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Short Communication

Expression of Interleukin-4 Receptor and CCL5 Chemokine is not Related to Insect Bite Hypersensitivity in Horses

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ABSTRACT

Insect bite hypersensitivity (IBH) in horses represents a type I or sometimes a type IV hypersensitivity to salivary antigens from numerous *Culicoides* spp and some other insects. Until now, there has been no curative treatment available, but there are clear indications that the susceptibility to IBH is partly heritable. Identification of equine genes that are associated with susceptibility to IBH could lead to the development of a marker-assisted selection method. The aim of this study was to investigate the expression of functional candidate genes in relation to IBH. Based on literature, the interleukin-4 receptor (*IL4R*) and chemokine CCL5 (*CCL5*) genes were selected for examination by quantitative reverse transcription-PCR (qRT-PCR). Expression levels were determined in 16 horses: 8 IBH-positive horses, all showing clinical IBH symptoms at sampling; and 8 IBH-negative horses, stabled at the same location (case-control set up). Peripheral blood mononuclear cells (PBMC) were isolated from whole blood samples, and expression levels of *IL4R* and *CCL5* were measured. No differential expression was found for *IL4R* and *CCL5* in PBMC between IBH-positive and IBH-negative horses ($P = .58$ and $P = .63$). Expression of *CCL5* and *IL4R* in PBMC is not related to insect bite hypersensitivity in warmblood horses. Research toward a marker-assisted selection procedures could reduce IBH prevalence in horse populations by means of selection.

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1. Introduction

Insect bite hypersensitivity (IBH) is an allergic reaction of horses to the bite of certain *Culicoides* species and sometimes black flies (*Simulium* spp) or stable flies (*Stomoxys calcitrans*) [1]. Presently, there is no curative treatment available, but there are clear indications that susceptibility to IBH is partly heritable [2]. A more pronounced Th2 response plays an important role in the development of allergies [3]. The Th2 cytokine interleukin-4 (IL-4) plays an important role in the stimulation and differentiation of Th2 cells. It promotes proliferation and differentiation of activated B cells, recruits circulating cells,

and suppresses Interferon gamma production by Th1 cells. IL-4 mediates its biological effects by binding to its receptor (IL4R), which is located on almost all hematopoietic cells and a large number of nonhematopoietic cells [4]. Numerous studies report a positive association with one or more alleles with atopic dermatitis, asthma, atopy, or total Immunoglobulin E level in humans [5–7]. Moreover, overexpression of *IL4R* mRNA was described in human patients with atopic dermatitis and atopic asthma [8–10]. CCL5 is a CC chemokine, which is a chemotactic cytokine that attracts eosinophils, lymphocytes, monocytes, and basophils to an infection through a concentration gradient [11]. CCL5 activates the release of histamine, platelet-activating factor, and prostaglandins by intradermal basophils and mast cells [12]. Various studies describe an association of polymorphisms in *CCL5* with (atopic) dermatitis, (atopic) asthma, bronchial hyper-responsiveness, skin prick test, or total IgE level in humans [7,13,14]. The CCL5 protein level is

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Table 1Primers for qRT-PCR to determine expression levels of interleukin-4 receptor (*IL4R*) and chemokine *CCL5*

Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')	Fragment Size (bp)
<i>ACTB</i>	CCAGCAGATGAAGATCAAG	GTGGACAATGAGGCCAGAAT	88
<i>B2M</i>	CGGGCTACTCTCCCTGACTG	TCTGACCGGTGCACTTTCATC	142
<i>RPL32</i>	GAAGCCCAATCGTCAAAAA	TTGACATTGTGGACCAGGAA	184
<i>GAPDH</i>	GATGCCCAATGTTTGTA	AAGCAGGATGATGTTCTGG	250
<i>HPRT1</i>	GGCAAAACAATGCAACCTT	CAAGGGCATATCTACGACAA	163
<i>IL4R</i>	GACTACATCAGCGCTCCAC	CTTGAAGGAGCTGTTCCAC	201
<i>CCL5</i>	CACTGCCACCTTCTGCACTC	CGGGAGATGTAGGCAAGCA	85

For every gene, the primer sequences are given in 5' > 3' direction, together with the resulting fragment size. The different housekeeping genes were β 2-microglobulin (*B2M*), hypoxanthine guanine phosphoribosyl transferase (*HPRT1*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), β -actin (*ACTB*) and ribosomal protein L32 (*RPL32*).

elevated in serum [15], in leukocytes [16], and in skin biopsy samples [17] of human patients with atopic dermatitis. Also, in patients with asthma, a higher *CCL5* level in plasma [18] and bronchoalveolar fluids [19] has been described. A higher expression of *CCL5* (mRNA level) was measured in patients with atopic dermatitis and asthma than in patients without clinical symptoms [17,20].

2. Materials and Methods

Based on these findings, *IL4R* and *CCL5* were selected as candidate genes for a differential expression study concerning IBH in horses. Sixteen horses were selected for this study (eight IBH-positive horses and eight IBH-negative

horses). All IBH-positive horses showed clinical symptoms at sampling, and negative horses were selected from the same stable to ensure *Culicoides* spp contact. Whole-blood samples (9 mL) were collected from all horses in K2-Ethylendiaminetetraacetic acid tubes (Venosafe, Terumo Europe NV, Leuven, Belgium) by jugular venipuncture during the summer of 2010. Isolation of peripheral blood mononuclear cells (PBMC) was performed within 6 hours after sampling using Ficoll-Paque Plus and RNA isolation was performed by using the phenol-chloroform method. The following five housekeeping genes were selected: *GAPDH*, *B2M*, *RPL32*, *HPRT1*, and *ACTB* [21,22]. They code for glyceraldehyde-3-phosphate dehydrogenase, β 2-microglobuline, ribosomal protein L32, hypoxanthine phosphoribosyl transferase, and β -actin, respectively. The primers used are listed in the Table 1.

RNA was reverse transcribed into cDNA by using avian myeloblastosis virus reverse transcriptase (Promega). qRT-PCR was performed in triplicate using SYBR Green (Fermentas Life Sciences) with the ABI StepOnePlus machine (Applied Biosystems). *GAPDH*, *ACTB*, and *RPL32* were selected as housekeeping genes [23]. Expression data were log transformed and analyzed with a univariate general linear model using Enterprise Guide version 4 (SAS, Cary, NC, USA).

3. Results and Discussion

No statistically relevant differences between expression levels in IBH-positive and -negative horses could be found ($P = .58$ for *IL4R* and $P = .63$ for *CCL5*) (Fig. 1).

Hatano et al [24] also reported no differential expression of *CCL5* in PBMC of patients with atopic dermatitis. Although differential expression of both genes is described in PBMC, some studies report differential expression of *CCL5* in other tissues and not in PBMC [20,25]. In addition, stimulation of cultured PMBC with *Culicoides* spp antigens might increase the chance to measure differential expression between cases and controls. For example, when PBMCs are cultured and stimulated with a specific allergen, the production of *CCL5* is elevated in atopic asthma patients [26]. Renz et al [8] reported differential expression of *IL4R* mRNA in PBMC but used Northern blotting. Moreover, mRNA expression was increased only in acute dermatitis but not in chronic dermatitis [9], and epithelial *IL4R* mRNA expression did not significantly differ between nonatopic asthmatic and nonatopic control subjects. Only significant differences in *IL4R* levels between atopic asthmatic and

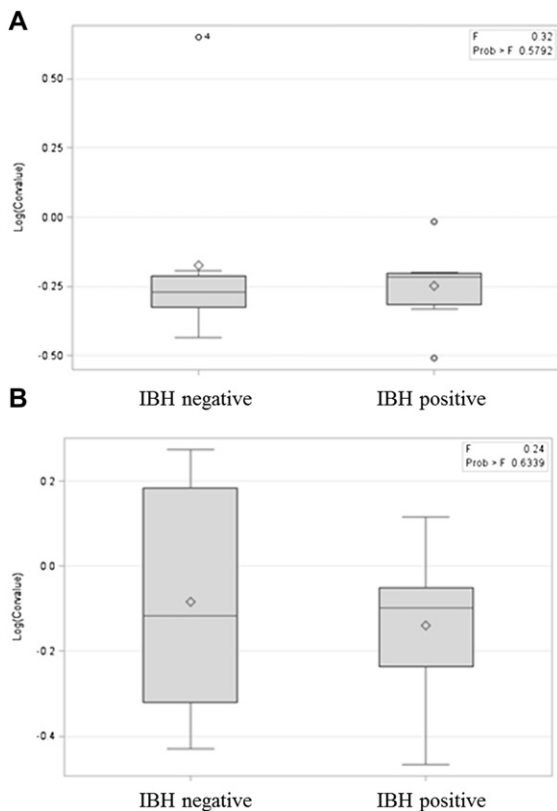


Fig. 1. Boxplots of log-transformed expression data for *IL4R* (A) and *CCL5* (B).

atopic control subject are described [10]. Finally, as preventive measures that reduce *Culicoides* contact are common in warmblood horses, *Culicoides* contact might be too low to measure expression differences in this breed.

4. Conclusions

In conclusion, expression of CCL5 and IL4R in PBMC is not related to IBH in warmblood horses. However, additional research should be performed because marker-assisted selection procedures could reduce IBH prevalence in horse populations by means of selection.

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