Protein Networks Mediating Airway Hyper-responsiveness in Equine Airways

CE Swiderski1, A Akkul1, JE Bowser1, AK Claude1, AJ Cooley3, LRR Costa1, AL Eddy1, CA Mochal1, B Nanduri2, ME Johnson2, RW Wills3, Zayas J1,4
Department of Clinical Sciences, (2) Department of Basic Sciences, (3) Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University and (4) College of Veterinary Medicine, Tuskegee University

HYPOTHESIS: Exacerbations of airway hyper-responsiveness (AHR) in horses with pasture asthma reflect increased activity in protein networks that augment airway smooth muscle contractility and proliferation.

Rationale:

- AHR is the keystone of asthmatic bronchoconstriction that drives novel therapeutic development, yet the processes that direct AHR are incompletely characterized. Airway smooth muscle (ASM) is a pivotal cell type mediating AHR in human asthma.1-4
- AHR is a pervasive characteristic of airways diseases (Recurrent airway obstruction, Inflammatory airway disease, Equine asthma). Exercise induced pulmonary hyperreactivity) that account for up to 80% of poor performance in horses.5-10 AHR is also a sequel to viral respiratory infections in other species.11-14
- Seasonal exacerbation/remission that characterizes pasture-associated equine asthma.15 It provides a unique opportunity to identify genes that link AHR and exacerbations of asthma-like disease.

Method:

Methacholine challenge was performed using a two minute tidal breathing protocol with serially increasing doubling doses of methacholine to increase lung resistance until a 40% increase in baseline lung resistance was identified. Airway smooth muscle was quantified from paired thoracic lung biopsies from 6 horses suffering from pasture asthma and 6 age and sex matched control horses co-housed on pasture.

Results:

Aim #1: Correlate disease severity & airway muscle mass to AHR magnitude in diseased & control horses

<table>
<thead>
<tr>
<th>Methacholine Dose (ug) Causing 40% Increase in Rl</th>
<th>No reaction to inhaled methacholine</th>
<th>11</th>
<th>34</th>
<th>54</th>
<th>60</th>
<th>66</th>
<th>92</th>
<th>113</th>
<th>332</th>
<th>141</th>
<th>188</th>
<th>359</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diseased Horses</td>
<td>Control Horses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exacerbation</td>
<td>Remission</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: The magnitude of AHR in horses with pasture asthma, quantified by the dose of methacholine causing a 40% increase in lung resistance (PD40RL), mirrors severe human asthma.16 AHR severity decreases significantly during seasonal disease remission (p<0.001), but the magnitude remains diagnostic of severe human asthma (PD40RL achieved at methacholine concentrations ≤1 mg/ml).

Figure 1: ASM area is positively correlated to the magnitude of AHR in horses with pasture asthma, ie as ASM area increases PD40RL decreases (p=0.047).

ASM area did not differ between diseased and control horses (p=0.4233) during seasonal exacerbations. ASM area was significantly greater (p<0.001) in diseased relative to control lung biopsies collected during seasonal disease remission (3-6 months following exacerbation in all horses). This reflected decreases in ASM area in control horses (p=0.003) and increases in ASM area in diseased horses (p=0.018) in remission versus exacerbation. This indicates altered regulation of ASM remodeling occurs during disease remission, influencing gene targets of interest.

Aim #2: Identify disease associated differentially expressed gene products and their relationship to AHR

Figure 2: EGF Ligands & Orphan Nuclear Receptors that Induce Smooth Muscle Proliferation are Differentially Expressed in the Lung of Horses with Pasture Asthma. 50 gene products with increased expression (FDR<0.05, log FC>2) during exacerbation of pasture asthma were modeled in String v10.5 as human orthologues.19 EGF signaling pathway was significantly over-represented (FDR=0.022). EGF ligands (HBBEGF, ARGE, CENH1A) known to increase airway smooth muscle proliferation were significantly increased in disease.15-18 Orphan nuclear receptors NRRA41 and NRRA43 were identified as novel targets for investigation based on, respectively, EGF1R binding properties and ability to induce smooth muscle proliferation outside the long.

Method:

Method: Methacholine challenge was performed using a two minute tidal breathing protocol with serially increasing doubling doses of methacholine from 0.0625mg/ml until a 40% increase in baseline lung resistance was identified. Airway smooth muscle was quantified from paired thoracic lung biopsies from 6 horses suffering from pasture asthma and 6 age and sex matched control horses co-housed on pasture.

Outcomes:

1. The magnitude of AHR in pasture asthma mirrors severe human asthma and is positively correlated to airway smooth muscle area. Altered regulation of ASM remodeling is relevant to the pathophysiology of AHR in pasture asthma.

2. Epidermal growth factor ligands and orphan nuclear receptors known to increase smooth muscle proliferation are increased in the lungs of horses with pasture asthma.

3. Aligned to anecdotal reports of poor glucocorticoid responsiveness in horses with pasture asthma, GCR staining is decreased in the epithelium of diseased horses.

Our work identifies shared characteristics of AHR between diseased horses as well as severe human asthma. The identified signaling homologies between pasture asthma affected horses and human asthma support broad relevance of protein networks identified from our analysis across domestic species.

Outputs: 1) We will participate as a collaborating training site for an animal genomics training program submitted by the University of Florida (USDA-NIFA-NRF). 2) Three oral presentations were delivered from this work in 2017: a) the genetics plenary at the 2017 Equine Science Symposium, b) one hour of continuing education at the 2017 ACVM Forum, and c) an invited lecture at the University of Florida Genomics Institute. Manuscripts from this work include: Swiderski CE, Hunter CL, Bowser JE, Costa LRR et al. Deciphering the role of airway hyper-responsiveness in equine pasture asthma. Journal of Equine Veterinary Science. 2017; 52:27-35. 2017.


References:

5. Hirota N, Risse PA, Novali M, McGovern T, Al-Alwan L, McCuaig S, Proud D, Hayden P, Hamid Q, Martin JG. Histamine may induce airway remodeling through release of epidermal growth factor receptor ligands from
6. Rettmer H, Hoffman AM, Lanz S, Oertly M, Gerber V. Owner-reported coughing and nasal discharge are associated with clinical findings, arterial oxygen tension, mucus score and bronchoprovocation in horses with

Acknowledgements: This project is supported by the Agriculture and Food Research Initiative (AFRI) Animal Health Program competitive grant no. 2015-67518-23172 from the USDA - National Institute of Food and Agriculture.