

# Genetic susceptibility to sarcoid in Arabian horses: associations with MHC class II and compound MHC class I/KLRA genotypes

Veterinary Research and Communications

Leona Vychodilova<sup>1</sup>, Martin Plasil<sup>2</sup>, Jan Futas<sup>1,2</sup>, Andrea Kopecka<sup>1</sup>, Dobromila Molinkova<sup>1</sup>, Tamara Wijacki<sup>3</sup>, Petr Jahn<sup>1</sup>, Ales Knoll<sup>3</sup>, Petr Horin<sup>1,2 \*</sup>

<sup>1</sup>Faculty of Veterinary Medicine, University of Veterinary Sciences, Brno, Czech Republic

<sup>2</sup>Ceitec Vetuni, University of Veterinary Sciences, Brno, Czech Republic

<sup>3</sup>Faculty of Agronomy, Mendel University, Brno, Czech Republic

Corresponding author email: [horin@med.muni.cz](mailto:horin@med.muni.cz)

**Table S1** Primers

| Primers       | Sequence                                              | PCR product length | Ta (°C) | Position NC_009163 CHr 20 EquCab 3.0 | Position Chr 20 TB-T2T   |
|---------------|-------------------------------------------------------|--------------------|---------|--------------------------------------|--------------------------|
| <i>COR112</i> | 5'ccccaagcagaccaaacttc3' /<br>5'agggtctgagaggaaaacg3' | 5406               | 62      | 34186982bp<br>34192388bp             | 36696389bp<br>36701793bp |

**Table S2** Haplotypes across the entire MHC or NKC region

| UMN-JH34-2 305-93 CZM001 CZM002 ABGe17402 TKY2933 |                  |                         | Sarcoid         |                 |                           |                         |
|---------------------------------------------------|------------------|-------------------------|-----------------|-----------------|---------------------------|-------------------------|
| <b>MHCI</b>                                       | <b>Absolute</b>  |                         |                 |                 |                           |                         |
| <b>Most common haplotypes</b>                     | <b>frequency</b> | <b>Alleles</b>          | <b>positive</b> | <b>negative</b> | <b>P<sub>uncorr</sub></b> | <b>P<sub>corr</sub></b> |
| ≥10%                                              |                  |                         |                 |                 |                           |                         |
| 1                                                 | 10               | 204 128 174 248 205 209 | 4               | 6               | NS                        | NS                      |
| 2                                                 | 14               | 214 128 180 234 209 221 | 4               | 10              | NS                        | NS                      |
| 4                                                 | 17               | 204 128 176 254 219 217 | 4               | 13              | NS                        | NS                      |
| 6                                                 | 8                | 208 126 176 246 211 217 | 0               | 8               | <b>0.039</b>              | 0.195                   |
| Number of different haplotypes                    |                  |                         | 24              |                 |                           |                         |

  

| CZM004 TKY3324 COR112 UM11 COR114 |                  |                     | Sarcoid         |                 |                           |                         |
|-----------------------------------|------------------|---------------------|-----------------|-----------------|---------------------------|-------------------------|
| <b>MHCII</b>                      | <b>Absolute</b>  |                     |                 |                 |                           |                         |
| <b>Most common haplotypes</b>     | <b>frequency</b> | <b>Alleles</b>      | <b>positive</b> | <b>negative</b> | <b>P<sub>uncorr</sub></b> | <b>P<sub>corr</sub></b> |
| ≥10%                              |                  |                     |                 |                 |                           |                         |
| 1                                 | 22               | 118 246 252 180 233 | 4               | 18              | NS                        | NS                      |
| 2                                 | 10               | 122 256 246 168 237 | 4               | 6               | NS                        | NS                      |
| 4                                 | 15               | 118 246 244 168 237 | 1               | 14              | <b>0.014</b>              | 0.055                   |
| 9                                 | 6                | 118 246 244 168 231 | 1               | 5               | NS                        | NS                      |
| Number of different haplotypes    |                  |                     | 23              |                 |                           |                         |

  

| CZM003 ABGe9019 HMS082        |                  |                | Sarcoid         |                 |                           |                         |
|-------------------------------|------------------|----------------|-----------------|-----------------|---------------------------|-------------------------|
| <b>MHCIII</b>                 | <b>Absolute</b>  |                |                 |                 |                           |                         |
| <b>Most common haplotypes</b> | <b>frequency</b> | <b>Alleles</b> | <b>positive</b> | <b>negative</b> | <b>P<sub>uncorr</sub></b> | <b>P<sub>corr</sub></b> |
| ≥10%                          |                  |                |                 |                 |                           |                         |
| 1                             | 10               | 285 304 207    | 4               | 6               | NS                        | NS                      |
| 2                             | 20               | 285 310 205    | 7               | 13              | NS                        | NS                      |
| 4                             | 22               | 285 292 205    | 7               | 15              | NS                        | NS                      |
| 5                             | 12               | 285 304 209    | 2               | 10              | NS                        | NS                      |

|                                    |   |             |   |   |    |    |
|------------------------------------|---|-------------|---|---|----|----|
| 6                                  | 7 | 282 298 207 | 1 | 6 | NS | NS |
| 7                                  | 6 | 282 308 209 | 1 | 5 | NS | NS |
| The number of different haplotypes |   | 10          |   |   |    |    |

  

| CZM005 CZM006 CZM007 CZM008        |                    |                 | Sarcoid  |          |                     |                   |
|------------------------------------|--------------------|-----------------|----------|----------|---------------------|-------------------|
| <i>NKC</i>                         | Absolute frequency | Alleles         | positive | negative | P <sub>uncorr</sub> | P <sub>corr</sub> |
| Most common haplotypes             |                    |                 |          |          |                     |                   |
| ≥10%                               |                    |                 |          |          |                     |                   |
| 1                                  | 18                 | 271 222 242 267 | 3        | 15       | NS                  | NS                |
| 2                                  | 11                 | 269 220 242 261 | 1        | 10       | NS                  | NS                |
| 3                                  | 16                 | 269 218 242 265 | 4        | 12       | NS                  | NS                |
| 6                                  | 9                  | 269 222 242 267 | 4        | 5        | NS                  | NS                |
| 4                                  | 6                  | 273 218 242 267 | 2        | 4        | NS                  | NS                |
| 5                                  | 6                  | 269 222 242 265 | 0        | 6        | NS                  | NS                |
| The number of different haplotypes |                    | 17              |          |          |                     |                   |

**Table S3** Associations of individual SNPs within the *COR112* region

| SNP position chrom. 20 in the |          | Allele | Effect | P <sub>uncorr</sub> | P <sub>corr</sub>     |
|-------------------------------|----------|--------|--------|---------------------|-----------------------|
| EquCab3                       | TB-T2T   |        |        |                     |                       |
| 34190845                      | 36700259 | G      | R      | 4*10 <sup>-5</sup>  | 9.75*10 <sup>-5</sup> |
| 34190924                      | 36700338 | T      | R      | 0.002               | 0.003                 |
| 34190992                      | 36700406 | A      | R      | 0.004               | 0.008                 |
| 34190996                      | 36700410 | A      | R      | 0.004               | 0.008                 |
| 34191971                      | 36701386 | G      | R      | 0.004               | 0.008                 |
| 34192067                      | 36701482 | A      | R      | 0.0007              | 0.002                 |
| 34192285                      | 36701710 | C      | R      | 0.0007              | 0.002                 |

R resistance

**Table S4** Associations of MHC class III microsatellites

| Microsatellite | FST   | CHI2<br>P |
|----------------|-------|-----------|
| MHCIII         | 0.000 | NS        |
| HMS 082        | 0.000 | NS        |
| ABGe 9019      | 0.000 | NS        |

NS – non significant

**Table S5** NKC association analysis

| Microsatellite | subregion | FST   | CHI2<br>P |
|----------------|-----------|-------|-----------|
| CZM011         | KLRA      | 0.004 | NS        |
| CZM006         | KLRA      | 0.015 | NS        |
| CZM005         | KLRA      | 0.002 | NS        |
| TKY1745        | CLEC      | 0.000 | NS        |
| CZM009         | KLRA      | 0.022 | NS        |
| CZM010         | KLRA      | 0.000 | NS        |
| CZM008         | KLRA      | 0.000 | NS        |
| CZM012         | KLRA      | 0.061 | NS        |
| CZM007         | KLRA      | 0.000 | NS        |
| CZM013         | KLRA      | 0.017 | NS        |
| ABGe3660       | KLRA      | 0.000 | NS        |

NS – non significant

**Table S6** Associations of MHC class II/KLRA microsatellites combinations with sarcoid in 43 Arabian horses

| Individual <i>MHCII</i><br>microsatellite | <b>P<sub>uncorr</sub></b> | <b>P<sub>corr</sub></b> | <i>MHCII/KLRA</i><br>allele combinations | <b>P</b>     | <b>R/S</b> |
|-------------------------------------------|---------------------------|-------------------------|------------------------------------------|--------------|------------|
| <i>TKY3324</i>                            | <b>0.006</b>              | <b>0.018</b>            | <i>COR112 /CZM007</i>                    | <b>0.006</b> | R          |
| 246                                       |                           |                         | 244/242                                  |              |            |
| <i>COR112</i>                             | <b>0.006/ 0.002/</b>      | <b>0.024/ 0.008</b>     | <i>COR112 / CZM005</i>                   | <b>0.001</b> | R          |
| 244/252/254                               | <b>0.024</b>              | 0.096                   | 244/269                                  |              |            |
|                                           |                           |                         | <i>TKY3324 / CZM005</i>                  | <b>0.001</b> | R          |
|                                           |                           |                         | 246/269                                  |              |            |
|                                           |                           |                         | <i>TKY3324 / CZM007</i>                  | <b>0.001</b> | R          |
|                                           |                           |                         | 246/242                                  |              |            |

R resistance

**Table S7** Associations between non-MHC microsatellites and presence of sarcoid

| <b>Msat</b>   | <b>Eca #</b> | <b>P<sub>uncorr</sub></b> | <b>P<sub>corr</sub></b> | <b>Msat</b>  | <b>Eca #</b> | <b>P<sub>uncorr</sub></b> | <b>P<sub>corr</sub></b> |
|---------------|--------------|---------------------------|-------------------------|--------------|--------------|---------------------------|-------------------------|
| <i>VHL20</i>  | 30           | <b>0.032</b>              | NS                      | <i>HMS3</i>  | 9            | <b>0.042</b>              | NS                      |
| <i>HTG4</i>   | 9            | NS                        | NS                      | <i>HMS2</i>  | 10           | NS                        | NS                      |
| <i>AHT4</i>   | 24           | NS                        | NS                      | <i>HTG7</i>  | 4            | NS                        | NS                      |
| <i>HMS7</i>   | 1            | NS                        | NS                      | <i>ASB17</i> | 5            | NS                        | NS                      |
| <i>HMS6</i>   | 4            | NS                        | NS                      | <i>LEX3</i>  | X            | <b>0.021</b>              | NS                      |
| <i>ASB23</i>  | 3            | <b>0.022</b>              | NS                      | <i>HMS1</i>  | 15           | <b>0.011</b>              | NS                      |
| <i>ASB2</i>   | 15           | NS                        | NS                      | <i>CA425</i> | 28           | NS                        | NS                      |
| <i>HTG 10</i> | 21           | NS                        | NS                      |              |              |                           |                         |

Msat microsatellite; NS non-significant

**Table S8** Sequence in COR112 region related to miRNA

| <b>miRNA</b> | <b>Accessions no</b> | <b>Eqca 20 location</b> |
|--------------|----------------------|-------------------------|
| eca-mir-9152 | MI0028375            | 36695841                |
| eca-mir-9013 | MI0028175            | 36695835                |
| eca-mir-9004 | MI0028164            | 36702611                |