leads to a V122A switch in the first extracellular loop near the transmembrane domain of this G-protein coupled receptor. The three-dimensional structure provided by the N-terminus and to

Table 3
Bovine CXCR1 SNP haplotypes.

TagSNP haplotypes	Frequency	AA haplotypes	Frequency
+621 +735 +816 +980[a]		365–621–735–980–995[a] 122–206–245–327–332[b]	
GCCA	31.8	TGCAA **V**WH**KH**[c]	32.4
AGCG	29.5	CAGGG AX	29.5
GCAG	19.3	TGCGG **V**WHRR	19.9
GGCG	18.2	CGGGG AW**Q**RR	18.2

[a] CXCR1 SNP position.
[b] CXCR1 amino acid position.
[c] Bold and underline indicates conserved amino acid across species.

a lesser degree the first extracellular loop is thought to contribute to initial ligand binding and strength (Leong et al., 1994; Monteclaro and Charo, 1996). Thus a change in shape could modify the response to ligand. This possibility is supported by the greater K_d observed in cows with a CC genotype or H_{245} amino acid at CXCR1c.+735 (former +777) when co-incubated with a CXCR2 inhibitor to block IL-8 binding to CXCR2 (Rambeaud and Pighetti, 2007). Because the CXCR1c.+735C nucleotide (H_{245}) and the CXCR1c.+365 T nucleotide (V_{122}) occur together (Table 3), this suggests the same observation could hold true for CXCR1c.+365 but would need to be tested independent of the CXCR1c.+735 SNP. The valine residue generated by the T nucleotide represents 52% of the bovine alleles and is conserved across human, swine, rabbit, and rat species. In the sample population, heterozygous cows were predominant (TC, 45%) over homozygous genotypes (TT, 30%; CC, 25%). The CXCR1c.+735C>G leads to a H245Q switch in the third extracellular loop and is equivalent to the previously reported +777 SNP (Youngerman et al., 2004b). The glutamine residue generated by the G nucleotide represents 48% of the bovine alleles in this study and is conserved among human, swine, mouse, rabbit, and rat species. Similar to the +365 SNP, heterozygous cows were most prevalent (GC, 45%) in our sample population when compared to homozygous genotype cows (CC, 30%; GG 25%). The conserved nature of this amino acid and its location near a G-protein binding site suggests it has the potential to influence signaling (Damaj et al., 1996). This SNP also has been associated with neutrophil functional activity and mastitis susceptibility. More specifically, GG genotypes at CXCR1c.+735 have been linked to greater neutrophil migration, CD11b/CD18 adhesion molecule expression, reactive oxygen species generation, calcium signaling, and subclinical mastitis versus the CC genotype (Youngerman et al., 2004a; Rambeaud and Pighetti, 2005, 2007; Rambeaud et al., 2006). In contrast, the CC genotype has been associated with greater survival against spontaneous apoptosis when compared to the GG genotype (Rambeaud et al., 2006).

The amino acid changes occurring as a result of SNP at CXCR1c.+980 and c.+995 were observed together in the 3rd linkage group and generated receptors expressing either $K_{327}H_{332}$ (~32%) or $R_{327}R_{332}$ (~67%) in the C-terminus. The SNP CXCR1c.+980A>G encodes for a K327R amino acid change with the GG genotype dominant in the sample population (50%) when compared to the AA (15%) and GA (35%) genotypes. The high frequency of the G allele (68%) and GG genotype was unexpected as the A nucleotide encodes for lysine which is conserved across the five species examined. Interestingly, the lysine at position 327 is found within the LL**K**IL and **K**ILAIHGLI motifs which have been linked to LASP-1/adaptin-2 and HSP-70 interacting protein (Hip) binding to CXCR2, respectively (Fan et al., 2001, 2002; Raman et al., 2010). Mutation of these motifs resulted in reduced adaptin-2 or Hip binding, CXCR2 internalization and chemotaxis. Specific mutation of lysine to arginine within the LLKIL motif of huCXCR2 greatly increased binding of the LIM domain of LASP-1 and would have the potential to influence downstream events such as migration (Raman et al., 2010). These findings are directly relevant to bovine CXCR1, as the C-terminus of CXCR1 and CXCR2 are 98–100% identical (Lahouassa et al., 2008). Although not directly examined by Leyva-Baca et al. (2008), the CXCR1c.+980 SNP is in the same linkage group as CXCR1c.−1606 (e.g. −1768 in the aforementioned paper) which was found to be associated with SCS in the Canadian Holstein population. In contrast to SNP CXCR1c.+980, the amino acid change H332R occurring with CXCR1c.+995A>G may not have as much impact on receptor function based on the large degree of amino acid variability at this position with histidine being present in human, swine and mouse species, arginine in rabbits, and missing in rats. Within the sample population, the GG genotype is dominant (50%), followed by the AG (35%) and AA (15%) genotypes.

Interestingly, the only haplotype containing the C allele of CXCR1c.+735 which has been associated with reduced neutrophil migration, adhesion molecule expression, calcium signaling and greater mastitis susceptibility (Youngerman et al., 2004a; Rambeaud and Pighetti, 2005, 2007; Rambeaud et al., 2006), also contains the T allele of CXCR1c.+735 and the A allele of CXCR1c.+980, generating valine at amino acid 122 and arginine at amino acid 327 (Table 3). However, as mentioned earlier, each of these amino acid changes has the potential to influence receptor activity through IL-8 binding (Leong et al., 1994;

Monteclaro and Charo, 1996), G-protein related signaling (Damaj et al., 1996), and/or receptor internalization (Fan et al., 2001, 2002). Thus it is difficult to pinpoint if the CXCR1c.+735 SNP is a causal mutation, merely served as a marker for one of the other SNP in CXCR1 or another gene, or it is the combined SNP (e.g. haplotype) that contributes to the functional changes observed in neutrophil activity and potentially disease resistance.

Perhaps the most intriguing finding was the introduction of an early stop codon with SNP CXCR1c.+621G>A at amino acid W206X when the A allele is present. This may allow receptor expression through the second intracellular loop or prevent expression completely. Either scenario would have the potential to significantly influence responses to its primary ligand IL-8. In our sample population the A allele only represented 29% of the sample population, resulting in the GG homozygous genotype representing 52% of the population and cows with AA (10%) and AG (38%) genotypes being less frequent. In keeping with this trend, the A allele occurred in only one of the four dominant haplotypes when determined by tag or amino acid based SNP: AGCG and CAGGG, respectively. However, it should be kept in mind the sample population in this study was selected based on having at least one complete lactation in order to assess association with mastitis and other production measures and may bias against animals who left the herd prior to this time.

In conclusion, the CXCR1 gene is highly polymorphic and has potential implications towards genetic selection and understanding host factors that increase the risk of infection. With respect to selection, CXCR1 SNP from two different linkage groups has been associated with at least one of the following traits: SCC/SCS, mastitis, and milk fat concentrations (Youngerman et al., 2004a; Leyva-Baca et al., 2008). This suggests that SNP haplotypes may be more effective than individual CXCR1 SNP in identifying at risk or resistant populations. Furthermore, at least one of the SNP has been associated with altered neutrophil function, suggesting the amino acid changes have the potential to influence CXCR1 activity. The combination of genetic selectivity, along with altered cellular activity and disease resistance, suggests CXCR1 haplotypes provide a needed model system to identify cellular and molecular processes that directly influence mastitis resistance.

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