Characterization of the equine 2'-5' oligoadenylate synthetase 1 (OAS1) and ribonuclease L (RNASEL) innate immunity genes

Jonathan J. Rios  
*Texas A & M University - College Station*

Andrey A. Perelygin  
*Georgia State University*

Maureen T. Long  
*University of Florida*

Teri L. Lear  
*University of Kentucky*, equigene@uky.edu

Andrey A. Zharkikh  
*Myriad Genetics, Inc.*

*See next page for additional authors*

Follow this and additional works at: https://uknowledge.uky.edu/gluck_facpub

Part of the Veterinary Medicine Commons

Right click to open a feedback form in a new tab to let us know how this document benefits you.

**Repository Citation**

Rios, Jonathan J.; Perelygin, Andrey A.; Long, Maureen T.; Lear, Teri L.; Zharkikh, Andrey A.; Brinton, Margo A.; and Adelson, David L., "Characterization of the equine 2'-5' oligoadenylate synthetase 1 (OAS1) and ribonuclease L (RNASEL) innate immunity genes" (2007). *Veterinary Science Faculty Publications.* 18. https://uknowledge.uky.edu/gluck_facpub/18

This Article is brought to you for free and open access by the Veterinary Science at UKnowledge. It has been accepted for inclusion in Veterinary Science Faculty Publications by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.
Authors
Jonathan J. Rios, Andrey A. Perelygin, Maureen T. Long, Teri L. Lear, Andrey A. Zharkikh, Margo A. Brinton, and David L. Adelson

Characterization of the equine 2'-5' oligoadenylate synthetase 1 (OAS1) and ribonuclease L (RNASEL) innate immunity genes

Notes/Citation Information
Published in BMC Genomics, v. 8, 313.

© 2007 Rios et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Digital Object Identifier (DOI)
http://dx.doi.org/10.1186/1471-2164-8-313
Research article

Characterization of the equine 2'-5' oligoadenylate synthetase 1 (OAS1) and ribonuclease L (RNASEL) innate immunity genes

Jonathan J Rios†1, Andrey A Perelygin†2, Maureen T Long3, Teri L Lear4, Andrey A Zharkikh5, Margo A Brinton2 and David L Adelson*6

Address: 1Department of Animal Science, Texas A&M University, 2471 TAMU, College Station, Texas 77843, USA, 2Biology Department, Georgia State University, 24 Peachtree Center Ave., Atlanta, Georgia 30302, USA, 3College of Veterinary Medicine, University of Florida, 2015 SW 16th Ave., Gainesville, Florida 32608, USA, 4Department of Veterinary Science, University of Kentucky, 108 Maxwell H. Gluck Equine Research Center, Lexington, Kentucky, 40546, USA, 5Bioinformatics Department, Myriad Genetics, Inc., 320 Wakara Way, Salt Lake City, UT, 84108, USA and 6School of Molecular and Biomedical Science, University of Adelaide, SA 5005, Australia

Email: Jonathan J Rios - jonathanrios@tamu.edu; Andrey A Perelygin - aperelygin@gsu.edu; Maureen T Long - LongM@mail.vetmed.ufl.edu; Teri L Lear - equigene@uky.edu; Andrey A Zharkikh - zharkikh@myriad.com; Margo A Brinton - biomab@langate.gsu.edu; David L Adelson* - david.adelson@adelaide.edu.au

* Corresponding author    †Equal contributors

Abstract

Background: The mammalian OAS/RNASEL pathway plays an important role in antiviral host defense. A premature stop-codon within the murine Oas1b gene results in the increased susceptibility of mice to a number of flaviviruses, including West Nile virus (WNV). Mutations in either the OAS1 or RNASEL genes may also modulate the outcome of WNV-induced disease or other viral infections in horses. Polymorphisms in the human OAS gene cluster have been previously utilized for case-control analysis of virus-induced disease in humans. No polymorphisms have yet been identified in either the equine OAS1 or RNASEL genes for use in similar case-control studies.

Results: Genomic sequence for equine OAS1 was obtained from a contig assembly generated from a shotgun subclone library of CHORI-241 BAC 100I10. Specific amplification of regions of the OAS1 gene from 13 horses of various breeds identified 33 single nucleotide polymorphisms (SNP) and two microsatellites. RNASEL cDNA sequences were determined for 8 mammals and utilized in a phylogenetic analysis. The chromosomal location of the RNASEL gene was assigned by FISH to ECA5p17-p16 using two selected CHORI-241 BAC clones. The horse genomic RNASEL sequence was assembled. Specific amplification of regions of the RNASEL gene from 13 horses identified 31 SNPs.

Conclusion: In this report, two dinucleotide microsatellites and 64 single nucleotide polymorphisms within the equine OAS1 and RNASEL genes were identified. These polymorphisms are the first to be reported for these genes and will facilitate future case-control studies of horse susceptibility to infectious diseases.
Background

The innate immune responses are the first line of host defense against a virus infection. An important component of the intracellular antiviral response is mediated by the 2′-5′ oligoadenylate synthetase (OAS)/ribonuclease L (RNase L) pathway. OAS genes are interferon-inducible and activated by binding to double-stranded RNA (dsRNA). dsRNA, present in virus infected cells, activates OAS proteins to catalyze the oligomerization of ATP to form 2′,5′-linked oligoadenylate chains (pppA(2′p5’A)n) [1-3]. Originally discovered as a low molecular weight inhibitor of protein synthesis, pppA(2′p5’A)n induces the activation of the latent endoribonuclease, RNase L, which degrades both cellular and viral RNA in a non-preferential manner [1,4-6]. The OAS/RNASEL pathway has also been implicated in the induction of apoptosis [7-11].

The murine flavivirus resistance gene, Flv, was positionally cloned and identified as Oas1b [12]. A cDNA sequence comparison among susceptible and resistant strains of mice identified a single nucleotide substitution that causes a premature stop codon in the Oas1b transcripts of susceptible mice [12,13].

The human OAS gene cluster, consisting of genes OAS1, OAS3 and OAS2, is located on chromosome 12q24.2 [14]. The small synthetases are transcribed from the OAS1 gene while the medium and large synthetases are encoded by the OAS2 and OAS3 genes, respectively. Alternative splicing was previously reported in both OAS1 and OAS2 transcripts [15,16]. For example, the human OAS1 transcript E16 corresponds to the p42 protein, which is translated from a 1.6 kilobase (kb) mRNA, while the alternatively spliced E18 transcript encoding the p46 protein is about 1.8 kb [17]. Both p42 and p46 proteins are identical in their first 346 N-terminal amino acids but differ at the C-terminus [18]. Variations in the human OAS1 gene that may be relevant to the outcome of virus infections have been reported [19-23].

The human RNASEL gene maps to chromosome 1q25 [24]. The 741 amino acid, 83,539 Dalton protein is translated from a ~2.8 kb transcript [25,26]. The RNase L protein consists of three domains: 1) an N-terminal domain of ankyrin repeats with P-loop motifs between the seventh and eighth repeat, 2) a serine/threonine protein kinase domain, and 3) a C-terminal ribonuclease domain [27]. RNase L activation requires binding of a single 2-5A molecule to the N-terminal ankyrin repeats 2–4 [28,29]. 2-5A binding reverses the naturally repressive state of the RNase L ankyrin repeats, ultimately producing a functional homodimer with ribonuclease activity [27,29-31].

Previously, the equine OAS gene cluster was mapped to horse (Equus caballus; ECA) chromosome 8p15 and shown to have an organization similar to that in the human genome: OAS1-OAS3-OAS2 [32]. Two clones were identified from segment 1 of the CHORI-241 equine BAC library, 77F4 (~200 kb) and 100110 (~130 kb), that contain complete OAS1 and OAS3 sequences. BAC clone 77F4 also contains nine 5′-terminal exons of OAS2 [32].

In this report, a subclone library generated from CHORI-241 BAC 100110 was sequenced and then used to construct a contig assembly spanning the OAS1 gene. The equine RNASEL gene was identified in multiple BAC clones of the CHORI-241 library and was FISH mapped on metaphase spreads to ECA5p17-p16. Equine RNASEL genomic sequence was obtained from BAC clone 159N12 and an assembly similar to that for OAS1 was constructed. Full-length RNASEL cDNA from 8 species were determined and compared in a phylogenetic analysis. Re-sequencing of genomic DNA from multiple horses of different breeds identified a total of 64 SNPs and 2 microsatellites within the OAS1 and RNASEL genes.

Results

BAC 100110 sequencing and OAS1 contig assembly

A shotgun subclone library was constructed from sheared fragments of CHORI-241 BAC 100110. Nine hundred subclones were bi-directionally sequenced, resulting in 513,390 bases with quality scores > 15, providing 3.95X coverage. The individual chromatogram files were analyzed by Phred, Phrap and Consed [33-37] and individual contigs were scaffolded on the human genome sequence using BLAST. The scaffold was further validated by the addition of multiple sequences from TraceDB [38] retrieved via BLAST searches using full length equine OAS1 mRNA [GenBank: AY321355]. The scaffold contained four genomic contigs spanning a substantial part of the equine OAS1 gene, including 4.5 kb of promoter sequence upstream of exon 1 and 1.6 kb of sequence downstream of exon 6, and was submitted to GenBank under accession number DQ536887. The genomic assembly also included sequence for the downstream equine OAS3 gene as well as an upstream gene orthologous to human RPH3A (data not shown). This assembly completely overlaps two whole genome shotgun sequences, AAWR01028567 (55,475 bp) and AAWR01028568 (31,407 bp), that were recently submitted to GenBank from the Broad Institute.

Identification of OAS1 microsatellites

The genomic sequence assembly identified two microsatellites, one located within the promoter and the other downstream of exon 6. The promoter GT-microsatellite is located 575 bp upstream of the ATG translation initiation site. A shorter GT-microsatellite is in the same relative position in the human OAS1 promoter and the flanking regions were well conserved between the two sequences...
(Figure 1). This microsatellite may affect the functions of flanking regulatory elements. Sequencing the OAS1 promoter regions of 13 horses established that this promoter microsatellite is polymorphic in length. The second polymorphic microsatellite was a GT-dinucleotide repeat located 43 bp downstream of exon 6 within the 3’ UTR. It has previously been reported that a 3’ UTR microsatellite can alter the level of synthesis of a mRNA. [39].

**OAS1 SNP identification**

The assembled OAS1 scaffold was aligned to the full length, 1.6 kb cDNA equine transcript [GenBank: AY321355] to delineate individual exons and flanking intron sequences from the genomic contigs. Genomic primers were designed within flanking intron sequences as well as for the proximal promoter (Table 1).

Sequence data obtained from the screening population and from CHORI BAC 100I10 were analyzed using Phred, Phrap and Consed programs [33-37]. Both visual analysis

---

**Figure 1**

**Local alignment of human and horse OAS1 promoters.** BLAST2 alignment of the 1000 bp upstream of the transcription start for human OAS1 and equine OAS1 genes. The following BLAST parameters were used: a mismatch penalty of -1 and word size of 7. Lower case masking of repeats was used. The alignment shows that the sequence from ~800 bp to ~-350 bp in the horse promoter is similar to a region of the human promoter interrupted by a 200 bp Alu repeat (~-811 bp ~-590 bp). The horse microsatellite is shown in underlined bold and corresponds to a smaller dinucleotide repeat in the human sequence. Numbering shown in the alignments is from the translation ATG start sites.
of the chromatogram data to identify heterozygotes and computer analysis using the Consed visualization tool identified 33 single nucleotide substitutions within the proximal promoter and exons of OAS1 (Table 2). Of these, 11 were within coding regions, 9 within non-coding regions and the remaining 13 within the proximal promoter upstream of exon 1. Four of the 9 non-coding polymorphisms were located within the 5' and 3' untranslated regions (UTR). Of the 11 coding polymorphisms, 4 were synonymous and 7 were non-synonymous. Five of the 7 non-synonymous SNPs resulted in substitutions of amino acids with different properties. Interestingly, the amino acids encoded by the major alleles of 4 of the 7 non-synonymous mutations were identical to the corresponding amino acids in the human OAS1 protein [UniProtKB: P00973]. The genotypes of each individual were used to identify potential haplotypes within equine OAS1 using PHASE v2.1 software [40,41]. Only those SNPs verified within multiple individuals were used for the haplotype analysis (minor allele frequency = 0.08). The best reconstruction produced 15 haplotypes from the 33 diallelic SNPs (Table 3). The polymorphic microsatellites were not included in the analysis.

Assembling full-length RNASEL mRNA sequences of cattle, dog, horse, cat, domestic pig, Guinea pig, elephant and opossum

A limited number of mammalian RNASEL mRNA sequences were previously deposited to GenBank and some of these sequences were predicted from whole genome annotations. However, this GenBank information was not sufficient to identify evolutionarily conserved regions in mammalian RNASEL sequences that could be used to design PCR primers to amplify equine RNASEL fragments. The predicted sequences of cattle [GenBank: XM_597290] and dog [GenBank: XM_547430] RNASEL ORFs were amplified from commercial cDNA (BioChain, Hayward, CA), directly sequenced and extended to full-length cDNA sequences by DNA walking. The full-length cattle and dog RNASEL sequences were submitted to GenBank under accession numbers DQ497162 and DQ497163, respectively. These two sequences as well as the human full-length RNASEL sequence NM_021133 were aligned and degenerate primers were designed from conserved regions (Table 4) and used to amplify the middle portions of equine RNASEL cDNA. This partial sequence was extended to the full-length sequence by DNA walking and submitted to GenBank under accession number DQ497159.

Several additional mammalian RNASEL sequences were also determined and subsequently used to perform a phylogenetic analysis. The GenBank feline Whole Genome Sequence (WGS) database was searched with the canine RNASEL sequence [GenBank: DQ497163]. Four genomic contigs, AANG01026257, AANG01026258, AANG01630549 and AANG01026248, were detected. These contigs contais the first, second and third, as well as the fifth and sixth feline RNASEL coding exons, respectively. No contigs containing the fourth coding exon of the feline RNASEL gene were found in GenBank. Two primers were designed based on the 3'-end AANG01026302 sequence and the 5'-end AANG01630549 sequence (Table 4) and used to amplify and sequence this region from a commercial cat genomic DNA (Novagen, Madison, Wisconsin). The sequence of

| Gene | Region | Forward Primer | Reverse Primer |
|------|--------|---------------|---------------|
| OAS1 | Promoter | AACCCACAGAATAAACACACCA | GTGATGCTCAGCAGGAC |
|     | Exon 1 | CCGAGCTCAGGCACGTTAAG | GTTTGCTCTCCTCCTCCT |
|     | Exon 2 | GTTATGTTGCTTGGTGTAGTG | AAACTGTGGGAGAAGATGG |
|     | Exon 3 | GTAACTTGGT GTGGTCCGAC | AGATGGGAGGAGCCCCA |
|     | Exon 4 | AGCGTGAAACCCACACACCA | TCCCACATCCTCATTCC |
|     | Exon 5 | CTCAGGGCTGTCTCCTCCT | CCTCAGAAGGGGTCAAA |
|     | Exon 6 | GCAGTGCGACGTCACAG | GGCAGCTGCCCAGTGATTA |
| RNASEL | Exon 1 | CATCTCCCTTTCCTCCTCCTG | TGGATGGGAGACTCTCCTG |
|      | Exon 2 | CAAAGTACCTTTCCTCCTCCAG | TCCGAAGAGCTTGGAAA |
|      | Exon 3 | AAGATCTCCTTCCGTAGATGG | GGCTTTTCTATCCTGCAATG |
|      | Exon 4 | CTCGTAGCTCCTCCACACCA | CAAGCAGTCCACACCA |
|      | Exon 5 | CCATCGTAAATTTCCTCTCATCCTTCAG | TCTTCACCTTCTGTAGG |
|      | Exon 6 | GTCTCTACATATTTCCTCTCATCCTTCAG | GTTTCCATCAATAATAGCAGA |
|      | Exon 7 | ATCTCTGAACCCGGGTCT | CACTACAAATGCCCCCTGAG |
|      | Exon 8 | CTCTGAGGTGCTTCTTCA | TCCAGCAGTCTTTTTGAG |

Forward and reverse primers for amplification of the OAS1 promoter and individual exons of equine OAS1 and RNASEL.

---

Table 1: Primers used to detect equine polymorphisms

| Gene | Region | Forward Primer | Reverse Primer |
|------|--------|---------------|---------------|
| OAS1 | Promoter | AACCCACAGAATAAACACACCA | GTGATGCTCAGCAGGAC |
|     | Exon 1 | CCGAGCTCAGGCACGTTAAG | GTTTGCTCTCCTCCTCCT |
|     | Exon 2 | GTTATGTTGCTTGGTGTAGTG | AAACTGTGGGAGAAGATGG |
|     | Exon 3 | GTAACTTGGT GTGGTCCGAC | AGATGGGAGGAGCCCCA |
|     | Exon 4 | AGCGTGAAACCCACACACCA | TCCCACATCCTCATTCC |
|     | Exon 5 | CTCAGGGCTGTCTCCTCCT | CCTCAGAAGGGGTCAAA |
|     | Exon 6 | GCAGTGCGACGTCACAG | GGCAGCTGCCCAGTGATTA |
| RNASEL | Exon 1 | CATCTCCCTTTCCTCCTCCTG | TGGATGGGAGACTCTCCTG |
|      | Exon 2 | CAAAGTACCTTTCCTCCTCCAG | TCCGAAGAGCTTGGAAA |
|      | Exon 3 | AAGATCTCCTTCCGTAGATGG | GGCTTTTCTATCCTGCAATG |
|      | Exon 4 | CTCGTAGCTCCTCCACACCA | CAAGCAGTCCACACCA |
|      | Exon 5 | CCATCGTAAATTTCCTCTCATCCTTCAG | TCTTCACCTTCTGTAGG |
|      | Exon 6 | GTCTCTACATATTTCCTCTCATCCTTCAG | GTTTCCATCAATAATAGCAGA |
|      | Exon 7 | ATCTCTGAACCCGGGTCT | CACTACAAATGCCCCCTGAG |
|      | Exon 8 | CTCTGAGGTGCTTCTTCA | TCCAGCAGTCTTTTTGAG |
Table 2: Equine OAS1 single nucleotide polymorphisms and microsatellites

| Region          | Accession | Alleles | Residue | Amino Acid Type | Frequency | Polymorphism |
|-----------------|-----------|---------|---------|-----------------|-----------|--------------|
| -               | 3640      | C       | -       | -               | 0.25      | Transition   |
|                 | 3687      | G       | -       | -               | 0.65      | Transition   |
|                 | 3718      | A       | -       | -               | 0.65      | Transition   |
|                 | 3724      | C       | -       | -               | 0.65      | Transition   |
|                 | 3825      | C       | -       | -               | 0.35      | Transition   |
|                 | 3830      | A       | -       | -               | 0.85      | Transition   |
|                 | 3973      | C       | -       | -               | 0.65      | Transition   |
|                 | 4032–4063 | -       | -       | -               | -         | GT repeat    |
|                 | 4234      | C       | -       | -               | 0.65      | Transition   |
|                 | 4333      | C       | -       | -               | 0.35      | Transition   |
|                 | 4455      | C       | -       | -               | 0.08      | Transversion |
|                 | 4487      | C       | -       | -               | 0.88      | Transition   |
|                 | 4501      | C       | -       | -               | 0.65      | Transition   |
|                 | 4531      | A       | -       | -               | 0.08      | Transition   |
| 5' UTR          | 4598      | C       | -       | -               | 0.65      | Transversion |
|                 | 4625      | A       | -       | -               | 0.35      | Transversion |
| Exon 1          | 4690      | A       | 18Tyr   | Uncharged Polar | 0.85      | Transition   |
|                 |           | G       | 18Cys   | Uncharged Polar | 0.15      |             |
|                 | 4783      | C       | 49Ala   | Nonpolar        | 0.35      | Transition   |
|                 |           | T       | 49Val   | Nonpolar        | 0.65      |             |
| Intron 1        | 5609      | C       | -       | -               | 0.67      | Transition   |
|                 |           | T       | -       | -               | 0.33      |             |
| Exon 2          | 5701      | C       | 77Leu   | Nonpolar        | 0.64      | Transition   |
|                 |           | T       | 77Leu   | Nonpolar        | 0.36      |             |
|                 | 5743      | C       | 91Phe   | Nonpolar        | 0.33      | Transition   |
|                 |           | T       | 91Phe   | Nonpolar        | 0.67      |             |
| Exon 2          | 5765      | A       | 99Lys   | Basic Polar     | 0.65      | Transition   |
|                 |           | G       | 99Glu   | Acidic Polar    | 0.35      |             |
| Exon 2          | 5776      | A       | 102Arg  | Basic Polar     | 0.38      | Transition   |
|                 |           | G       | 102Arg  | Basic Polar     | 0.62      |             |
| Exon 2          | 5786      | A       | 106Lys  | Basic Polar     | 0.38      | Transition   |
|                 |           | G       | 106Glu  | Acidic Polar    | 0.62      |             |
| Exon 2          | 5920      | G       | 150Pro  | Nonpolar        | 0.08      | Transversion |
|                 |           | T       | 150Pro  | Nonpolar        | 0.92      |             |
| Exon 3          | 9374      | C       | 209Arg  | Basic Polar     | 0.85      | Transition   |
|                 |           | T       | 209Cys  | Uncharged Polar | 0.15      |             |
| Exon 4          | 12714     | C       | 264Asn  | Uncharged Polar | 0.59      | Transversion |
|                 |           | G       | 264Lys  | Basic Polar     | 0.41      |             |
| Intron 4        | 12810     | C       | -       | -               | 0.64      | Transition   |
|                 |           | T       | -       | -               | 0.36      |             |
| Intron 4        | 12853     | A       | -       | -               | 0.55      | Transition   |
|                 |           | G       | -       | -               | 0.45      |             |
| Intron 5        | 13628     | A       | -       | -               | 0.55      | Transversion |
|                 |           | T       | -       | -               | 0.45      |             |
this exon was submitted to GenBank under accession number EF062998. Using this sequence as well as the other exon sequences derived from GenBank (see above), the predicted full-length mRNA sequence of the feline RNASEL gene was assembled.

The TIGR porcine database [42] was searched using the cattle sequence [GenBank: DQ497162] and five partial RNASEL sequences were found. The TC212507 and TC212872 sequences correspond to the 5'-end of porcine RNASEL mRNA, while the TC218317, TC237301, and TC236970 sequences represent the 3'-end. An additional 5'-end cDNA sequence, 20060611S-038813, was detected in the Pig EST Data Explorer [43]. A pair of primers were designed based on the partial sequence (Table 4) and used to amplify pooled cDNA (kindly provided by Dr. Jonathan E. Beever, University of Illinois at Urbana-Champaign). The middle portions of the porcine RNASEL cDNA were directly sequenced. The partial sequence was then extended to the full-length sequence by DNA walking and submitted to GenBank under accession number DQ497160.

Table 3: Equine OAS1 and RNASEL haplotypes

| Gene  | Haplotype Sequence | Count | Frequency |
|-------|--------------------|-------|-----------|
| OAS1  | CTGTCATTCTGGGACCCCTAGTGCTACCTT | 0.08  |           |
|       | CTGTTTTCCTGGGACCCCTAGTGCTACCTT | 0.04  |           |
|       | CTGTTTTCCTGGGACCCCTAGTGCTACCTT | 0.08  |           |
|       | CTGTTTTCCTGGGACCCCTAGTGCTACCTT | 0.04  |           |
|       | TGACTACCTGCGGAGCTTCGAGCTACCTT   | 0.19  |           |
|       | TGACTACCTGCGGAGCTTCGAGCTACCTT   | 0.04  |           |
|       | TGACTACCTGCGGAGCTTCGAGCTACCTT   | 0.04  |           |
|       | TGACTACCTGCGGAGCTTCGAGCTACCTT   | 0.04  |           |
|       | TGACTACCTGCGGAGCTTCGAGCTACCTT   | 0.11  |           |
|       | TGACTACCTGCGGAGCTTCGAGCTACCTT   | 0.08  |           |
|       | TGACTACCTGCGGAGCTTCGAGCTACCTT   | 0.08  |           |
|       | TGACTACCTGCGGAGCTTCGAGCTACCTT   | 0.04  |           |
|       | TTGTACCTGCTGGACCCCTAGTGCTACCTT  | 0.08  |           |
|       | TTGTACCTGCTGGACCCCTAGTGCTACCTT  | 0.04  |           |
| RNASEL | GACTGCAAAGGGACCCGCTGGGCAGTTTTCTTT | 0.07  |           |
|       | GATCGCAAGGGACCGGCGGTGGGACACCCCC  | 0.04  |           |
|       | GATCGCAAGGGACCGGCGGTGGGACACCCCC  | 0.12  |           |
|       | GATCTACAGGAGGCGGCTGGCCACACCTCC   | 0.12  |           |
|       | GATCTACAGGAGGCGGCTGGCCACACCTCC   | 0.12  |           |
|       | GATCTACAGGAGGCGGCTGGCCACACCTCC   | 0.07  |           |
|       | GATCTACAGGAGGCGGCTGGCCACACCTCC   | 0.23  |           |
|       | GATCTACAGGAGGCGGCTGGCCACACCTCC   | 0.07  |           |
|       | GATCTACAGGAGGCGGCTGGCCACACCTCC   | 0.04  |           |
|       | GATCTACAGGAGGCGGCTGGCCACACCTCC   | 0.12  |           |

Haplotypes were assembled using PHASE v2.1 [40, 41] under default settings. The haplotypes identified from the best reconstruction are shown with their corresponding frequencies among the 13 horses screened for both OAS1 and RNASEL SNPs. Both OAS1 microsatellites were omitted from the haplotype reconstruction.
The GenBank Guinea Pig whole genome sequence database was searched using both mouse [GenBank: NM_011882] and rat [GenBank: NM_182673] full-length RNASEL sequences. Two Guinea pig sequences, AAKN01052053 and AAKN01424676, showed significant similarity to the 5’ and 3’ regions of the rodent RNASEL sequences, respectively. These two sequences were used to design primers (Table 4) to amplify commercial cDNA (BioChain, Hayward, CA) and directly sequence the middle portions of Guinea pig RNASEL cDNA. This partial sequence was extended to the full-length sequence by DNA walking, and submitted to GenBank under accession number DQ497161.

Cattle, dog, horse and pig RNASEL sequences were used to search the GenBank elephant genome trace archive using the discontiguous Mega BLAST program. The same sequences were also used to search the GenBank elephant whole genome sequence database using the BLASTN program. The sequences for all potential exons of the elephant RNASEL gene were identified. Based on these sequences, five primer pairs (Table 4) were designed to amplify genomic DNA (kindly provided by Drs. Alfred L. Roca and Stephen J. O’Brien, National Cancer Institute) and directly sequence each of the elephant RNASEL exons. The resulting sequence was submitted to GenBank under accession number DQ497164.

The RNASEL ORF sequence of the laboratory opossum (Monodelphis domestica) was predicted by searching the UCSC genome browser [44] using the BLAT program. No sequence traces similar to RNASEL were detected in frog (Xenopus tropicalis) or several fish species (Danio rerio, Takifugu rubripes and Tetraodon nigroviridis).

Phylogenetic analysis of vertebrate RNASEL gene sequences

Only sequences of human and mouse RNASEL genes were previously reported [26]. Sequences of orthologous rat (GenBank: AM0492248) genes were recently submitted to GenBank but have not been reported in any publications. In addition, annotations of chimpanzee, orangutan and rhesus macaque genomes using a GNOMON method resulted in predicted RNASEL sequences in these three species. Primate, rodent and avian RNASEL sequences were downloaded from GenBank and aligned to orthologous sequences described above to build a phylogenetic tree (Figure 2). Rodents showed the highest rate of nucleotide substitutions, while primates showed the lowest rate of evolution. Evolution rates were found to be fairly uniform in the three different RNase L domains: ankyrin repeats, serine/threonine protein kinase domain, and ribonuclease domain. The percent identity between the RNASEL ORFs of horse and the other species compared is shown in Table 5.

Assignment of the RNASEL gene to horse chromosome ECA5p17-p16

The horse CHORI-241 BAC library was searched with a probe derived from the partial equine RNASEL cDNA fragment. Twelve positive clones were identified and two of them, 108P15 and 189I19, were FISH mapped to assign the RNASEL gene to the horse chromosomal location ECA5p17-p16 (Figure 3).

Exon/intron structures of vertebrate RNASEL genes

Partial sequence for the equine RNASEL gene was obtained by sequencing PCR fragments of BAC 159N12. The mRNA sequence [GenBank: DQ497159] was used as a reference for determining intron/exon junctions. Sufficient genomic sequence was obtained to build a scaffold as described for the equine OAS1 gene. The scaffold was verified using sequences from TraceDB [38] and submitted to GenBank under accession number EF070193. This scaffold completely overlaps the whole genome shotgun sequence AAWR01030439 (193510 bp) that was recently submitted to GenBank from the Broad Institute. Comparison of genomic and mRNA sequences revealed six coding and one 5’-terminal non-coding exon in the equine RNA-
Figure 2
**Phylogenetic tree of RNASEL genes.** RNASEL ORF sequences from 15 vertebrate species were aligned and the njtree program was used for tree construction.

Table 5: Lengths of coding exons (bp) within the ORFs of vertebrate RNASEL genes and percent identity between horse and other species RNASEL ORFs

| Species      | Exon A | Exon B | Exon C | Exon D | Exon E | Exon F | Identity |
|--------------|--------|--------|--------|--------|--------|--------|----------|
| Horse        | 1480   | 86     | 206    | 133    | 134    | 130    | 100.0    |
| Cat          | 1477   | 86     | 206    | 133    | 134    | 139    | 83.0     |
| Dog          | 1477   | 86     | 206    | 133    | 134    | 139    | 81.1     |
| Cattle       | 1474   | 86     | 206    | 130    | 131    | 145    | 79.2     |
| Elephant     | 1510   | 86     | 206    | 133    | 137    | 187    | 75.1     |
| Human        | 1480   | 86     | 206    | 133    | 134    | 187    | 81.9     |
| Chimpanzee   | 1480   | 86     | 206    | 133    | 134    | 187    | 79.7     |
| Orangutan    | 1480   | 86     | 206    | 133    | 134    | 187    | 81.2     |
| Rhesus       | 1480   | 86     | 206    | 133    | 134    | 187    | 79.7     |
| Mouse        | 1474   | 86     | 206    | 133    | 137    | 172    | 66.3     |
| Rat          | 1489   | 86     | 206    | 133    | 131    | 172    | 65.5     |
| Guinea Pig   | 1462   | 86     | 206    | 133    | 134    | 187    | 69.7     |
| Opossum      | 1453   | 86     | 206    | 129    | 131    | 139    | 56.5     |
| Chicken      | 1402   | 89     | 191    | 124    | 122    | 136    | 37.3     |

Figure 3
**FISH mapping of equine RNASEL.** FISH map position ECA5p17-p16 of horse RNASEL gene (orange) on DAPI counterstained metaphase chromosomes (blue).
SEL gene. This exonic composition is similar to that of a number of other mammalian RNASEL genes. However, two and three 5'-terminal non-coding exons were found in the chicken and mouse RNASEL genes, respectively. The coding vertebrate RNASEL exons were designated A through F. Comparison of the genomic and mRNA sequences of vertebrate RNASEL genes revealed significant length variation in both the 5'- (1402–1510 bp) and 3'-terminal (130–187 bp) coding exons (Table 5).

SNP identification in the horse RNASEL gene
After identification of the equine RNASEL introns, exon-specific genomic primers were designed (Table 1). Exon-specific sequencing of DNA from the screening population identified 31 SNPs within the RNASEL gene (Table 6). Of the 10 non-coding polymorphisms, one was within the second intron and the others were located in the 5’ and 3’ UTRs. Seventeen of the 31 SNPs were located within the ankyrin repeat-encoding exon 2, 13 of which are non-synonymous, with 10 resulting in substitutions of amino acids with different properties. Three non-synonymous polymorphisms were identified within exons 3 and 5. The remaining exons, including the non-coding exon 1 were invariant among these horses. The amino acids encoded by the major allele of 11 of the 16 non-synonymous mutations were identical to the corresponding human RNase L amino acid [UniProtKB: Q05823]. Using MOTIF Search [45] to identify putative transcription factor binding motifs in the TRANSFAC database, the promoter SNP was found to be located within a potential cAMP-response element binding site (Score: 90) upstream of the first exon. Haplotypes were assembled in the same manner as for the equine OAS1 gene. The best reconstruction from Phase analysis produced 10 haplotypes among the 31 verified diallelic SNPs with minor allele frequencies = 0.08 (Table 3). As with OAS1, only good quality, unambiguous resequencing data were used for the haplotype analysis.

Identifying single nucleotide polymorphisms by sequencing DNA from multiple individuals enhances the possibility of artifacts either from PCR or sequencing error. The 64 SNPs identified from the equine OAS1 and RNASEL genes were considered valid if each allele was identified in at least two individuals. Eight additional SNPs were identified but could not be verified in more than one individual (minor allele frequency < 0.08). Within the 3,864 and 5,406 base pairs re-sequenced during the SNP identification for OAS1 and RNASEL, respectively, equine OAS1 contained an average of one polymorphism per 117 bases, while equine RNASEL averaged one polymorphism per 174 bases.

Discussion
Sequence characterization of the horse OAS1 gene in CHORI-241 BAC 100I10 enabled a partial genomic sequence assembly [GenBank: DQ536887] and comparison among multiple equine individuals. We identified 2 polymorphic microsatellites and 33 single nucleotide polymorphisms from a group of 13 individuals and CHORI-241 BAC 100I10 (Table 2). In an attempt to identify potential structural and/or functional consequences of the coding non-synonymous SNPs, each was analyzed using PolyPhen software [46-48]. Each polymorphic variant identified in equine OAS1 was predicted to cause benign effects at their respective residue position. However, the single mutation resulting in an Arg209Cys substitution may significantly change OAS1 enzymatic activity. Arg209 in the equine OAS1 protein corresponds to Arg544 in the human OAS2 protein, which is located in the donor binding domain. Substitution of Arg544 with either Ala or Tyr significantly decreased enzymatic activity of the OAS2 protein [49]. In addition, the equine OAS1 promoter SNP at position 4531 is located in an interferon stimulating response element [29]. Inactivation of this regulatory element by a single nucleotide substitution may alter expression of the equine OAS1 gene.

RNASEL enzymatic activity was previously reported in reptiles, birds, and mammals [50]. However, no RNASEL genes have been found for amphibians or fishes to date. Interestingly, the same classes of vertebrates also do not have OAS genes[51].

The horse RNASEL gene was FISH mapped to chromosomal location ECA5p17-p16. Orthologous genes are located on primate chromosome 1 (human, chimpanzee and rhesus macaque), cattle chromosome 16, dog chromosome 7, mouse chromosome 1, rat chromosome 13 and chicken chromosome 8 [52]. Using comparative chromosome painting (Zoo-FISH), similarities between human chromosome 1 and horse chromosome 5 [53], mouse chromosome 1, rat chromosome 13 [54], dog chromosome 7 [55,56] and cattle chromosome 16 [57] were previously established. Our results further confirm the conservation of RNASEL-containing syntenic chromosomal segments in horses.

Thirty one SNPs were identified for equine RNASEL (Table 6). Interestingly, all but three of the 20 coding SNPs identified are located within exon 2. The RNase L protein contains 9 N-terminal ankyrin repeats responsible for binding 2-5A molecules that are essential for activation [27]. Exon 2 of the human RNASEL gene encodes the entire ankyrin repeat region (amino acid 24 to 329). The high frequency of non-synonymous polymorphisms within exon 2 suggests that a single SNP or haplotype could ablate 2-5A binding and/or other RNase L interac-
Table 6: Equine RNASEL single nucleotide polymorphisms

| Region       | Accession  | Alleles | Residue | Amino Acid Type | Frequency | Polymorphism |
|--------------|------------|---------|---------|-----------------|-----------|--------------|
|              | EF070193   |         |         |                 |           |              |
| -            | 143        | C       | -       | -               | 0.12      | Transversion |
|              | 1857       | A       | -       | -               | 0.55      | Transversion |
| 5' UTR       |            |         |         |                 |           |              |
|              | 1991       | C       | 27His   | Basic Polar     | 0.54      | Transition   |
|              |            | T       | 27Tyr   | Uncharged Polar | 0.46      |              |
| Exon 2       | 2020       | C       | 36Gly   | Uncharged Polar | 0.92      | Transition   |
|              |            | T       | 36Gly   | Uncharged Polar | 0.08      |              |
| Exon 2       | 2021       | G       | 37Asp   | Acidic Polar    | 0.69      | Transition   |
|              |            | T       | 37Tyr   | Uncharged Polar | 0.31      |              |
| Exon 2       | 2118       | A       | 69Asn   | Uncharged Polar | 0.29      | Transition   |
|              |            | C       | 69Thr   | Uncharged Polar | 0.71      |              |
| Exon 2       | 2121       | A       | 70Tyr   | Uncharged Polar | 0.92      | Transition   |
|              |            | G       | 70Cys   | Uncharged Polar | 0.08      |              |
| Exon 2       | 2316       | A       | 135Lys  | Basic Polar     | 0.75      | Transition   |
|              |            | C       | 135Thr  | Uncharged Polar | 0.25      |              |
| Exon 2       | 2332       | A       | 140Ala  | Nonpolar        | 0.35      | Transition   |
|              |            | G       | 140Ala  | Nonpolar        | 0.65      |              |
| Exon 2       | 2374       | G       | 154Arg  | Basic Polar     | 0.83      | Transition   |
|              |            | T       | 154Ser  | Uncharged Polar | 0.17      |              |
| Exon 2       | 2635       | A       | 241Thr  | Uncharged Polar | 0.21      | Transition   |
|              |            | G       | 241Thr  | Uncharged Polar | 0.79      |              |
| Exon 2       | 2680       | C       | 256Ser  | Uncharged Polar | 0.43      | Transition   |
|              |            | G       | 256Ser  | Uncharged Polar | 0.57      |              |
| Exon 2       | 2771       | A       | 287Lys  | Basic Polar     | 0.57      | Transition   |
|              |            | G       | 287Glu  | Acidic Polar    | 0.43      |              |
| Exon 2       | 3144       | A       | 411Asn  | Uncharged Polar | 0.19      | Transition   |
|              |            | G       | 411Glu  | Acidic Polar    | 0.81      |              |
| Exon 2       | 3152       | C       | 414Arg  | Basic Polar     | 0.81      | Transition   |
|              |            | T       | 414Ser  | Uncharged Polar | 0.19      |              |
| Exon 2       | 3281       | A       | 457Lys  | Basic Polar     | 0.19      | Transition   |
|              |            | G       | 457Glu  | Acidic Polar    | 0.81      |              |
| Exon 2       | 3301       | A       | 463Lys  | Basic Polar     | 0.19      | Transversion |
|              |            | C       | 463Asn  | Uncharged Polar | 0.81      |              |
| Exon 2       | 3311       | C       | 467Pro  | Nonpolar        | 0.19      | Transition   |
|              |            | T       | 467Ser  | Uncharged Polar | 0.81      |              |
| Exon 2       | 3372       | A       | 487Gln  | Uncharged Polar | 0.58      | Transition   |
|              |            | G       | 487Arg  | Basic Polar     | 0.42      |              |
| Intron 2     | 3404       | A       | -       | -               | 0.19      | Transition   |
|              |            | G       | -       | -               | 0.81      |              |
| Exon 3       | 5108       | A       | 513Lys  | Basic Polar     | 0.11      | Transition   |
|              |            | G       | 513Glu  | Acidic Polar    | 0.89      |              |
| Exon 3       | 5111       | C       | 514Pro  | Nonpolar        | 0.82      | Transition   |
|              |            | T       | 514Ser  | Uncharged Polar | 0.18      |              |
| Exon 5       | 7314       | A       | 598Asn  | Uncharged Polar | 0.87      | Transition   |
|              |            | G       | 598Asp  | Acidic Polar    | 0.13      |              |
| 3' UTR       | 9994       | C       | -       | -               | 0.15      | Transversion |
|              |            | G       | -       | -               | 0.85      |              |
| 3' UTR       | 9999       | A       | -       | -               | 0.88      | Transversion |
|              |            | T       | -       | -               | 0.12      |              |
| 3' UTR       | 10247      | C       | -       | -               | 0.31      | Transition   |
|              |            | T       | -       | -               | 0.69      |              |
| 3' UTR       | 10914      | C       | -       | -               | 0.38      | Transition   |
|              |            | T       | -       | -               | 0.62      |              |
| 3' UTR       | 11105      | C       | -       | -               | 0.88      | Transition   |
|              |            | T       | -       | -               | 0.12      |              |
| 3' UTR       | 11146      | C       | -       | -               | 0.35      | Transition   |
|              |            | T       | -       | -               | 0.65      |              |
| 3' UTR       | 11184      | C       | -       | -               | 0.35      | Transition   |
tions. As well, the SNP identified within the promoter upstream of the first exon is located within a potential cAMP-response element binding site. Mutations within this promoter element have been shown to affect gene expression [58-60]. PolyPhen analysis was also conducted on the non-synonymous coding SNPs identified within equine RNASEL. All but 4 of the RNase L SNPs were predicted to have benign effects. However, the SNP at residue 287 was predicted to change hydrophobicity at a buried site within the RNase L protein and the effect of this on protein function is unknown. The predictions provided by PolyPhen analysis are based on functional effects identified using human nsSNPs and may differ for the horse RNase L. Four SNPs within the ankyrin repeat region in exon 2 (residues 414, 463, 467 and 487) were predicted to have a negative effect on function. These data support our hypothesis that a single SNP or haplotype could affect 2-5A binding within the equine RNase L ankyrin repeats.

A number of SNPs were detected within the 3’UTR region of the equine RNASEL gene. Of the eight SNPs found within this region, six result in transitions. The 3’UTR regions of mRNAs contain regulatory regions capable of protein and microRNA binding that control mRNA stability, translation and localization. A simple analysis of octamer motifs containing equine 3’ UTR SNPs identified SNP 10247 as being within a human miRNA target site [61]. If this target site is conserved in horses, this SNP could significantly affect the synthesis of RNase L. However, this particular octamer motif was not found in human or rodent RNASEL 3’UTRs. Furthermore, cross-species sequence comparison using mVISTA[62,63] also revealed no significant longer range conservation in this region between species (data not shown).

Genotype analysis using PHASE v2.1 [40,41] identified 15 and 10 haplotypes among equine OAS1 and RNASEL genes, respectively, and suggested the existence of haplotype blocks spanning most of each gene (Table 3). Even if efforts to show an association between viral-induced disease susceptibility and OAS1 and/or RNASEL SNPs are successful, it may prove difficult to unambiguously identify a single causal SNP because of potential linkage disequilibrium at these loci. As determined from our screening population, a single haplotype occurred more frequently than any other, with a frequency of 0.19 and 0.23 in OAS1 and RNASEL, respectively (Table 3).

The frequency of SNP identification in this study in two equine genes was high considering the previously estimated equine SNP frequency of 1 per 1500 bp [64]. In dogs, the estimated SNP rate is ~1 per 1600 bp (based on entire genome re-sequencing), but a higher frequency of ~1 per 900 bp was estimated between breeds [65]. Re-sequencing of specific genes in several breeds of the domestic dog identified polymorphisms at frequencies comparable to our estimates, with 1 SNP per ~250–330 bp [S. Canterbury, personal communication]. Furthermore, re-sequencing within an Elk (Cervus elaphus nelsoni) putative promoter region, which is highly conserved between mule deer, cow and sheep, detected an average SNP frequency of 1 per 69 bp [unpublished data].

The microsatellite identified within the promoter region in this study may also alter expression of the equine OAS1 gene. The alleles observed to date indicate that dinucleotide repeat lengths of 9 and 18 may represent the major alleles at this locus. The over-representation of these alleles may be due to the fact that they correspond to one complete rotation of the DNA helix. If this microsatellite separates cis-regulatory elements, alterations in its length could affect the binding of transcriptional regulators to these elements and significantly alter gene expression [66-71]. In support of this hypothesis, there is a high degree of conservation between human and horse OAS1 promoters in the regions flanking the microsatellite (Figure 1). As well, recent micro-array data provide evidence of an inverse relationship between gene expression and dinucleotide microsatellite length, supporting the significantly higher frequency with which we identified the (GT), allele within the individuals screened[66]

Conclusion

We report the genomic sequences of the equine OAS1 and RNASEL genes and identify 64 single nucleotide polymorphisms and 2 polymorphic microsatellites in these genes. On the basis of the allelic variants characterized, we conclude that a number of these are plausible candidates for regulatory or structural mutations which may influence transcription or enzymatic activity of OAS1 and RNase L proteins. Also, RNASEL cDNA sequences were determined for 8 mammals and utilized in a phylogenetic analysis. The chromosomal location of the RNASEL gene was assigned by FISH to ECA5p17-p16.
Methods

**RNASEL cDNA and FISH**
Preparation of horse cDNA was described previously [32]. Partial RNASEL sequences were extended using a DNA Walking SpeedUp Kit (Seegene USA, Del Mar, CA) according to the manufacturer’s protocol. Four high-density filters for segment 1 of the CHORI-241 equine genomic BAC library were purchased from the Children’s Hospital Oakland Research Institute (CHORI), Oakland, CA. These filters were screened using a P32-labeled equine RNASEL cDNA probe according to the supplier’s protocol. Two positive equine BAC clones were purchased from CHORI. Each of these BAC clones was grown individually in 500 mL of LB media. BAC DNA was isolated using the NucleoBond BAC Maxi Kit (BD Biosciences Clontech, Palo Alto, CA) and used as the template for direct partial sequencing with a BigDye terminator v1.1 Cycle Sequencing Kit on an ABI 3100 Genetic Analyzer according to the manufacturer’s recommendations. DNA from equine BAC clones 108P15 and 189I19 was FISH mapped as described previously [67]. International cytogenetic nomenclature of the domestic horse [68] was used to identify individual horse chromosomes.

The njtree program was used to construct a phylogenetic tree as described previously [51] and tree topology was inferred by the Neighbor-Joining algorithm. The bootstrap algorithm with 1000 replications was used to estimate the confidence of each node. The njtree program is available upon request.

**Construction of subclone library**
BAC clone 100I10 was isolated from segment 1 of the CHORI-241 equine BAC library at Texas A&M University and confirmed by PCR as containing OAS1. The colony-isolated clone was cultured and BAC DNA was isolated by standard alkaline/lysis miniprep using Millipore Solutions and treated with Plasmid-Safe ATP-dependent DNase (Epicentre, Madison, WI). BAC DNA was fragmented using a HydroShear DNA Shearing Device (GeneMachine, San Carlos, CA) at Speed Code 8 for an estimated fragment size of 2.5 kb. The fragmented product was analyzed by agarose gel electrophoresis stained with ethidium bromide and gel extracted using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). Extractions were eluted in water according to the manufacturer’s protocol. Purified fragments were cloned into vector pCR®-4Blunt-TOPO® using the TOPO® ShotGun Subcloning Kit (Invitrogen, Carlsbad, CA) following the manufacturer’s protocol. Ligation reactions were incubated 30 minutes at room temperature and electroporated into E. coli. Colonies were screened for lack of β-galactosidase activity and selected for ampicillin resistance on LB-agarose plates containing 50 µg/mL ampicillin. White colonies were cultured and screened for appropriate insert size by PCR using vector-sequence M13 primer sites flanking the cloned insert, prior to sequencing.

**Sequencing of clones**
Individual OAS1 inserts were amplified directly from individual colonies by PCR using vector-sequence M13 primer sites flanking the cloned insert. Amplification products were purified by centrifugation with the PSI-Clone PCR 96 kit (Princeton Separations, Adelphia, NJ) according to manufacturer’s protocol. Purified products were sequenced in separate reactions with each M13 primer using a cycle sequence of 96C, 10 sec; 50C, 5 sec; 60C, 4 min with BigDye Terminator Mix v1.1 (Applied Biosystems, Foster City, CA). Sequencing reactions were analyzed using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Primers were designed to amplify the immediate promoter and exons of OAS1 and RNASEL genes from 13 individual horses by PCR (Table 1). Sequencing was carried out in the same manner as used for the library subclones. Sequences obtained were compared between individuals to identify SNPs within the amplified regions.

**Sequence analysis and contig assembly**
Sequences were assembled and analyzed using Phrap assembly software [33,34,37] and viewed with the Consed visualization tool [35-37]. Contig and singleton reads were assembled by scaffolding onto the human genome using BLASTN [69-71].

Additional sequences were added to the assembly data and re-analyzed with Phrap and BLAST until the consensus sequence spanned the genes from the promoter to the 3’ UTR. The genomic equine consensus sequence was confirmed using data from the Equine Genome Sequencing Project (2x) [38] and intron/exon boundaries were assigned by local alignment to the full-length equine OAS1 [GenBank: AY321355] and RNASEL [GenBank: DQ497159] cDNAs. The equine genomic sequences of OAS1 and RNASEL were submitted to GenBank and assigned the accession numbers DQ536887 and EF070193, respectively.

**Genotyping population**
Blood samples were collected at the Texas A&M University Equestrian Center in accordance with ethical standards. The sampled set used for screening consisted of 13 horses, including 10 geldings/stallions and 3 mares, ranging in age from 21 months to 20 years. Breeds represented include American Quarter Horse (9), Arabian (1), American Paint Horse (1), Appaloosa (1) and Thoroughbred (1).
Authors’ contributions

JJR and DLA provided the OAS1 and RNASEL genomic sequence and assembled the haplotypes for both genes. AAP and MAB contributed all of the cDNA sequences for RNASEL. AAZ completed the phylogeny analysis. TLL completed the FISH analysis for RNASEL. JJR, AAP, MAB and DLA contributed to the identification of polymorphisms and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work was supported by Grant Cl000216 from the National Center for Infectious Diseases, Centers for Disease Control and Prevention to MAB and AAP.

References

1. Baglioni C, Minks MA, Maroney PA: Interferon action may be mediated by activation of a nuclease by pppA2p5'Ap5'A. Nature 1978, 273(5646):684-687.

2. Clemens MJ, Williams BR: Inhibition of cell-free protein synthesis by pppA2p5'Ap5'A: a novel oligonucleotidase synthesized by interferon-treated L cell extracts. Cell 1978, 13(1):565-572.

3. Kerr IM, Brown RE: pppA2p5'Ap5'A: an inhibitor of protein synthesis synthesized with an enzyme fraction from interferon-treated cells. Proc Natl Acad Sci USA 1978, 75(1):256-260.

4. Hovanessian AG, Brown RE, Kerr IM: Synthesis of low molecular weight inhibitor of protein synthesis with enzyme from interferon-treated cells. Nature 1977, 268(5620):537-542.

5. Kerr IM, Brown RE, Hovanessian AG: Nature of inhibitor of cell-free protein synthesis formed in response to interferon and double-stranded RNA. Nature 1977, 268(5620):540-542.

6. Roberts WK, Hovanessian A, Brown RE, Clemens MJ, Kerr IM: Interferon-mediated protein kinase and low-molecular-weight inhibitor of protein synthesis. Nature 1976, 264(5585):477-480.

7. Castelli JC, Hassel BA, Morian A, Gonzalez OY, Belmont J, Gibbs RA, Tweardy DJ: Single nucleotide polymorphisms in genes for 2'-5'-oligoadenylate synthetase and RNase L in patients hospitalized with West Nile virus infection. J Infect Dis 2005, 192(6):923-33.

8. Yakoub I, Lillibridge KM, Morian A, Gonzalez OY, Belmont J, Gibbs RA, Tweardy DJ: Single nucleotide polymorphisms in genes for 2'-5'-oligoadenylate synthetase and RNase L in patients hospitalized with West Nile virus infection. J Infect Dis 2005, 192(6):923-33.

9. Mullan PB, Hosey AM, Buckley NE, Quinn JD, Hele D, Ban VW, Matsushita I, Yanai H, et al.: Polymorphisms of interferon-inducible genes OAS-1 and MxA associated with SARS in the Vietnamese population. Biochem Biophys Res Commun 2003, 309(4):1234-1239.

10. He J, Feng D, de Vlas SJ, Wang H, Fontanet A, Zhang P, Plancoulaine S, Tang F, Zhan L, Yang H, et al.: Association of SARS susceptibility with single nucleic acid polymorphisms of OAS1 and MxA genes: a case-control study. BMC Infect Dis 2006, 6:106.

11. Knapp S, Yes Lj, Frodsham AJ, Hennig B, Hellier S, Zhang L, Wright M, Chiaromonte M, Graves M, Thomas HC, et al.: Polymorphisms in interferon-induced genes and the outcome of hepatitis C virus infection: roles of MxA, OAS-1 and PKR. Genes Immun 2003, 4(6):411-419.

12. Squire J, Zhou A, Hassel BA, Nie H, Silverman RH: Localization of the interferon-induced, 2-5A-dependent RNase gene (RNS4) to human chromosome 1q25. Genomics 1994, 19(1):174-175.

13. Squire J, Meurs EF, Chong KL, McMillan NA, Hovanessian AG, Williams BR: Localization of the human interferon-induced, dsRNA activated p68 kinase gene (PRKR) to chromosome 2p21-p22. Genomics 1993, 16(3):768-770.

14. Zhou A, Hassel BA, Silverman RH: Expression cloning of 2-5A-dependent RNAses: a uniquely regulated mediator of interferon action. Cell 1993, 72(5):753-765.

15. Dong B, Silverman RH: A bipartite model of 2-5A-dependent RNases: a uniquely regulated mediator of interferon action. J Biol Chem 1995, 270(8):4133-4137.

16. Ewing B, Green P: Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res 1998, 8(3):175-185.

17. Buiting K: Viewing and Editing Assembled Sequences Using Consed. In Current Protocols in Bioinformatics Edited by: Baxevanis AD, Davison DB. New York: John Wiley & Co; 2004:11.12.11-12.43.
36. Gordon D, Abajian C, Green P: Consed: a graphical tool for sequence finishing. Genome Res 2000, 10(11):1046-1047.

37. Laboratory of Phil Green [http://www.phrap.org]

38. NCBI Trace Archive [http://www.ncbi.nlm.nih.gov/Traces/trace.cgi]

39. Chen TM, Kuo PL, Hsu CH, Tsai SJ, Chen MJ, Lin CW, Sun HS: Microsatellite in the 3′ untranslated region of human fibroblast growth factor 9 (FGF9) gene exhibits pleiotropic effect on modulating FGF9 protein expression. Human mutation 2007, 28(1):98-100.

40. Stephens M, Scheet P: Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. Am J Hum Genet 2005, 76(3):449-462.

41. Stephens M, Smith NJ, Donnelly P: A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001, 68(4):978-989.

42. TIGR Institute for Genomic Research [http://compbio.dfci.harvard.edu] and The Institute for Genomic Research [http://www.tigr.org]

43. UCSC Genome Bioinformatics [http://www.genome.ucsc.edu]

44. MOTIF Search [http://motif.genome.jp]

45. Ramensky V, Bork P, Sunyaev S: Human non-synonymous SNPs: Server and survey. Nucleic acids research 2002, 30(17):3894-3900.

46. Emelianov S, Kim H, Lonardi S, Quackenbush J, Pollack JR, Ziaie B: Comparative methods for DNA microarray data analysis. Methods 2004, 33(3):27-35.

47. Sunyaev S, Ramensky V, Bork P: TMRH: A tool for detecting cancer-driver transcriptional regulatory variants. Genome Res 2006, 16(2):221-228.

48. Sunyaev S, Ramensky V, Koch I, Lathe W 3rd, Kondrashov AS, Bork P: Prediction of deleterious human alleles. Human molecular genetics 2001, 10(6):591-597.

49. Sarkar SN, Miyagi M, Crabb JW, Sen GC: Identification of the substrate-binding sites of 2′-5′-oligoadenylate synthetase. J Biol Chem 2002, 277(27):24321-24330.

50. Cayley PJ, White RF, Antoniw JF, Walesby NJ, Kerr IM: Distribution of the ppp(Ap)5pA-binding protein and interferon-related enzymes in animals, plants, and lower organisms. Biochem Biophys Res Commun 1982, 108(3):1243-1250.

51. Poy YL, Zharkikh A, Abajian C, Green P: VISTA: a graphical tool for visualizing genome scale DNA sequence alignments of arbitrary length. Bioinformatics (Oxford, England) 2000, 16(11):1046-1047.

52. Lander ES, Linton LM, Birren BW, Nusbaum C, Zody MC, Baldwin J, L derail P, Littau V, Barden E, Dalrymple B, et al: Initial sequencing and analysis of the human genome. Nature 2001, 409(6822):860-921.

53. Raudsepp T, Fronicke L, Scherthan H, Gustavsson I, Chowdhary BP: Genetic comparison of rodent and human chromosomes 8-10. Cytogenet Cell Genet 2006, 112(3):243-250.

54. Nilsson S, Helou K, Walentinsson A, Szpirer C, Nerman O, Stahl F: Chromosomal homology and high-resolution zoo-FISH. Chromosome Res 1996, 4(3):287-298.

55. Kardos I, Frischek L, Scherthan H, Gustavsson I, Chowdhary BP: Zoo-FISH delineates conserved chromosomal segments in horse and man. Chromosome Res 1996, 4(3):218-225.

56. Nilsson S, Helou K, Walentinsson A, Szpirer C, Nerman O, Stahl F: Rat-mouse and rat-human comparative maps based on gene homology and high-resolution zoo-FISH. Genomics 2001, 74(3):287-294.

57. Solinas-Toldo S, Lengauer C, Fries R: Comparative genome map of human and cattle. Genomics 1995, 27(3):489-496.

58.eng Y, Goulet AC, Nelson MA: Identification and characterization of the human Cdc212 gene promoter. Gene 2004, 334(1):75-82.

59. Ogasawara K, Terada T, Asaka J, Kishikawa T, Inui K: Human organic anion transporter 3 gene is regulated constitutively and inducibly via a CAMP-response element. J Pharmacol Exp Ther 2006, 319(1):317-322.

60. Rani CS, Qiang M, Ticku MK: Potential role of CAMP response element-binding protein in ethanol-induced N-methyl-D-aspartate receptor 2B subunit gene transcription in fetal mouse cortical cells. Mol Pharmacol 2005, 67(6):2126-2136.

61. Patrocles Targets Database [http://www.patrocles.org]

62. Frazer KA, Pachter L, Poliakov A, Rubin EM, Dubchak I: VISTA: computational tools for comparative genomics. Nucleic acids research 2004, 32(Web Server):W273-279.

63. Mayor C, Brudno M, Schwartz JR, Poliakov A, Rubin EM, Frazer KA, Pachter LS, Dubchak I: VISTA: visualizing global DNA sequence alignments of arbitrary length. Bioinformatics (Oxford, England) 2000, 16(11):1046-1047.

64. Lander ES, Linton LM, Birren BW, Nusbaum C, Zody MC, Baldwin J, L derail P, Littau V, Barden E, Dalrymple B, et al: Initial sequencing and analysis of the human genome. Nature 2001, 409(6822):860-921.

65. Sharma VK, Kumar N, Brahmacari SK, Ramachandran S: Abundance of Dinucleotide Repeats and Gene Expression are Inversely Correlated: a role for gene function in addition to intron length. Physiol Genomics 2007.

66. Perelygin AA, Zharkikh AA, Brinton MA: Comparative analysis of vertebrate EIF2AK2 (PKR) genes and assignment of the equine gene to ECA15q24-q25 and the bovine gene to BTA11q12-q15. Genet Sel Evol 2006, 38(5):551-563.

67. Bowling AT, Breen M, Chowdhary BP, Hirota K, Lear T, Millon LV, Ponce de Leon FA, Raudsepp T, Stranzinger G: International system for cytogenetic nomenclature of the domestic horse. Report of the Third International Committee for the Standardization of the domestic horse karyotype, Davis, CA, USA, 1996. Chromosome Res 1997, 5:433-443.

68. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: Basic local alignment search tool. J Mol Biol 1990, 215(3):403-410.

69. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic acids research 1997, 25(17):3389-3402.

70. National Center for Biotechnology Information [http://www.ncbi.nlm.nih.gov/]

71. Sir Paul Nurse, Cancer Research UK

Your research papers will be:
- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp

Publish with BioMed Central and every scientist can read your work free of charge