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# Detection of new allotypic variants of bovine λ-light chain constant regions in different cattle breeds

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ABSTRACT

In the cattle breeds German Black Pied (GBP), German Simmental (GS), Holstein Friesian (HF), Aubrac (A) three transcribed allotypic variants in isotype IGLC2 and five allotypic variants in isotype IGLC3 were identified. Substitutions within the putative interface to CH1 at position 11 and 79 were noted. In IGLC2$^{b}$, K79E led to a charge conversion. In IGLC3$^{b}$ and IGLC3$^{c}$, the E79N replacement removed the charge while the T11K substitution resulted in a positively charged amino acid residue. In addition, D15 and T16 were found in IGLC2$^{c}$, IGLC3$^{b}$, and IGLC3$^{c}$. Substitutions located on the outer site of the molecule were observed in IGLC2$^{b}$ (V40, H45.5), IGLC2$^{c}$ (A1, V40, D77), IGLC3$^{b}$ (A1, D77, D109, P127), IGLC3$^{c}$ (A1, G45.5, D77, D109, P127), IGLC3$^{d}$ (D109), and IGLC3$^{e}$ (A1). Amino acid residues P83 (IGLC2$^{c}$, IGLC3$^{b}$, IGLC3$^{c}$), N93 (IGLC2$^{b}$), D93 (IGLC3$^{b}$), and G93 (IGLC3$^{c}$) were positioned in cavities but seemed to be accessible for solvents.



## 1. Introduction

Diversity of a jawed vertebrate species antibody repertoire is defined by the relative contribution of germline based combinatorial diversity and post-combinatorial mechanisms such as somatic hypermutations and gene conversion (Aitken et al., 1999; Butler, 1998; Davis, 2004; Lucier et al., 1998; Maizels, 2005; Market and Papavasiliou, 2003; Parng et al., 1995, 1996; Reynaud et al., 1995; Saini et al., 2003; Saini and Kaushik, 2002).

Two distinct immunoglobulin light chain isotypes (λ and κ) have been described in mammals (Das et al., 2008a; Korngold and Lipari, 1956; Pilstrom, 2002). Bovine λ-light chains are expressed in a proportion of 95% (Arun et al., 1996) and the locus was assigned to *Bos taurus* autosome 17 (BTA17) (Tobin-Janzen and Womack, 1996). About 5% of the heavy chains are associated with κ-light chains (Arun et al., 1996). Their genetic information is located on BTA11 (Ekman et al., 2009). Similarly, horse (Ford et al., 1994) and sheep (Broad et al., 1995; Foley and Beh, 1992; Griebel and Ferrari, 1994) carry a functional kappa system, but their light chain repertoire is similar to cattle dominated by λ-light chain expression (Home et al., 1992; Hood et al., 1967).

In cattle there are at least four light chain constant region (IGLC) genes (Chen et al., 2008; Ekman et al., 2009; Parng et al., 1995, 1996; Pasman et al., 2010). Two of them (IGLC2 and IGLC3) are functional with preferential expression of IGLC3 during rearrangement while the other two genes (IGLC1 and IGLC4) are pseudogenes (Chen et al., 2008; Pasman et al., 2010). In addition, a fifth bovine IGLC gene was detected, which was not yet mapped to a bovine chromosome (Ekman et al., 2009). IGLC1 and IGLC2 described by Ekman et al. (2009) corresponded to IGLC2 mentioned by Chen et al. (2008), as both genes presented the same coding sequences but differ in their 3′UTR. Studies in sheep revealed only two IGLC genes. The ovine IGLC2 gene presents a premature stop codon (Jenne et al., 2006). In horse DNA four to seven IGLC genes were identified. Three of these genes are functional, whereas the others seem to be pseudogenes (Das et al., 2008b; Home et al., 1992; Sun et al., 2010).

In general, light chains contribute to antigen binding and enlarge variability of the antibodies. The light chains (or their surrogate) allow the expression of the heavy-chains in pre-B-cells and therefore are responsible for the expression of B-cell receptors as well as of secreted antibodies (Max, 2008). Beside antigen recognition, a structural function of immunoglobulin light chain constant regions is the stabilization of the variable region and the association to the constant region of heavy chain isotypes (Chen et al., 2008; Padlan et al., 1986). Padlan et al. (1986) observed specific amino acid residues that formed the inter-domain interface of constant regions of the light chains and the first constant region of the heavy chains in four different Fabs derived from murine and human monoclonal antibodies. Allotypic markers of human light chains were associated with the susceptibility of different

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infectious diseases (Giha et al., 2009; Granoff et al., 1984; Pandey, 2000; Pandey et al., 1995, 1979). Observations regarding complement activation, age-dependent expression, and influences on the effector function in defending *Haemophilus somnus* and *Tritrichomonas foetus* were found for allotypes of bovine IgG2 (Bastida-Corcuera et al., 2000, 1999b; Corbeil et al., 1997). To date there is no description of allotypes in bovine λ-light chain isotypes, which is necessary for the examination of their possible influence in the immune response.

The present study focused on the comparative analyses of bovine IGLC sequences and investigated the identification of different alleles and allotypic variants. Using comparative 3D modeling with known crystal structures, variable allotypic locations of amino acid residue substitutions within the molecule were examined. Their putative influence within the molecule was discussed. We based our analyses on the findings of Chen et al. (2008) because the complete IGLJ-IGLC cluster from Holstein cattle breed has been sequenced and annotated. Four cattle breeds (German Black Pied, German Simmental, Holstein Friesian, and Aubrac) with different population sizes, distribution areas, and intended use were examined with respect to the distribution of the detected allotypic variants.

## 2. Materials and methods

### 2.1. Breed selection, isolation of B-cells and cDNA-synthesis

For the analyses of breed specific expression of IGLC genes the four cattle breeds German Black Pied (GBP), German Simmental (GS), Holstein-Friesian (HF), and Aubrac (A) were chosen. Holstein Friesian and GS represent breeds commonly used in global commercial milk and meat production, whereas GBP and A are breeds of local importance that are raised in smaller populations.

Blood samples were collected from 10 randomly chosen animals per breed. The animals selected from the herd of breed A were composed of seven French and three German animals, whereas the sample of breed GS included one Austrian bull. Lymphocytes were isolated individually from 10 ml peripheral EDTA blood using Ficoll gradients (GE Healthcare Europe GmbH, Germany) according to the manufacture's protocol. Viable B-cells were counted by staining with trypan blue. Total RNA was isolated from $1 \times 10^7$ cells by using the RNeasy® Mini Kit (Qiagen, Germany). The first-strand cDNA was synthesized using pd$(N)_6$-primers from 3 μg of total RNA in a total volume of 20 μl (First-Strand cDNA Synthesis Kit, GE Healthcare Europe GmbH, Germany).

### 2.2. PCR amplification of the immunoglobulin λ-light chain repertoire

PCR amplification of λ-light chain cDNA was performed using primers binding within the leader region and 3′UTR of λ-light chains. For the λ-leader region, two different gene specific primers were designed on the basis of database sequence information due to polymorphisms in this sequence area referring to Accession numbers: BC102189 and BC112657. The annealing site of forward primer bIgL_Leader_1 (5′-ATGGCCTGGTCCCCTCTG-3′) started at position 54 of BC102189, whereas primer bIgL_Leader_2 (5′-ATGGCCTTGGCCCCTCTG-3′) started at position 55 in BC112657. Using the reverse primer bIgL_3′UTR (5′-TCAGGGGTCCATGGAGAG-3′) the expected product size of the λ-light chain including leader, variable, and constant regions was 778 bp (BC102189) and 781 bp (BC112657).

A total reaction volume of 50 μl contained 0.67 μl of cDNA, 200 μM dNTPs (Bioline, Germany), 5 μl of 10× PCR buffer (75 mM Tris HCl pH 9.0; 2 mM $MgCl_2$; 50 mM KCl; 20 mM $(NH_4)_2SO_4$), 0.4 μM of each primer, and 2 units DNA polymerase (Biotools, Spain). PCR was performed under cycling conditions of 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 59.4 °C for 1 min, 72 °C for 2 min terminated with elongation at 72 °C for 10 min. Length and purity of the PCR products were evaluated by electrophoresis on 1% agarose gels.

### 2.3. Cloning and purification of PCR products

PCR-products were purified using a MiniElute Gel Extraction Kit (Qiagen, Germany) according to the manufacture's protocol. QX1 buffer replaced QG buffer. Samples were eluted with 13 μl EB buffer (10 mmol Tris/HCl; pH 8, 5) and were stored at 4 °C. DNA products were cloned into the pCR® 2.1-TOPO® 3.9 kb TA vector (Invitrogen™, Karlsruhe, Germany) and transformed into chemically competent One Shot TOP10 *Escherichia coli* cells (Invitrogen™, Karlsruhe, Germany). Transformants were plated on LB-agar containing 0.3 mM ampicillin, 40 μl 2.44 μM X-gal (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside), and 40 μl 1 M IPTG (isopropyl β-D-1-thiogalactopyranoside) for blue–white selection. After incubation at 37 °C overnight cultures of randomly chosen white transformants were grown in 5 ml LB-ampicillin broth. Plasmids were isolated using the MiniPrep Kit (Qiagen, Germany). In order to assess the insert size, plasmid DNA was either cleaved with *Eco*RI (New England Biolabs, Germany) or a colony-PCR was carried out. For this reason a 25 μl mixture containing 2 μl cell culture, 0.4 μM of vector specific primers M13 (-20) Forward and M13 Reverse (Invitrogen, Germany), respectively, and one PCR-bead (GE Healthcare Europe GmbH, Germany) were used in a hot start PCR at 95 °C for 5 min, denaturation at 95 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 2 min up to a total of 30 cycles. A final extension at 72 °C for 10 min was included after the final cycle before PCR mixtures were cooled down to 4 °C. The size of the resulting fragments and of the PCR products was confirmed by agarose gel electrophoresis.

### 2.4. Sequence analysis of PCR products

Using an ABI Prism 3100 Analyzer, eight clones per animal and per amplified IGLC were sequenced (Applied Biosystems Deutschland GmbH, Germany). The M13 (-20) Forward and M13 Reverse (Invitrogen, Germany) vector specific primers as well as the

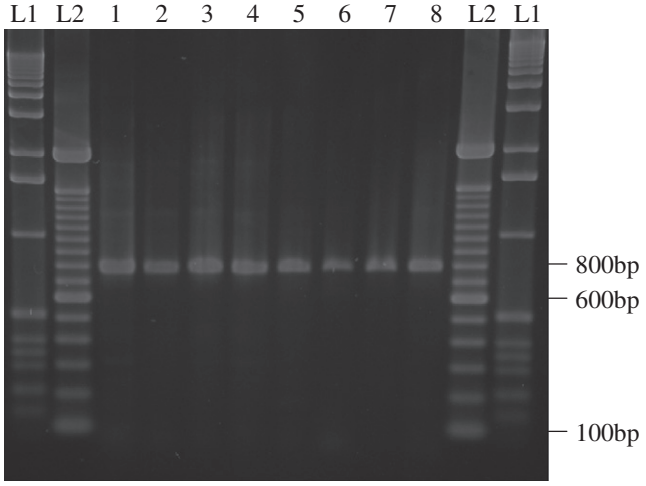


**Fig. 1.** Amplification of λ-light chains with primer pairs Igλ_leader_1 (1–4) and Igλ_leader_2 (5–8)/Igλ_3′UTR and cDNA from four different cattle breeds. 1, 5: German Black Pied, 2, 6: German Simmental, 3, 7: Holstein Friesian, 4, 8: Aubrac, L1: 1 kb ladder, L2: 100 bp ladder. A no-template control was present in all PCR amplifications (data not shown). The expected product sizes were 778 bp and 781 bp, respectively.