Identification of Genetic Markers for Ventricular Septal Defects in Arabian Horses

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Introduction

Ventricular septal defects (VSDs) are the most common congenital heart defects reported in horses. VSDs are characterized by an abnormal communication between the left and right ventricles that varies in size, location, and clinical relevance. Even small, high velocity defects known as restrictive VSDs can lead to impaired cardiac function if the cusps of the aortic valve are drawn into the defect, resulting in aortic insufficiency. When severe, these lesions result in volume overload in the left heart and ultimately congestive heart failure.

Arabian horses are overrepresented for incidence of congenital heart defects including VSDs, and a genetic basis for VSDs in the breed is considered likely. Identification of genetic markers associated with VSDs would facilitate the isolation of a VSD causative mutation and ultimately enable the development of a genetic test for Arabian breeding managers to promote the health of the breed and limit the prevalence of this defect.

Hypothesis

Ventricular septal defects (VSDs) in Arabian horses are associated with a single genetic locus identifiable through a genome-wide association study (GWAS).

Materials and Methods

Cardiac auscultation and echocardiographic evaluations were used to phenotype 13 affected and 38 normal Arabian horses. DNA was extracted from whole blood samples and genotyped using the Assimil® Equine Genotyping Array that contains 670,000 SNPs across the equine genome.

Results

Mixed linear model analysis (EEMAX) was performed to further reduce the effects of population stratification. QQ plot improved: results displayed. Chromosome 25 significance persisted after 330,000 permutations.

Discussion

Despite the suspected heritability of VSDs in Arabian horses, no prior studies have undertaken to identify SNPs, haplotypes, and genes associated with VSDs in this breed. Case vs. control GWAS was successful in identifying a chromosomal region of interest.

Chromosome 25 has the strongest association with VSDs and withstood Bonferroni correction. Phenotypic diversity is a well-known feature of VSDs in many species. A specific form of VSD as a component of a more severe congenital malformation known as tetralogy of Fallot is rarely diagnosed in Arabian horses and other horse breeds. Our data suggests that tetralogy of Fallot is geographically distinct from the VSD variety tested in this study (perimembranous).

Further defining the identified region of interest and investigating variants in candidate genes through whole genome sequencing will ideally guide future translational research and aid in the understanding of VSD pathogenesis.

Phenotype

Auscultation: A VSD is associated with two possible heart murmurs. The shunt itself causes a loud, holosystolic murmur with a point of maximal intensity over the right thorax. A murmur of relative pulmonic stenosis is often auscultated as well. Though the right ventricular outflow tract may be anatomically normal, the increased volume of blood leaving the right ventricle results in a holosystolic crescendo-decrescendo murmur most audible over the pulmonary valve region on the left thorax.

Echocardiogram: Echocardiography is used to definitively diagnose a VSD and classify its location. With cardiac ultrasound, the defect is visualized as a communication between the two ventricles. The velocity and direction of flow through the shunt is measured to determine if the defect is restrictive (hemodynamically insignificant) or non-restrictive. The downstream effects of volume overload, such as dilation of the pulmonary artery and left atrium and eccentric hypertrophy of the left ventricle, are assessed. This study targets perimembranous VSDs.

Acknowledgements

References


Table 1. The SNPs most strongly associated with VSDs from the EEMAX analysis are detailed by location in EquCab2 and test statistics.

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<thead>
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<th>Location</th>
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Figure 1. A 2D and color Doppler echocardiogram still image is provided of a 2 year old Arabian gelding. The image is obtained from the right thorax. The VSD (arrow) is visualized with turbulent flow passing into the right ventricle, just below the aortic valve (arrowhead).

Figure 2. Spectral Doppler echocardiographic image depicts blood flow across the VSD. The flow measures 5.5 m/s toward the transducer in systole. This confirms the shunt direction is right to left and the velocity confirms that this VSD is hemodynamically insignificant (restrictive).

Figure 3. Manhattan plot illustrating the most significant SNP association on chromosome 25 in the EEMAX analysis. Bonferroni adjusted and raw P values are displayed.

Figure 4. Additive association model with 330,000 permutations. The pink line denotes a genome-wide significant value of P<0.05.

Figure 5. QQ plot demonstrating that population stratification has been corrected after adjustment by BSA and EEMAX.

Figure 6. Haplotype analysis. Complex VSD represents tetralogy of Fallot - a genetically distinct disease complex in humans.