Analysis of Suppressor of Cytokine Signaling 2 Gene (SOCS2) Polymorphism in Different Dog Breeds

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Abstract

SOCS2 is a negative regulator of growth hormone signaling. The deletion of SOCS2 in mice results in a 30-50% increase in post-natal growth. The aim of the paper was to identify of suppressor of cytokine signaling 2 gene (SOCS2) polymorphism in different dog breeds. The material involved 77 dogs from 14 different breeds. Canine genomic DNA was isolated from saliva by modified method with using DNAzol® (Molecular Research Center) and linear polyacrylamide (LPA) carrier and from blood by using NucleospinBlood (Macherey-Nagel) and used in order to estimate SOCS2 genotypes by PCR-RFLP method. The PCR products were digested with TaqI restriction enzyme. The T allele was distributed among large dog breeds (Czech pointer, Golden retriever, Rottweiler) with an allele frequency ranging from 0.2857 to 1.00. In the population of Czech pointer we detected all genotypes. There were detected homozygote genotype GG with frequency 0.5476, heterozygote genotype GT with frequency 0.3333 and homozygote genotype TT with frequency 0.1191. Results point out that frequency of G allele was high and was represented 0.7143. Frequency of T allele was 0.2857. In Rottweiler was detected homozygote genotype TT. Genotypes GG and GT has not been observed. In Golden retriever we detected only heterozygote genotype GT.

Keywords: Czech pointer, dog, PCR-RFLP, SOCS2.

1. Introduction

Cytokines consist of a large family of secreted proteins that regulate a diverse array of physiologic and pathologic responses, including body growth, hematopoiesis, immune response, inflammation, and development of the nervous system. Suppressors of cytokine signaling (SOCS) are a group of intracellular proteins that have the ability to regulate the magnitude and duration of cytokine signaling [1,2].

Activity of SOCS2 has been predominantly linked to growth hormone (GH) and insulin-like growth factor 1 (IGF-1) signaling but may also contribute to several biological processes including metabolism, bone formation, neuronal development, cancer, infection and other cytokine-dependent pathways [3].

Growth hormone (GH) has numerous effects in the body and is most commonly known for its role in regulating metabolism and body growth. Because GH is involved in many aspects of cell function, its signaling is tightly controlled by s everal pathways at both the extracellular and intracellular level. Suppressor of cytokine signaling-2 (SOCS2) is one such intracellular regulator of GH signal transduction. Expression of SOCS2 is tightly regulated and alteration of its levels leads to marked abnormalities in metabolism and growth [4]. A spontaneous deletion in mouse chromosome 10 disrupts expression of SOCS2 and results in the *high growth* mouse phenotype which is characterized by a 30-50% increase in postnatal body growth without altering overall body composition [2,5].

The nature of the nucleotide change and the effect on the protein together with the finding of a QTL related to body size in the same CFA15 region by other researchers suggest canine

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SOCS2 as a potential candidate gene for body size in dogs [2].

2. Materials and methods

The material involved 77 dogs from 14 different breeds. Canine genomic DNA was isolated from saliva by modified method with using DNAzol$^{®}$ (Molecular Research Center), which base is guanidine thiocyanate; and linear polyacrylamide (LPA) carrier to a nucleic acid precipitation [6] and from blood by using NucleospinBlood (Macherey-Nagel) and used in order to estimate SOCS2 genotypes by PCR-RFLP method.

DNA primers described by Rincón et al. (2007) [2] were used to PCR amplification: forward primer 5´- TGT TGC CAA GTA TTT GCC CTA - 3´ and reverse primer 5´ - TTT ACA TAG CTG CAT TC GGA GA - 3´.

The PCR reaction elaborated by Rincón et al. (2007) [2] was modificated.

The reaction mixture in the total volume 25 µl containing 50 ng DNA, 1 U Taq polymerase (FERMENTAS),1X PCR buffer (750 mM Tris-HCl, pH 8.8, 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20), 3 mM $MgCl_2$, 200 µM dNTP, 10 pM of each primer. The following amplification parameters were applied: 95°C for 3 minutes followed by 34 cycles: 95°C for 10 seconds, 59°C for 30 seconds, 72°C for 40 seconds. The reaction was completed by the final synthesis:

72 °C for 5 minutes.

The PCR products of 604 bp were digested with the *Taq*I restriction enzyme (Fermentas). Restriction digestion fragments were loaded on 3 % agarose gel (Invitrogen) containing GelRed^{TM} (Biotium) and the gel were analyzed in the UV rays.

3. Results and discussion

*Taq*I digestion of the PCR product was analyzed by 3% agarose-gel electrophoresis. Allele G produced 87 bp, 238 bp and 279 bp fragments, and allele T produced 238 bp and 366 bp fragments.

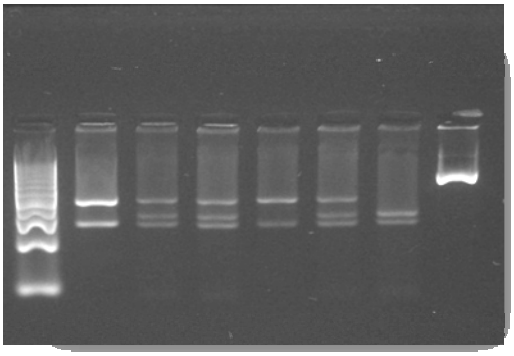
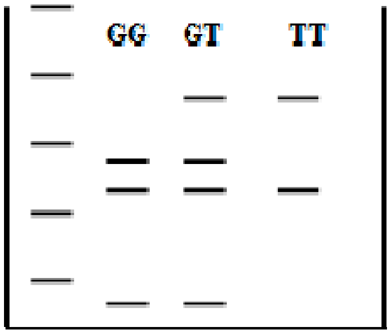


Figure 1. Representatively results of analysis PCR-RFLP for *SOCS2* gene by *Taq*I on 3 % agarose gel.

1 –DNA Ladder 100 bp (Fermentas); 2,5 – genotype TT (238 bp, 366 bp); 3,4,6 – genotype GT (87 bp, 238 bp, 279 bp, 366 bp); 7 – genotyp GG (87 bp, 238 bp, 279 bp); 5 – PCR product (604 bp)



Scheme 1. Schematic image of PCR-RFLP product *SOCS2* gene.

1 – DNA ladder 100 bp; 2 – genotype GG (87 bp, 238 bp, 279 bp); 3 – genotype GT (87 bp, 238 bp, 279 bp; 366 bp); 4 – genotype TT (238 bp, 366 bp)

In the population of Czech pointer we detected all genotypes. There were detected homozygote genotype GG (23 dogs), heterozygote genotype GT (14 dogs) and homozygote genotype TT (5 dogs). In Rottweiler was detected homozygote genotype TT. Genotypes GG and GT has not been observed. In Golden retriever we detected only heterozygote genotype GT. In other breeds (six group of small breed and five group of large breed) all dogs showed only homozygote genotype GG. Detailed genotype and gene frequencies per breed are presented in Table 1.

Table 1. Frequency of genotypes and alleles of *SOCS2* gene in the population of dog breeds

BREED	***DOGS***	***GENOTYPES FREQUENCY***			***ALLELES FREQUENCY***	
		GG	**GT**	**TT**	**G**	**T**
Beagle*	2	1	0	0	1	0
Bernese Mountain Dog•	1	1	0	0	1	0
Chihuahua*	1	1	0	0	1	0
Czech pointer•	42	0.5476	0.3333	0,1191	0.7143	0.2857
Doberman•	1	1	0	0	1	0
French bulldog*	9	1	0	0	1	0
German Shepherd•	4	1	0	0	1	0
Golden Retriever•	1	0	1	0	0.5	0.5
Irish setter•	5	1	0	0	1	0
Pinscher*	2	1	0	0	1	0
Rottweiler•	1	0	0	1	0	1
Slovak Tchouvatch•	1	1	0	0	1	0
West Highland White Terrier*	6	1	0	0	1	0
Yorkshire Terrier*	1	1	0	0	1	0
TOTAL	**77**					

Small breeds*, Large breeds•

Our results are similary to those as reported by Rincón et al. (2007) [2], who described predominant allele T in Rottweiler and Great Danes with a frequency of 0.68 and 0.72, respectively. They also reported occurrence of allele T in rest of the large breeds with frequencies ranged from 0.04 in the Bernese Mountain Dog to 0.22 in the Mastiff. In the small breed group only three standard Dachshunds showed the T allele.

4. Conclusions

The T allele was distributed among large dog breeds (Czech pointer, Golden retriever, Rottweiler) with an allele frequency ranging from 1.00 to 0.2857. In the population of Czech pointer we detected all genotypes. In Rottweiler was observed homozygote genotype TT. In Golden retriever we detected only heterozygote genotype GT. In other breeds (six small breed group and five big breed group) all dogs showed only homozygote genotype GG.

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3. The Slovakian Club of Czech Pointer Breeders.

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