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# GENETIC VARIATION OF TWO HORSE BREEDS IN CPG ISLANDS OF *OAS1* LOCUS*

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**Abstract**

**Allelic and haplotypic variations at 27 SNP sites identified in four CpG islands of *OAS1* were described in a group of Anglo-Arabian and Hucul horses. Variation in the type of less frequent alleles was the source of variability among breeds. A number of putative LD blocks were identified which could be used to study changes in the genetic structure between generations of both breeds concerning susceptibility to flaviviral infections. Some of the identified SNPs may have an impact on the transcriptional activity of *OAS1* or could lead to amino acid substitution influencing proper function of *OAS1* enzyme. In the light of recent studies, the described genetic variability of investigated CpG islands might be important in view of the effectiveness of viral incorporation into the host genome.**

**Key words: *OAS1*, genetic variation, CpG islands, horses**

Studies of molecular features of the immune system are important to find efficient ways of prevention and treatment during pathogenic infections. One of major genetic components of the immune system are interferon signalling pathways. These involve genes which are responsible for proper creation of immune response and the preservation of antiviral state of cells. Around 300 interferon inducible genes are involved in direct fight with pathogens on the cellular level. One of the best known is *OAS1* gene which belongs to the 2'–5' oligoadenylate synthetase (OAS) gene family. The transcription of OAS members is actively induced by interferon after virus in-

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fection (Melchjorsen et al., 2009). Polymorphic variants of equine *OAS1* gene were investigated in case-control mode of naturally occurring susceptible and resistant horses to lethal West Nile encephalitis (WNE). All of the associated *OAS1* polymorphisms were located within the promoter region (Rios et al., 2010). In turn, studies in other species like human and mouse allowed identifying polymorphisms located inside the *OAS1* locus to be responsible for higher susceptibility to West Nile Virus (WNV) and other flaviviral infections (Lucas et al., 2003; Lim et al., 2009). Splice variants of biological importance of *OAS1* have been found in the case of human WNV disease (Lim et al., 2009). Also the nonsense mutations in *OAS1* ortholog were described in mouse strains with greater susceptibility to flaviviral infections (Lucas et al., 2003). In turn, polymorphism in the 3'-untranslated region of *OAS1* was responsible for increased affinity for hepatitis C infections in humans which also belongs to the flavivirus family (Knapp et al., 2003). In this work allelic and haplotypic variation at 27 SNP sites of *OAS1* locus were described in a group of Anglo-Arabian and Hucul horses. Both horse breeds possess different breeding history and differ in conformation traits, types of utility, nutrition requirements and health. Therefore, they are a good subject for searching numerous genetic variants in substantially distinct allelic pools. The target of polymorphism identification were four CpG islands (CGIs) placed in the *OAS1* region. CpG islands located at the 5' ends near the gene transcription start sites often distribute regulatory features that are a target for binding of transcriptional complexes resulting in hypomethylated state of CGIs (Deaton and Bird, 2011). One of the features of CpG islands is their ability to accumulate a large number of mutations due to spontaneous deamination of methylated cytosines (Hellman and Chess, 2010). Therefore, CG rich sequences are a good source of potential genetic markers to conduct association based studies. Another issue is that CpG islands were found to be attractive sites of retroviral incorporation into the host genome (Derse et al., 2007; Gillet et al., 2013). The comparison of polymorphic rate (minor allele frequency) of identified SNPs was conducted for markers located in coding and non-coding parts of the gene.

## Material and methods

Detection of sequences with increased CG content in proximity to the region of *OAS1* gene was conducted with EMBOSS Cpgplot software (Larsen et al., 1992). *OAS1* promoter region was predicted using MatInspector software (Cartharius et al., 2005). Blood samples of 30 Anglo-Arabian and 30 Hucul horses were the source of DNA which was isolated using Wizard Genomic DNA purification kit (Promega). DNA samples were amplified with a set of PCR primers corresponding to identified CGIs using hot start Taq DNA polymerase (Qiagen) and then sequenced with SANGER method using BigDye Terminator sequencing chemistry (Life Technologies) (Table 1). Sequencing reads were inspected for polymorphic sites in the investigated DNA stretches. To achieve this, SEQUENCHER software (Sequencher® version 5.2) was implemented. All SNPs were submitted to the NCBI GeneBank. Se-

quencing stretches of particular SNP variants were checked for putative amino acid changes of encoded protein using Blastx software (NCBI). Allele frequencies were calculated and the test for genetic equilibrium (Guo and Thompson, 1992) was performed using Arlequin software (Excoffier, 2010). Minor allele frequency (MAF), observed (HObs) and expected heterozygosity (HExp) were calculated to evaluate the polymorphic rate of SNP sites (CERVUS software) (Summers and Amos, 1997). Haplotypes were determined with the Expectation-Maximization (EM) algorithm using Arlequin software (Excoffier and Slatkin, 1995).

## Results

Four CGIs identified in the 17320 bp region (genomic contig NW_001870614) of *OAS1* locus (NCBI GeneID: 100034147) were a subject of this study (Table 1). Sequencing of all CGIs revealed the presence of 32 SNP sites from which 27 were in genetic equilibrium. Exonic SNPs were comprised of 4 markers located in exon 1 (SNP5 – SNP8) and 5 markers in exon 2 (SNP12 – SNP16) (Table 1). Putative amino acid changes were detected due to polymorphisms at the SNP 7 and 15 (Table 2). Allele G at the SNP 7 resulted in non-conservative amino acid change. In turn, the presence of allele G at the SNP15 led to conservative substitution (Table 2).

Table 1. Nucleotide positions of identified CpG islands in relation to the OAS1 locus and location of corresponding PCR products in the genomic contig NW_001870614

| CpG island | CGI start..stop | CG% | Investigated markers | PCR oligos (5'-3') | PCR product start..stop | Size (bp) | Ta (°C) |
|---|---|---|---|---|---|---|---|
| CGI2 | 25142..25798 (upstream to 5'end) | 47.7 | SNP1 | F:ctggaaaattacaaaacatttggag R:tccctctctgagttcagatctt | 25142..25798 | 657 | 59 |
| CGI3 | 23719..24695 (promoter and exon 1) | 48.9 | SNP2 – SNP8 | F:gaggatcgtgggtgatctttac R:cccttcctctctggaaactctt | 23728..24484 | 757 | 60 |
| CGI4 | 22692..23503 (intron and exon 2) | 53.8 | SNP9 – SNP18 | F:ctggggagtcactgtttctgcag R:caggcaggacatcaaattccac | 22692..23503 | 812 | 60 |
| CGI6 | 4677..5199 (downstream to 3'end) | 54.5 | SNP19 – SNP27 | F:agcatgctcttatcacctgagc R:caggtgagctgtttgctatctg | 4605..5223 | 619 | 61 |

Table 2. Allele frequencies of 27 SNP sites, p-values of HW test, observed (HObs) and expected (HExp) heterozygosities, amino acid changes

| Marker | Allele frequency | | | | HW | | HObs | | HExp | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | AA | | HC | | AA | HC | AA | HC | AA | HC | Amino acids substitution |
| snp1 (ss#836189208) | A (0.147)* | G (0.853) | A (0)* | G (1) | 1.00000 | - | 0.294 | - | 0.258 | - | - |
| **snp2 (ss#836189225)** | T (0.176) | C (0.824) | T (0.1) | C (0.9) | 1.00000 | 1.00000 | 0.353 | 0.2 | 0.299 | 0.185 | - |
| **snp3 (ss#836189226)** | C (0.176) | T (0.824) | C (0.125) | T (0.875) | 1.00000 | 1.00000 | 0.353 | 0.25 | 0.299 | 0.224 | - |
| **snp4 (ss#836189227)** | C (0) | G (1) | C (0.425) | G (0.575) | - | 0.35451 | - | 0.65 | - | 0.501 | - |
| snp5 (ss#836189230) | G (0.176) | C (0.824) | G (0.125) | C (0.875) | 1.00000 | 1.00000 | 0.353 | 0.25 | 0.299 | 0.224 | - |
| snp6 (ss#836189231) | A (0.176) | C (0.824) | A (0.125) | C (0.875) | 1.00000 | 1.00000 | 0.353 | 0.25 | 0.299 | 0.224 | - |
| snp7 (ss#836189232) | G (0.147) | A (0.853) | G (0.35) | A (0.65) | 1.00000 | 0.32814 | 0.294 | 0.6 | 0.258 | 0.467 | 18Cys (G allele) >Tyr (A allele) |
| snp8 (ss#836189210) | C (0.206) | T (0.794) | C (0.125) | T (0.875) | 0.53636 | 1.00000 | 0.294 | 0.25 | 0.337 | 0.224 | - |
| snp9 (ss#836189233) | T (0.353) | C (0.647) | C (0.2) | T (0.8) | 1.00000 | 0.54797 | 0.471 | 0.4 | 0.471 | 0.328 | - |
| snp10 (ss#836189211) | T (0.353) | C (0.647) | C (0.225) | T (0.775) | 1.00000 | 0.52896 | 0.471 | 0.45 | 0.471 | 0.358 | - |
| snp11 (ss#836189212) | T (0.353) | C (0.647) | C (0.2) | T (0.8) | 1.00000 | 0.54860 | 0.471 | 0.4 | 0.471 | 0.328 | - |
| snp12 (ss#836189213) | T (0.353) | C (0.647) | C (0.225) | T (0.775) | 1.00000 | 0.52864 | 0.471 | 0.45 | 0.471 | 0.358 | - |
| snp13 (ss#836189214) | C (0.353) | T (0.647) | T (0.225) | C (0.775) | 1.00000 | 0.52860 | 0.471 | 0.45 | 0.471 | 0.358 | - |
| snp14 (ss#836189215) | G (0.353) | A (0.647) | A (0.25) | G (0.75) | 1.00000 | 0.27646 | 0.471 | 0.5 | 0.471 | 0.385 | - |
| snp15 (ss#836189216) | A (0.353) | G (0.647) | G (0.225) | A (0.775) | 1.00000 | 0.52762 | 0.471 | 0.45 | 0.471 | 0.358 | 99Glu (G allele) > Lys (A allele) |
| snp16 (ss#836189217) | A (0.353) | G (0.647) | G (0.225) | A (0.775) | 1.00000 | 0.52977 | 0.471 | 0.45 | 0.471 | 0.358 | - |
| snp17 (ss#836189234) | T (0.324) | G (0.676) | G (0.45) | T (0.55) | 0.60814 | 0.64992 | 0.529 | 0.6 | 0.451 | 0.508 | - |
| snp18 (ss#836189235) | T (0.471) | C (0.529) | T (0.125) | C (0.875) | 0.64409 | 1.00000 | 0.588 | 0.25 | 0.513 | 0.224 | - |
| snp19 (ss#836189236) | C (0.324) | T (0.676) | T (0.45) | C (0.55) | 0.60943 | 0.64946 | 0.529 | 0.6 | 0.451 | 0.508 | - |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| snp20 (ss#836189218) | A (0.324) | C (0.676) | C (0.45) | A (0.55) | 0.60928 | 0.64896 | 0.529 | 0.6 | 0.451 | 0.508 | - |
| snp21 (ss#836189237) | T (0.324) | A (0.676) | A (0.45) | T (0.55) | 0.60775 | 0.64979 | 0.529 | 0.6 | 0.451 | 0.508 | - |
| snp22 (ss#836189238) | A (0.471) | G (0.529) | A (0.1) | G (0.9) | 0.64348 | 1.00000 | 0.588 | 0.2 | 0.513 | 0.185 | - |
| snp23 (ss#836189239) | C (0.324) | T (0.676) | T (0.45) | C (0.55) | 0.60755 | 0.64974 | 0.529 | 0.6 | 0.451 | 0.508 | - |
| snp24 (ss#836189240) | A (0.324) | C (0.676) | C (0.45) | A (0.55) | 0.60835 | 0.64940 | 0.529 | 0.6 | 0.451 | 0.508 | - |
| snp25 (ss#836189219) | A (0.324) | G (0.676) | G (0.45) | A (0.55) | 0.60855 | 0.64986 | 0.529 | 0.6 | 0.451 | 0.508 | - |
| snp26 (ss#836189220) | A (0.324) | C (0.676) | C (0.4) | A (0.6) | 0.60871 | 0.38552 | 0.529 | 0.6 | 0.451 | 0.492 | - |
| snp27 (ss#836189221) | C (0.324) | G (0.676) | G (0.45) | C (0.55) | 0.60759 | 0.64912 | 0.529 | 0.6 | 0.451 | 0.508 | - |

* First columns include minor allele frequencies (MAF); the highest values of H indices are underlined; SNPs located in the promoter are is bold.

Single loci were monomorphic for both breeds while MAF was in the range from 0.147 to 0.471 for AA horse group and from 0.1 to 0.45 for Hucul breed. Numerous alleles of different SNPs demonstrating the same frequency were identified. AA horses were characterized by the presence of four different groups of alleles with equal frequencies between loci. Groups were comprised of the following SNPs: 1st group: SNP1, 2, 3, 5, 6, 7; 2nd: SNP9, 10, 11, 12, 13, 14, 15, 16; 3rd: SNP17, 19, 20, 21, 23, 24, 25, 26, 27; 4th: SNP18, 22. For Hucul breed, five groups of equal interlocus allelic frequency were identified (1st including SNP2, 22; 2nd: SNP3, 5, 6, 8, 18; 3rd: SNP9, 11; 4th: SNP10, 12, 13, 15, 16; 5th: SNP17, 19, 20, 21, 23, 24, 25, 27) (Table 2). Both breeds differed in the presence of less frequent allele for a total of 17 SNPs (Table 2). Concerning relative small sample sizes of AA and HC horses, only haplotype frequencies above 10% were considered as the most probable (Table 3). Three of them were present in the AA horse group and two in the Hucul breed. One haplotype was common for both breeds (Table 3). Small differences between allelic frequencies of particular markers were consistent with small differences between observed and expected heterozygosity indices (Table 2). The degree of differences between the frequencies of both alleles at each of the investigated SNP sites were in general lower for variants detected in AA horses (Figure 1). Eight markers showed lack of differences between observed and expected H values for the AA group (Figure 2). The values of mean MAF and heterozygosity indices across all 27 SNP markers were higher for the Anglo-Arabian horses (Table 4).

Table 3. Haplotype variation among two horse breeds

| Haplotype No. | Sequence predicted using EM algorithm | Frequency | |
|---|---|---|---|
| | | AA | HC |
| 1 | ACTGCCATTTTTCGAAGTTCAATCGCG | 0.029 | 0 |
| 2 | ACTGCCATTTTTCGAATCCATGCAAAC | 0.118 | 0 |
| 3 | GCCGGAACCCCCTAGGTCCATGCAAAC | 0 | 0.025 |
| 4 | GCTCCCATTTTTCAAATCCATGCAAAC | 0 | 0.025 |
| 5 | GCTCCCATTTTTCGAATCCATGCAAAC | 0 | 0.375 |
| 6 | GCTCCCATTTTTCGAATCCATGTCGCG | 0 | 0.025 |
| 7 | GCTGCCACCCCCTAGGGTTCAATCGCG | 0.029 | 0.025 |
| 8 | GCTGCCATCCCCTAGGGTTCAATCGCG | 0.324 | 0.05 |
| 9 | GCTGCCATCCCCTAGGTCCATGCAAAC | 0.088 | 0 |
| 10 | GCTGCCATTCTCTAGGGTTCAATCGCG | 0 | 0.025 |
| 11 | GCTGCCATTTTTCGAAGCTCAGTCGCG | 0.059 | 0 |
| 12 | GCTGCCATTTTTCGAATCCATGCAAAC | 0.029 | 0 |
| 13 | GCTGCCGTCCCCTAGGTCCATGCAAAC | 0.029 | 0 |
| 14 | GCTGCCGTTTTTCGAAGCTCAGCAAAC | 0 | 0.025 |
| 15 | GCTGCCGTTTTTCGAAGCTCAGTCGAG | 0 | 0.025 |
| 16 | GCTGCCGTTTTTCGAAGCTCAGTCGCG | 0.118 | 0.25 |
| 17 | GCTGCCGTTTTTCGAAGTTCAGTCGCG | 0 | 0.025 |
| 18 | GCTGCCGTTTTTCGAATCCATGCAAAC | 0 | 0.025 |
| 19 | GTCGGAACCCCCTAGGGCTCAGTCGCG | 0.029 | 0.025 |
| 20 | GTCGGAACCCCCTAGGGTTCAATCGCG | 0.088 | 0 |
| 21 | GTCGGAACCCCCTAGGTCCATGCAAAC | 0.059 | 0.075 |

Table 4. Values of the mean MAF and expected heterozygosity

| | AA | HC |
|---|---|---|
| Mean of minor allele frequency (MAF) | 0.292 | 0.273 |
| Mean of expected heterozygosity | 0.4038 | 0.3645 |

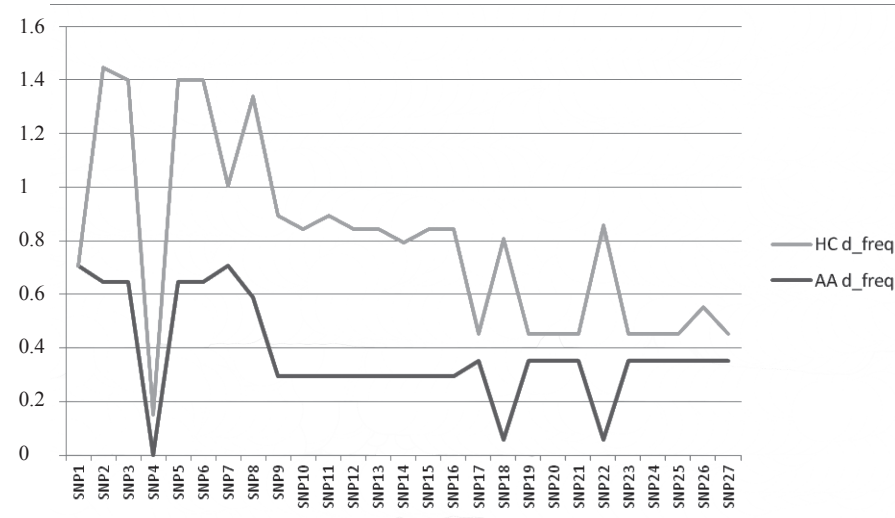


Figure 1. Level of differences between frequencies of both alleles at each of 27 SNP sites calculated for AA and HC horse groups

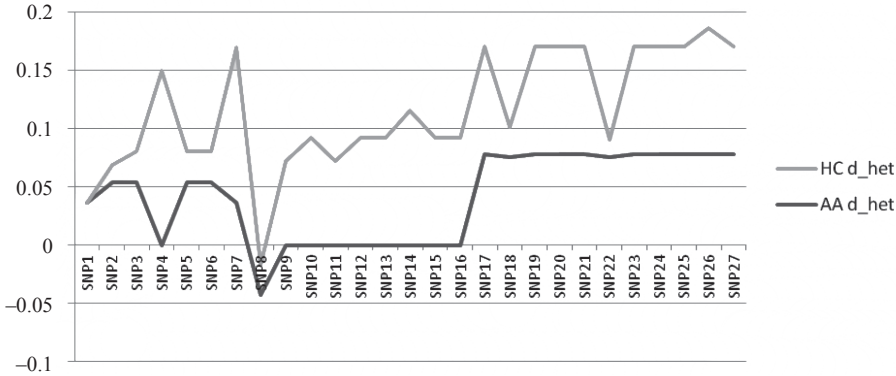


Figure 2. Level of differences between observed and expected heterozygosities calculated for each of 27 SNP markers in the AA and HC horse groups

## Discussion

*OAS1* is an IFN inducible gene which is activated in the presence of viral RNA in infected cells. Therefore, its role is mainly connected with the defence against viral

infections. A number of studies in humans, mice and horses documented the role of *OAS1* genetic variants in the susceptibility to flaviviral infections (Lucas et al., 2003; Lim et al., 2009; Rios et al., 2010). Three of identified polymorphic sites in this study are located in the promoter region of *OAS1* and one of them occurs only in the Hucul horse group. The identified promoter SNPs enlarge the panel of markers putatively important for the transcriptional activity of *OAS1* gene (Rios et al., 2010). Among the SNP sites located in the coding parts of the gene two are the source of potential amino acid substitution and these sites were reported previously (Rios et al., 2007). One of them could change the protein function and might impair the activity of the OAS1 enzyme. It is known that some point mutations in this locus found in humans led to the decrease of OAS enzymatic activity which correlated with increased risk of infections (Lim et al., 2009). High mutation rates in CG rich sequences are a great source of polymorphisms in comparison to other genomic regions. This is the case for investigated CGIs around *OAS1* locus. Sequencing of only four CGI fragments ranging from 657 to 812 bp allowed identifying comparable number of informative SNP sites to a set of 27 informative loci recognized after sequencing of thirty PCR fragments that covered the region of 18000 bp of the equine *TERT* gene (Ząbek et al., 2012). Characterization of sequence variability of CpG islands might be important for the studies on mechanisms of viral incorporation into the genome during infection. It was found that a number of recombining viruses prefer to integrate within certain parts of the host genome. During the initial phase viral genomes are mostly integrated into the actively transcribed genes or near their transcription start sites. The specific structure of virus-encoded integrases is the major factor of target site selection (Derse et al., 2007; Gillet et al., 2013). It was shown that during the initial stage of infection a majority of sites of viral integration are located near CpG islands of transcribed regions (Gillet et al., 2013). In this sense, observed variation of CpG sites would be significant for the occurrence of specific entries in the host genome "being recognized by viral particles". Identification of SNP variants with equal frequencies between particular loci of four CpG islands may indicate perfect linkage disequilibrium (LD) between them. Based on the frequency data of 27 SNPs the occurrence of four putative LD blocks in AA horses and five LDs in the Huculs can be assumed which could be then used to track trans-generational changes in the genetic structure of both horse breeds in terms of potential susceptibility to flaviviral infections. Some of SNPs of particular LD blocks could be then used as tag SNPs in GWAS like projects to find subsequent variants associated with increased risk of viral infections. From the 27 investigated loci, 19 SNPs in AA horses and 15 SNPs in HC horses (all of which belonged to three putative LD blocks) reveal MAF values greater than 0.2, which predispose them to be efficient genetic markers. For example, MAF value for SNP loci selected to develop DNA microarray suitable for scanning of the genome of cattle ranged between 0.24 and 0.27 (Matukumalli et al., 2009). According to Werner et al. (2004), MAF greater than 0.1 served as a criterion for choosing SNP markers for parentage tests for cattle. Breeds differentiation concerning the types of minor alleles was the source of the variability of predicted haplotypes. Lower number of haplotypes and lower mean heterozygosity for the Hucul horse group are contrary to the data reported so far (Ząbek et al., 2005). It is possible they

could be biased by greater relatedness among Huculs, because of relatively small sample sizes used in this study.

*OAS1* locus includes a number of genetic variants important for the VWN and other flaviviral diseases and presented findings would contribute to further extending the knowledge about genetic component of susceptibility to diseases caused by viruses in horses. Description of polymorphic variants of CpG islands would also be important to study the hypothesis of effectiveness of viral integration into the host genome during initial stages of infection.

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