Investigation of a Missense Mutation in DDB2 Associated with Squamous Cell Carcinoma in Haflinger and Belgian Horses

S. Vigl, J. Liu, M. Lassaline, T.M. Michaud, C.M. Reilly, E. Bentley, R. Bellone

1University of California Davis, School of Veterinary Medicine, 2University of California Davis, Department of Population Health and Reproduction, 3University of California Davis, Ophthalmology Service, 4BluePearl, Specialty and Emergency Pet Hospital, Tampa FL, 5University of Wisconsin College of Veterinary Medicine, Department of Surgical Sciences

Introduction

Squamous cell carcinoma (SCC) is the most common ocular cancer1 and the second most common systemic cancer overall of the horse.2 Ocular SCC occurs most often on the nictitating membrane and/or nasal canthus, limbus, and eyelid. It is often invasive and can be difficult to treat, although it has a low rate of metastasis.3,4 Marked by a consistent increase in incidence, most cases of equine SCC occur in the Percheron breed of horse.5 Lower risk breeds, such as the Haflinger breed, have been documented to share a common ancestor with Percherons.6 Gatti et al. identified a mutation in DDB2 that is associated with nictitating membrane SCC in Percherons.7,8 Lassaline et al. identified the same mutation in a Haflinger and a Belgian horse.9,10 Moreover, the mutation was also associated with nictitating membrane SCC in Haflingers. Horses homozygous for the T/T allele had a five times greater risk for SCC than horses with either C/C or C/T alleles.11

The Haflinger breed of horse is overrepresented in the squamous cell carcinoma literature.12 A pedigreed analysis supported a genetic basis with a recessive mode of inheritance for those horses affected with SCC.9 A genome-wide association study (GWAS) identified a locus on equine chromosome 12 as the most strongly associated with disease status (P(perm) = 0.04). Within this associated locus, damage-specific DNA Binding Protein 2 (DDB2) was chosen as a candidate gene because it functions in the repair of DNA damage by UV radiation.12,13 DNA Sequencing of DDB2 identified a non-synonymous misincorporation (C>T, p. Thr338Met) which was predicted to be deleterious. A strong but not perfect association for this variant was detected in a phenotyped sample set (N=67, p=3.41x10^-10), and horses homozygous for the T allele had a 5 times greater risk for SCC than those who were not (C/C or C/T).9 Allelic frequency was examined in thirteen breeds reported with cases of SCC, and the associated T allele was detected in 4/13 breeds. The highest frequencies were present in Haflingers, Belgians, and Percherons, three breeds which have been documented to share a close genetic relationship.10

Results

Pedigree analysis: All but one Haflinger with nictitating membrane SCC (18/19) traced back to the same sire identified as the last common ancestor for SCC in Haflingers. The remaining horse, RF-15-88, traced back to one on only one side (Figure 1). This supports the same genetic mechanism for both breeds of SCC.

Association Testing: Among Haflinger horses with nictitating membrane SCC, 80% (18/22) were homozygous for the risk allele (Thr338Met). The other horses affected had the T/T genotype (Fisher’s Exact Test P = 0.0023, Table 2).

DNA quality analysis: FFPE extracted DNA yielded genotyping results for 67% (12/18) of the horses for which FFPE samples were tested. Disregarding samples from before 1990 and two samples whose extraction did not have measurable DNA by spectrophotometry modified that percentage to 80% (12/15). Although the quantity of DNA was very low, the cleaned and concentrated DNA had more intense bands of small molecular weight on the Bioanalyzer gel, and there were 19 peaks detected compared to 0 peaks in the same sample before cleaning (Figure 3).

Discussion

This study is significant because it means the genetic test used for SCC in horses needs to be reevaluated. Additionally, the increased risk for SCC in Haflingers is also valid for nictitating membrane SCC in this breed. In addition, it may apply to Belgian horses although additional testing is warranted to confirm this finding. The more accurate test can be used to advise owners to be especially vigilant in protecting at-risk (T/T) horses from UV exposure and having routine ocular exams performed for those horses. Breeders should avoid mating horses that could result in offspring with this genotype.

For the pedigree analysis, it is possible that the one Haflinger horse with nictitating membrane SCC who only traced back to the last common ancestor on one side would have traced back to him on both sides if more extensive pedigree records were available. Given that the mutation has been identified in both breeds, the most recent common ancestor identified can be negated as the ancestor in which the mutation arose.

The reasons why some FFPE samples may have yielded results while others did not are not clear. Age appears to play a role, since all the samples that were successful were collected in or after the year 1997. However, age is probably not the only cause of failure since one sample from 2013 was also unsuccessful. Other possible causes are the quality and quantity of tissue embedded in the block, the protocol used for genotyping, and the presence of fragmented DNA. The appearance of more intense bands and more peaks on the cleaned sample suggests that the additional clean and concentrate step had the desired effect, even though genotyping of the cleaned samples did not result in variant genotypes. In general, samples which had the most high molecular weight peaks on the Bioanalyzer were also the samples which were successful for genotyping.

The Belgian dataset analyzed for age of onset of SCC in Belgians is limited by its small sample size, and because it was determined retrospectively based on their VMTV membership. However, within these limits, the most recent common ancestor identified can be negated as the ancestor in which the mutation arose. A more strict cutoff than 11 years for unaffected Belgians would strengthen the validity of the phenotyping, although it was not possible for this study because it would have decreased the sample size.

Conclusions

This study substantiated previous work that associated a missense mutation in DDB2 with ocular SCC in Haflingers, and expanded that association to a second breed of ocular SCC. The proposed mechanism by which the mutation may be causal is based on DDB2’s role in recognition and repair of UV-damaged DNA. Three highly conserved residues, Phe336, Glu372 and Gln378, are highly conserved across species. While a small shift in the amino acid of equine DDB2 may alter DNA-binding in the tyrosine kinase domain, this is unlikely to disrupt the function of the gene. A more simple mechanism may be that Thr338 plays a role in UV-damage recognition, which is a key step in DNA repair. The identification of this mutation as the cause of SCC in Haflingers and Belgians is a significant advancement in understanding this common disease in horses.

Acknowledgements

We gratefully acknowledge the technical assistance of Mara Mark and give thanks to all of the horse owners who participated in the study. This work was supported by the School of Veterinary Medicine Endowment Fund and the Research Center for Equine Health Grant (Grant 015-15).

References