Investigation of genetic risk factors for squamous cell carcinoma of the equine eye

Kari Hancock, Kelly Knickelbein, Zachary Lounsbury, Moriel Singer-Berk, Mary Lassaline, Rebecca Bellone, Veterinary Genetics Laboratory, School of Veterinary Medicine, University of California-Davis, Davis, CA; a Veterinary Medical Teaching Hospital, University of California-Davis, Davis, CA; b Department of Surgical and Radiological Sciences, School of Veterinary Medicine, c Department of Population Health and Reproduction, School of Veterinary Medicine, University of California-Davis, Davis, CA.

Abstract

Squamous cell carcinoma (SCC) has a high prevalence in equine horses indicating a genetic predisposition. A missense variant on equine chromosome 12 (ECA12) (c.103 C>T p.Thr33Met) in damage specific DNA binding protein 2 (DDB2) explains 76% of ocular SCC-affected Haflingers.1,2 This variant was not perfectly concordant with SCC status, as 24% of the affected horses were not homozygous for the risk allele. Further investigation of the 1.5 Mb region on ECA12 via serval methodologies did not identify another variant that was more strongly associated with the disease phenotype.3 It is hypothesized that genetic heterogeneity contributes to cancer risk. To investigate this hypothesis, whole genome Illumina sequencing data from four Haflingers (two ocular SCC affected horses without the DDB2 risk variant and two clinically unaffected horses) were evaluated to identify variants segregating with disease status. Thirty one variants were selected for further investigation based on genomic location, estimated allelic frequency difference between cases and controls, gene function, and SNPeff predicted effects.1 Assessment of these variants within a sample set of 76 horses (14 ocular SCC-affected horses not homozygous for the DDB2 risk variant and 62 unaffected controls) identified two alleles that warrant further evaluation.

Background

- Squamous cell carcinoma (SCC), the most common cancer of the equine eye, has a high prevalence in Haflinger horses indicating a genetic predisposition.
- Potential contributors to ocular SCC include UV radiation, viral exposure, hormonal regulation, coat color and breed predispositions.1
- A recently identified recessive missense variant (c.103 C>T p.Thr33Met) in a damage specific DNA binding protein 2 (DDB2) explains 76% of ocular SCC-affected Haflingers.1,2 This variant was not perfectly concordant with SCC status, as 24% of the affected horses were not homozygous for the risk allele. Further investigation of the 1.5 Mb region on ECA12 via serval methodologies did not identify another variant that was more strongly associated with the disease phenotype.3 It is hypothesized that genetic heterogeneity contributes to cancer risk. To investigate this hypothesis, whole genome Illumina sequencing data from four Haflingers (two ocular SCC affected horses without the DDB2 risk variant and two clinically unaffected horses) were evaluated to identify variants segregating with disease status. Thirty one variants were selected for further investigation based on genomic location, estimated allelic frequency difference between cases and controls, gene function, and SNPeff predicted effects.1 Assessment of these variants within a sample set of 76 horses (14 ocular SCC-affected horses not homozygous for the DDB2 risk variant and 62 unaffected controls) identified two alleles that warrant further evaluation.

Materials & Methods

Sample Collection

- Horses were phenotyped by complete ocular examination and included as cases with histopathologic confirmation of SCC. Controls had no evidence of ocular disease per examination by a board-certified veterinary ophthalmologist and were at least 13 years of age.2
- DNA was extracted from blood or hair by routine extraction using the Puregene whole-blood extraction kit (Qiagen Inc., Valencia, CA) or from formalin-fixed, paraffin-embedded tissue using the Quick-DNA FFPE kit (Zymo Research, Irvine, CA).

Variant Identification

- Whole genome sequencing data available from four Haflingers (Table 1)3 were mapped to EquCab2.04 and variants called using FreeBayes.4
- Variant caller files were filtered to prioritize polymorphism based on (1) location in an associated genomic locus from previous GWAS published2 and unpublished data (2) allele distribution between cases and controls for different genetic models (Table 2)3 (3) predicted functional effect1,6, and (4) the biologic relevance of the gene in cancer.

Genotyping Methods

- Twenty nine variants from the ranked variant list were used to design a multiplex SNP genotyping assays for genotyping 76 horses (14 cases and 62 controls) on the Agena MassArray platform.
- KRTR4 (T>G p.Val338Gln) and NOTCH1 (A>G p.Ile157Val) were genotyped via a PCR restriction fragment length polymorphism testing using PVUI and HpyCH4IV enzymes, respectively. Products were separated and visualized on the ABI3730 (ThermoFisher Scientific, Inc).
- Association testing was performed by Chi-squared test under several different models with predetermined significance level of α=0.05.

Results

- 31 variants from 7 chromosomes were selected in filters 1 through 4 (Table 3).
- 24 variants were successfully genotyped either by RFLP or multiplex SNP genotyping assays.
- Basic allelic association testing identified a SNP on ECA6 (p=3.0X10^-3) and a SNP on ECA11 (p=1.0X10^-4) warranting closer examination as genetic risk factors for ocular SCC (Figure 1).
- The associated missense variant on ECA6 (COP5B G>C p.Val174Leu) is predicted to alter protein function. Allelic frequencies significantly differed between cases and controls (Table 4).
- All of the cases were homozygous for the KRTR4 alternate allele (ECA11 SNP 1, KRTR4, A>G p.Ser75Gly), suggesting that homozygosity for this variant may contribute to cancer risk. (Table 5).

Table 1: Haflinger samples selected for high-throughput sequencing based on phenotype and DDB2 c.103 C>T genotype.

<table>
<thead>
<tr>
<th>Horse</th>
<th>Ocular SCC Status</th>
<th>DDB2 genotype c.103 C&gt;T*</th>
<th>c.103 C/T</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF-14-62</td>
<td>Affected</td>
<td>C/C</td>
<td></td>
</tr>
<tr>
<td>HF-14-55</td>
<td>Affected</td>
<td>C/C</td>
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<tr>
<td>HF-13-17</td>
<td>Unaffected</td>
<td>C/C</td>
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<tr>
<td>HF-13-33</td>
<td>Unaffected</td>
<td>T/T</td>
<td>* The T Allele was previously associated with ocular SCC phenotype.1,2</td>
</tr>
</tbody>
</table>

Acknowledgements

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References