Analysis of Single Nucleotide Polymorphism (SNP) rs22114085 Associated with Canine Atopic Dermatitis by PCR-RFLP Method

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Abstract

Canine atopic dermatitis (cAD) is a common inflammatory skin disease that is considered to be a naturally occurring, spontaneous model of human atopic dermatitis (eczema). The aim of the paper was to identify of the SNP rs22114085 in different dog breeds. The material involved 52 dogs from 5 different breeds. Canine genomic DNA was isolated from saliva by modified method with using DNAzol® and linear polyacrylamide (LPA) carrier and from blood by using commercial kit NucleospinBlood and used in order to estimate rs22114085 SNP genotypes by PCR-RFLP method. The PCR products were digested with *Dde*I restriction enzyme. The C allele was distributed in Czech Pointer, Chihuahua, German Wirehaired Pointer with an allele frequency ranging from 0.4545 to 1.00. In the population of Czech Pointer we detected all genotypes CC, CT and TT with frequency in male 0.25, 0.5833 and 0.1667, and in female 0.2728, 0.3636 and 0.3636, subsequently. In German Wirehaired Pointer was detected homozygote genotype CC in male and heterozygote genotype CT in female with frequency 1 and 1. In Chihuahua was observed homozygote genotype CC and heterozygote genotype CT with frequency 0.3333 and 0.6667, subsequently. In Golden retriever and Pincher we detected genotype TT with frequency 1.

Keywords: canine atopic dermatitis, dog, PCR-RFLP, rs22114085, SNP

1. Introduction

Canine atopic dermatitis (cAD) is a common inflammatory skin disease that is considered to be a naturally occurring, spontaneous model of human atopic dermatitis (hAD). The pathogenesis of the disease in both humans and dogs is strongly associated with immunological hyper-reactivity, although skin barrier function, microbial colonisation and infection are also considered contributing factors [1]. Currently, the inherited predisposition for AD in humans is believed to be complex and under polygenic and heterogenic control [2]. Similar to human AD, the canine AD phenotype is likely to be determined by various genetic and non-genetic factors [3].

Canine atopic dermatitis (CAD) is estimated to affect 15% to 30% of the canine population [4].

The heritability of atopy has been studied by Shaw *et al* (2004) [4]. When considering guide dogs as a whole, a heritability of 0.47 was found.

Early studies suggested that AD was transmitted as an autosomal dominant disorder but subsequent analyses have suggested an autosomal recessive mode of inheritance for this and other atopic disorders [5].

Wood et al. (2010) [1] as the first described the study to perform a genome-wide association study in canine atopic dermatitis (cAD) using the Illumina Canine SNP20 array, containing 22,362 single -nucleotide polymorphisms (SNPs). They identified SNP RS22114085 as a susceptibility locus to cAD. This SNP is located in intergenic regions on chromosome 10.

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2. Materials and methods

The material involved 52 dogs from 5 different breeds (Czech Pointer, German Wirehaired Pointer, Chihuahua, Golden Retriever and Pincher).

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 rs22114085 genotypes by PCR-RFLP method.

DNA primers used to PCR amplification (forward primer 5´- CCA AGC TCC ACG ATG AAC AG - 3´ and reverse primer 5´ - CTG TGA GCT ACG AAT GGT TTC C - 3´) were designed using the program BatchPrimer3 v1.0 [7].

The PCR 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), 1.5 mM $MgCl_2$, 200 µM dNTP, 10 pM of each primer. The following amplification parameters were applied: 95°C for 3 minutes followed by 30 cycles: 95°C for 10 seconds, 53 °C for 30 seconds, 72°C for 30 seconds. The reaction was completed by the final synthesis: 72°C for 5 minutes.

The PCR products of 152 bp were digested with the *Dde*I restriction enzyme (Fermentas). Restriction digestion fragments were loaded on 3 % agarose gel (Invitrogen) containing GelRed™ (Biotium) in 1 × SB buffer [8] at 180 V for 15 minutes and the gel were analyzed in the UV rays and the documentary system Olympus C-7070 were used to record the results.

3. Results and discussion

*Dde*I digestion of the PCR product was analyzed by 3% agarose-gel electrophoresis. Allele C produced 152 bp fragment, and allele T produced 104 bp and 48 bp fragments (Figure 1).

In the population of Czech Pointer we detected all genotypes. There were detected homozygote genotype CC (12 dogs – 3 male, 9 female), heterozygote genotype CT (19 dogs – 7 male, 12 female) and homozygote genotype TT (14 dogs – 2 male, 12 female).

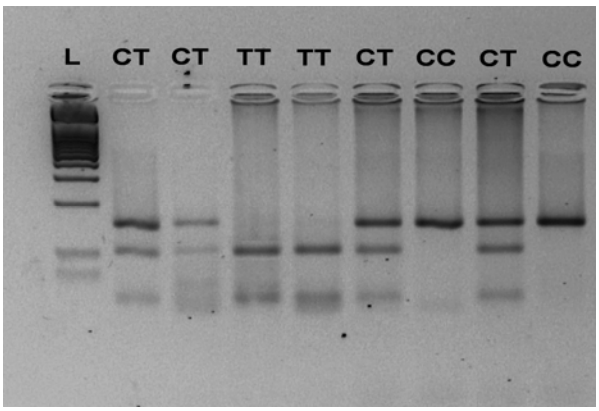

Figure 1. Representatively results of analysis PCR-RFLP for SNP rs22114085 by *Dde*I on 3% agarose gel
1–MassRuler™ Low Range DNA Ladder (Fermentas); 2, 3, 6, 8–genotype CT (152 bp, 104 bp, 48 bp); 4, 5–genotype TT (104 bp, 48 bp); 7, 9–genotyp CC (152 bp)

In German Wirehaired Pointer were detected one dog (male) with homozygote genotype CC and one dog (female) with heterozygote genotype CT. In Chihuahua was observed homozygote genotype CC (1 female) and heterozygote genotype CT (2 female). In Golden retriever and Pincher we detected only genotype TT. Detailed genotype and gene frequencies per breed are presented in Table 1.

The C allele was presents in all dogs with cAD with an allele frequency ranging from 0.4545 to 1.00, which included breeds Czech pointer, German Wirehaired Pointer and Chihuahua. This suggests that the C allele might be associated with cAD. In Pincher, which showed cAD phenotype we observed genotype TT. This may be due to the fact that has been tested only one dog of this breed or genetic contribution to cAD from clinical observations of breeds associations.

According to several authors, breeds predisposed to cAD include West Highland White Terriers and Boxers [3,5,9,10]. In contrast, sight hounds (e.g., Greyhounds and related breeds) are rarely affected suggesting a genetic component to cAD resistance [11]. Wood et al. (2010) [1] described association of SNP rs22114085 with cAD in eight dog breeds (Boxer, German Shepherd Dog, Labrador, Golden Retriever, Shiba Inu, Shih Tzu, Pit Bull, and West Highland white terrier).

Table 1. Frequency of genotypes and alleles of rs22114085 SNP in the population of dog breeds

		GENOTYPE FREQUENCIES			ALLELE FREQUENCIES	
BREED	DOGS	CC	CT	TT	C	T
Czech Pointer		0.2667	0.4222	0.3111	0.4778	0.5222
Male	12	0.25	0.5833	0.1667	0.5417	0.4583
Female	33	0.2728	0.3636	0.3636	0.4545	0.5455
German Wirehaired Pointer		0.5	0.5	0	0.75	0.5
Male	1	1	0	0	1	0
Female	1	0	1	0	0.5	0.5
Chihuahua		0.3333	0.6667	0	0.6667	0.3333
Female	3	0.3333	0.6667	0	0.6667	0.3333
Golden Retriever		0	0	1	0	1
Male	1	0	0	1	0	1
Pincher		0	0	1	0	1
Male	1	0	0	1	0	1
TOTAL	52					

4. Conclusions

The C allele was presents in all dogs with cAD (Czech Pointer, German Wirehaired Pointer and Chihuahua) with an allele frequency ranging from 0.4545 to 1.00. This suggests that the C allele might be associated with cAD.

In the population of Czech Pointer we detected all genotypes. In German Wirehaired pointer and Chihuahua were detected homozygote genotype CC and heterozygote genotype CT. In Golden Retriever and Pincher we detected only genotype TT.

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