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31653 Refinement of the Equine Cerebellar Abiotrophy Locus on ECA2 by Haplotype Analysis

L. Brault* and M.C.T. Penedo, Veterinary Genetics Laboratory, University of CA, Davis, CA, USA

INTRODUCTION

CA is a neurological condition found almost exclusively in Arabian horses.^{1,2} Symptoms generally appear around six weeks of age and include head tremors, ataxia and a lack of balance equilibrium.^{1,2} Affected horses may show exaggerated action of the forelegs, a wide-based stance, and a lack of menace response. The physical manifestations of the disease are due to a post-natal degeneration of the Purkinje cells and associated granular neurons of the cerebellum.^{3,4} The death of the Purkinje cells appears to result from a failure of these neurons to migrate correctly throughout the cerebellum.⁴ Segregation analysis in carrier x carrier and carrier x affected matings support the hypothesis that CA is inherited as a recessive trait.

We mapped the CA locus to ECA2 by linkage analysis in four families composed of 33 horses segregating for CA. This region included the genes *DMAPI* and *PRNPIP*, both of which are expressed in brain tissue. Sequencing of *DMAPI* and *PRNPIP* yielded no causative mutations. We have since added 56 additional horses segregating for CA to our mapping resource. The aim of this research was to develop additional markers throughout this region to identify a conserved haplotype associated with CA and to better delineate the interval breakpoints.

MATERIALS AND METHODS

Animal Resource

DNA samples were prepared from blood or hair root samples collected from 89 horses segregating for CA. These horses comprised 20 affected horses, 48 carriers, and 21 unaffected horses. Based on pedigree records, all affected and carrier horses shared common ancestors within 9

generations. The cerebellum from one affected foal in each of the families was harvested post-mortem and submitted to the Anatomic Pathology Service, Veterinary Medicine Teaching Hospital, University of California, Davis for histological confirmation of the neurological defect.

Marker Development

STR markers were identified from genomic sequence in the region of interest, which spanned approximately 6.2 Mb. Limited sequencing of selected genes in this region generated 6 SNPs in addition to the 2 SNPs discovered in previous research. Genotypes were collected for the 89 horses at all 34 loci and haplotypes were constructed from this data. All markers were also genotyped on a control group of Arabian horses drawn from the general population.

RESULTS

Previous research had identified a conserved haplotype spanning 91 Kb in the region of *DMAPI* and *PRNPIP*. The addition of 11 affected horses, as well as the development of 25 new STRs and 6 SNPs, has refined this location. From the 34 markers spanning 6.2 Mb, a region of conserved homozygosity, consisting of two STRs, was identified among all 22 affected individuals. These STRs span approximately 904 Kb.

Allele frequencies were counted within the affected population ($n = 22$) and the control population ($n = 41$) at 8 selected loci in the vicinity of the conserved CA region. A χ^2 test was performed using a 2 x 2 contingency table with 1 degree of freedom. A p value was assigned based on the null hypothesis that the alleles of each marker were randomly distributed throughout both populations. All of the 8 markers tested exhibited allele frequencies in the affected population that were significantly different ($p < .001$) from the control population.

DISCUSSION

Haplotype analysis of affected horses in the region of CA has identified conserved homozygosity for two STRs spanning 904 Kb. Allele frequencies for eight selected markers in affected horses were significantly higher than in control Arabians, which presumably represents the ancestral haplotype in which the CA mutation originally occurred.

This region contains a number of genes expressed in brain tissue. Sequencing of the coding regions of *POMGNT1*, a transmembrane protein linked to Muscle-Eye-Brain (MEB) disease,⁵ and *FAAH*, which encodes a protein that is responsible for the hydrolysis of a number of fatty acids,⁶ has been completed and has yielded no causative mutations. Continued sequencing of the introns of these genes is currently underway, as well as sequencing of additional candidate genes in the area.

Because horse owners are understandably reluctant to disclose that their breeding stock has produced a foal

affected with a neurological disease, the incidence of CA remains unknown. As knowledge of the disease becomes more widespread, more affected foals are being identified and reported. Pedigree analyses of the horses in the CA families indicate that the mutation was present in related horses that were breeding in the 1930's and that are common ancestors in many Arabian bloodlines. Therefore, the frequency of carriers in the breed may not be negligible. The cost of an affected foal to an Arabian breeder in terms of lost revenue can be significant, as many of these foals come from valuable breeding stock. Genetic screening of breeding stock will allow breeders to avoid mating two carriers together, thus eliminating the chances of an affected foal.

Keywords: Arabian; Disease; Cerebellum; Purkinje cell; Abiotrophy; Homozygosity mapping; Haplotype

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31838 Equine Gene Structure Annotation by RNA Sequencing

Stephen Coleman,*¹ Kai Wang,² Zheng Zeng,² Michael Mienaltowski,¹ Jinze Liu,² and James MacLeod¹, ¹University of Kentucky Department of Veterinary Science, Lexington, KY, USA, ²University of Kentucky Department of Computer Science, Lexington, KY, USA

INTRODUCTION

Analyses of the equine whole-genome sequence³ resulted in the prediction of 20,322 protein-coding genes (Ensembl 52.2b, http://www.ensembl.org/Equus_cabalus/index.html). Because the annotation of these predictions relied primarily on the projection of gene

structure from other mammalian species with only limited equine expression data available (35,504 ESTs and 1,141 mRNA sequences, UCSC Genome Browser 2009, <http://genome.ucsc.edu>), many are likely to contain structural inaccuracies. RNA sequencing is a powerful new technology that can generate full-length mRNA sequence information useful for the annotation of protein-coding gene structure.

MATERIALS AND METHODS

Messenger RNA was isolated from total RNA of 8 equine tissue samples (articular cartilage, synovial membrane, testes, placental villous, cerebellum, 34-day embryo, LPS-stimulated articular cartilage, and LPS-stimulated synovial membrane). The selected mRNA was processed and sequenced according to Illumina's mRNA-Seq protocol^{1,2,4} generating 35 basepair sequence tags. Tags were mapped to the equine genome sequence (EquCab2) by ELAND (efficient localization of nucleotide data, Illumina Analysis Pipeline v0.3) and filtered to select for single unique alignments. The tag alignment data were then used to predict gene structure.

RESULTS

RNA sequencing of 8 equine tissue samples generated 293,758,105 tags, equaling 10.28 giga-basepairs of total sequence data and 76.7X coverage of the estimated equine transcriptome (calculated as 5% of the equine genome). Of those, 144,031,045 were uniquely mapped to the equine genome sequence. Analysis of the mapped tags resulted in the identification of 150,502 exon-structures and assembly of 18,532 transcriptional units with exon-intron structure (putative protein-coding genes). At least 688 of these had no corresponding prediction in the Ensembl equine gene set.

DISCUSSION

Accurate annotation of equine gene structure is an important resource for equine genetic research. RNA sequencing data enabled refinement of structural annotation for the equine gene predictions, while at the same time, identifying previously unannotated transcriptional activity.

Keywords: RNA sequencing; Gene annotation; Genomics

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