Brief Note

Exclusion of adrenoceptor alpha 2 variants in a horse insensitive to medetomidine

Isabelle Schmutz¹, Vidhya Jagannathan¹, Sabina Dìez Bernal², Simone Lanz³, Ted Kalbfleisch⁴, Tosso Leeb^{1,*}, Claudia Spadavecchia²

- ¹ Institute of Genetics, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland
- ² Division of Anaesthesiology, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland
- ³ Swiss Institute of Equine Medicine, University of Bern and Agroscope, 3001 Bern, Switzerland
- ⁴ School of Medicine, University of Louisville, 40202 Louisville KY, USA

Running title: Equine alpha 2 adrenoceptor genes

*Address for correspondence

Tosso Leeb Institute of Genetics Vetsuisse Faculty University of Bern Bremgartenstrasse 109a 3001 Bern Switzerland

Phone: +41-31-6312326 Fax: +41-31-6312640 E-mail: Tosso.Leeb@vetsuisse.unibe.ch

Keywords: Equus caballus, WGS, pharmacogenetics, anaesthesia, adrenergic receptor

Background: Patients may react in different ways to drugs and genetic factors have to be considered when observing variability in drug responses.¹ Drugs acting as agonists for the alpha 2 adrenoceptor (formerly called α_2 adrenergic receptor), such as xylazine, detomidine, medetomidine, or romifidine are regularly used for sedation, premedication and analgesia in veterinary medicine.^{2,3} Three genes encode for separate subtypes of alpha 2 adrenoceptors, *ADRA2A*, *ADRA2B*, and *ADRA2C*.⁴ Alpha 2 adrenoceptors modulate the regulation of blood pressure, renal function, insulin release, cognition, memory and behaviour.⁵ *Adra2a* mutant mice (α_{2A} D79N) are resistant to sedation with dexmedetomidine.^{6,7}

Own analysis: A 9-year old Swiss Warmblood horse was presented due to anorexia. Since the horse was highly aggressive, clinical examination was only deemed possible under general anaesthesia. Therefore, tiletamine-zolazepam (2mg/kg) and medetomidine (0.04 mg/kg) were administered intramuscularly by blowpipe darting. While the drug-related side effects such as sweating, polyuria, tremor and ataxia were observed, the sedative effect remained absent. Therefore 35 minutes later a second dart containing tiletamine-zolazepam (1 mg/kg) and medetomidine (0.04 mg/kg) was shot, again without noticeable sedative effects. We sequenced the genome of this horse at 28x coverage as described (study accession PRJEB14779, sample accession SAMEA104357351).⁸ We called private variants with respect to 80 genomes from other horses of different horse breeds (Table S1). This analysis yielded 26,416 private variants, 222 of them predicted to be protein-changing (Table S2). During this analysis we recognized that ADRA2A, ADRA2B and ADRA2C contain gaps and/or are not correctly annotated in the current EquCab 2 assembly. Therefore, our automated bioinformatics pipeline for variant detection would not necessarily have detected all possible variants within these genes. Based on preliminary data from the ongoing efforts to produce an EquCab 3 assembly we designed PCR primers for the amplification of the entire ADRA2A, ADRA2B and ADRA2C genes (Table S3). We Sanger sequenced these genes from the medetomidine-resistant horse and a control horse and deposited curated reference sequences for these 3 genes in the European Nucleotide Archive (accessions LT935786 – LT935788). We did not detect any protein-changing variant in the medetomidine-resistant horse.

Comments: Coding variants in *ADRA2A*, *ADRA2B* and *ADRA2C* can be excluded for the observed insensitivity to medetomidine in a Swiss Warmblood horse. We provide new genomic reference sequences for these three genes.

Acknowledgements: We thank Nathalie Besuchet-Schmutz, Muriel Fragnière and Sabrina Schenk for excellent technical assistance. We thank the Next Generation Sequencing Platform of the University of Bern for performing the whole genome sequencing experiment, and the Interfaculty Bioinformatics Unit of the University of Bern for providing high performance computing infrastructure.

2

References

- 1. Belle D.J. & Singh H. (2008) Am Fam Physician 77, 1553-60.
- 2. Hobo S. et al. (1995) J Vet Med Sci 57, 507-10.
- 3. England G.C.W. & Clarke K.W. (1996) British Veterinary Journal 152, 641-57.
- 4. Bylund D.B.et al. (1994) Pharmacol Rev 46, 121-36.
- 5. Belfer I. et al. (2005) J Hum Genet 50, 12-20.
- 6. Altman J.D. et al. (1999) Mol Pharmacol 56, 154-61.
- 7. Lakhlani P.P. et al. (1997) Proc Natl Acad Sci U S A 94, 9950-5.
- 8. Murgiano L. et al. (2016) G3 (Bethesda) 7, 1315-21.

Supplementary data

 Table S1. Accession numbers of horse genome sequence data.

Table S2. Private variants in the medetomidine-resistant Swiss Warmblood horse.

Table S3. Primer sequences for the amplification of the equine *ADRA2A*, *ADRA2B*, and *ADRA2C* genes.