Analysis of Single Nucleotide Polymorphism (SNP) Rs23472497 Associated with Canine Atopic Dermatitis by ACRS-PCR Method

Martina Miluchová, Michal Gábor, Anna Trakovická, Jana Hanusová, Radovan Kasarda

Slovak University of Agriculture in Nitra, 949 76 – Nitra, Tr. A Hlinku, 2, Slovakia

Abstract
The aim of the paper was to identify of the SNP rs23472497 associated with canine atopic dermatitis (cAD). cAD is a common inflammatory skin disease that is considered to be a naturally occurring, spontaneous model of human atopic dermatitis (eczema). The material involved 60 dogs from 6 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 rs23472497 SNP genotypes by ACRS-PCR method. The PCR products were digested with NlaIII restriction enzyme.

In the population of Czech Pointer and Slovak Wirehaired Pointer we detected all genotypes AA, AG and GG with frequency 0.0732, 0.5122 and 0.4146 for Czech Pointer and 0.1818, 0.5455 and 0.2727 for Slovak Wirehaired Pointer. In Border Collie was observed heterozygote genotype AG and homozygote genotype GG with frequency 0.6667 and 0.3333, subsequently. In German Wirehaired Pointer, Australian Shepherd dog and American Staffordshire terrier we detected only genotype AG with frequency 1. The A allele was distributed with an allele frequency ranging from 0.3293 to 0.5. The G allele was distributed with an allele frequency ranging from 0.5 to 0.6707.

Keywords: ACRS-PCR, canine atopic dermatitis, dog, rs23472497, 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 rs23472497 as a protective locus to cAD. This SNP is located on chromosome 29.

2. Materials and methods

The material involved 60 dogs from 6 different breeds (Czech Pointer, German Wirehaired Pointer, Slovak Wirehaired Pointer, Border Collie, Australian Shepherd dog, American Staffordshire terrier).

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 rs23472497 SNP genotypes by ACRS-PCR (ACRS - amplification created restriction site) method.

DNA primers used to PCR amplification (forward primer 5’- TTT GTA GAA ACC TGT CAA GGT CTT GTC - 3’ and reverse primer 5’ - CCT TAT CCC CAA ACC CTT CC - 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 (NH4)2SO4, 0.1% Tween 20), 1.5 mM MgCl2, 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, 56°C for 20 seconds, 72°C for 30 seconds. The reaction was completed by the final extension: 72°C for 5 minutes.

The PCR products of 182 bp were digested with the NlaIII restriction enzyme (Fermentas). Restriction digestion fragments were loaded on 3% agarose gel (Invitrogen) containing GelRedTM (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

NlaIII digestion of the PCR product was analyzed by 3% agarose-gel electrophoresis. Allele G produced 182 bp fragment, and allele A produced 152 bp and 30 bp fragments (Figure 1).

In the population of Czech Pointer and Slovak Wirehaired Pointer we detected all genotypes AA, AG and GG with frequency 0.0732, 0.5122 and 0.4146 for Czech Pointer and 0.1818, 0.5455 and 0.2727 for Slovak Wirehaired Pointer.
In Border Collie was observed heterozygote genotype AG and homozygote genotype GG with frequency 0.6667 and 0.3333, subsequently. In German Wirehaired Pointer, Australian Shepherd dog and American Staffordshire terrier we detected only genotype AG with frequency 1. The A allele was distributed with an allele frequency ranging from 0.3293 to 0.5. The G allele was distributed with an allele frequency ranging from 0.5 to 0.6707. Detailed genotype and gene frequencies per breed are presented in Table 1.

Table 1. Frequency of genotypes and alleles of rs23472497 SNP in the population of dog breeds

<table>
<thead>
<tr>
<th>BREED</th>
<th>DOGS</th>
<th>GENOTYPE FREQUENCIES</th>
<th>ALLELE FREQUENCIES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>AG</td>
</tr>
<tr>
<td>Czech Pointer</td>
<td>41</td>
<td>0.0732</td>
<td>0.5122</td>
</tr>
<tr>
<td>German Wirehaired Pointer</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Slovak Wirehaired Pointer</td>
<td>11</td>
<td>0.1818</td>
<td>0.5455</td>
</tr>
<tr>
<td>Border Collie</td>
<td>3</td>
<td>0</td>
<td>0.6667</td>
</tr>
<tr>
<td>Australian Shepherd dog</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>American Staffordshire terrier</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 rs23472497 with cAD in eight dog breeds (Boxer, German Shepherd Dog, Labrador, Golden Retriever, Shiba Inu, Shih Tzu, Pit Bull, and West Highland white terrier). All Golden Retrievers showed a significant association with rs23472497, but individual breed associations with this SNP were not seen in the other breeds. This may have been due to the low sample sizes in some breeds affecting statistical power.

4. Conclusions

In the population of Czech Pointer and Slovak Wirehaired Pointer we detected all genotypes AA, AG and GG. In Border Collie was observed heterozygote genotype AG and homozygote genotype GG. In German Wirehaired Pointer, Australian Shepherd dog and American Staffordshire terrier we detected only genotype AG. Due to the low number of individuals for each breed and incomplete clinical records was not possible to clearly establish the association of SNP rs23472497 to alopecia.

Acknowledgements

This work has been supported by:
1. The Slovak Research and Development Agency under the contract No. LPP-0220-09 and No. APVV-0636-11
2. Excellence Centre for Agrobiodiversity and Benefit project (ECOVA, ITMS: 26220120015) implemented under the Operational Programme Research and Development financed by European Fund for Regional Development.
3. The Slovakian Club of Czech Pointer Breeders.

References

American Journal of Veterinary Research, 2004, 65, pp. 1014-20
6. Gábor, M., Genetické markery kvality mäsa hovädzieho dobytka a oviec, Doktorandská dizertačná práca (PhD.), SPU, Nitra, 2009, pp. 199