Journal of Veterinary Internal Medicine
Open Access

*Standard Article*

*J Vet Intern Med* 2018;32:465–468

# Investigation of Known Genetic Mutations of Arabian Horses in Egyptian Arabian Foals with Juvenile Idiopathic Epilepsy

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**Background:** The carrier status of lavender foal syndrome (LFS), cerebellar abiotrophy (CA), severe combined immunodeficiency (SCID), and occipitoatlantoaxial malformation (OAAM1) in foals with juvenile idiopathic epilepsy (JIE) is unknown.

**Hypothesis/Objectives:** To determine the carrier status of LFS, CA, SCID, and OAAM1 in foals with JIE.

**Animals:** Ten foals with JIE.

**Materials and Methods:** Archived DNA samples were tested for known genetic mutations causing LFS, CA, SCID, and OAAM1. The inclusion criteria consisted of having been diagnosed with JIE by ruling out other causes of seizures in foals and supported by electroencephalographic examination.

**Results:** Ten Egyptian Arabian horses (5 females and 5 males) were phenotyped as foals with JIE by electroencephalography (EEG). All foals were negative for the genetic mutations that cause LFS, CA, SCID, and OAAM1 except for 1 foal that was a carrier of CA.

**Conclusions and Clinical Importance:** Juvenile idiopathic epilepsy of Egyptian Arabian foals and LFS appear to be phenotypically and genetically distinct disorders. There was no apparent association between JIE and LFS, CA, SCID, and OAAM1.

**Key words:** Brain; Equine; Genetics; Seizures.

Juvenile idiopathic epilepsy (JIE) is a self-limiting epileptic syndrome described in Egyptian Arabian foals.[1] This disorder is characterized by recurrent generalized tonic-clonic seizures with no apparent precipitating events or underlying disease with an early onset in life (median age, 2 months).[1] Affected foals are clinically normal between seizures.[1,2] Seizures have been reported in large animals,[3–5] but JIE is the only well-characterized epileptic disorder based on clinical, neurologic, and electroencephalographic examination.[1] Electroencephalography and semiology of seizures have been used to define, classify, and distinguish seizures and epileptic syndromes in humans.[6–8] Electroencephalography has been used as a diagnostic aid in dogs, cats, horses, and ruminants.[3,9–14] Foals with JIE usually display paroxysmal activity (spikes, sharp waves, and complexes of multiple spikes and sharp waves) in the parietal and central regions with spread and generalization to other areas of the brain.[1] Photic stimulation has not been associated with epileptiform activity in foals with JIE.[1] Juvenile idiopathic epilepsy resembles some neonatal and infant epileptic disorders in human medicine.[15] Some of the hereditary epileptic disorders in humans involve specific voltage-gated calcium channels as well as subunits of potassium, sodium, nicotinic acetylcholine, and GABA receptors, among others.[16] Based on semiology, similarities with some benign familial seizure disorders in infants such as benign familial neonatal convulsions and neonatal epilepsy; a candidate gene approach targeting voltage-gated potassium channels (*KCNQ2*, *KCNQ3*) as a potential cause of JIE in Arabian foals proved unproductive.[17]

Based on history at breeding farms of Egyptian Arabian horses, JIE appears to have a genetic basis with affected animals typically having an affected parent (mare or stallion).[1] Although a dominant mode of inheritance is suspected, a recessive mode has not been entirely excluded.[1] Genetic disorders have been reported in various pure-bred horses.[18] Disorders caused by known genetic mutations that are present in Arabian horses include lavender foal syndrome (LFS), cerebellar

**Abbreviations:**

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| CA | cerebellar abiotrophy |
| EEG | electroencephalography |
| GBED | glycogen branching enzyme deficiency |
| HERDA | hereditary equine regional dermal asthenia |
| HYPP | hyperkalemic periodic paralysis |
| JIE | juvenile idiopathic epilepsy |
| LFS | lavender foal syndrome |
| MH | malignant hyperthermia |
| OAAM | occipitoatlantoaxial malformation |
| PSSM | polysaccharide storage myopathy |
| SCID | severe combined immunodeficiency |

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*The study was performed at the University of California at Davis.*

*The study was supported by gifts from anonymous donors to the Equine and Comparative Neurology Research Group at UCD.*

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*Submitted May 23, 2017; Revised September 22, 2017; Accepted October 12, 2017.*



*DOI: 10.1111/jvim.14873*

abiotrophy (CA), severe combined immunodeficiency (SCID), and occipitoatlantoaxial malformation (OAAM1).[19–27] Lavender foal syndrome is a lethal disorder of Egyptian Arabian foals characterized by foals born with a dilute coat color and profound neurologic dysfunction.[19–21] It is an autosomal recessive inherited disorder caused by a frameshift mutation in exon 30 of the myosin Va gene (*MYO5A*) that results in premature termination of transcription.[20,27] Myosin Va protein is essential for the normal function of melanocytes and neurons.[20] A study reporting foals with LFS described a mare that produced a filly with LFS that also produced a filly with epilepsy when bred to a different stallion.[21] Furthermore, a stallion that produced a filly with LFS also produced a foal with epilepsy when bred to a different mare.[21] Therefore, a possible association between the 2 disorders was considered.[21]

Based on our studies and personal experience (MA), the phenotypes of both disorders are different.[1] From our archived database, mares and stallions producing foals with JIE with characteristic electroencephalographic phenotype have not produced foals with LFS.[1] Therefore, the purpose of our study was to investigate if foals with JIE are carriers of the *MYO5A* LFS mutation. Furthermore, the presence of co-mutations in pure-bred horses causing multiple disorders or exacerbating disease processes is possible as described in Quarter Horses.[28,29] As a second objective, we wished to investigate if known genetic mutations reported in Arabian horses occur in foals with JIE.

## Materials and Methods

The database of the Equine and Comparative Neurology Research Group was searched for Arabian horses diagnosed with JIE. Their records were identified, and stored frozen DNA samples at −80°C were retrieved for genetic testing of LFS, CA, SCID, and OAAM1. The inclusion criteria consisted of samples from foals phenotyped exclusively by 1 of the authors (MA). The phenotype was determined by clinical, neurologic, and electroencephalographic examinations. In brief, clinical and neurologic examinations were determined to be normal between seizures in Egyptian Arabian foals. Paroxysmal activity consisting of sharp waves (70–200 ms[30]), and spikes (<70 ms) and waves on central and parietal cortical areas of the brain based on electroencephalography were part of the inclusion criteria. The central cortical area of the brain was defined as the area between the frontal and parietal cortical areas as described previously.[1] Other extracranial and intracranial causes of seizures in foals were ruled out as previously described.[1] Examples of extracranial causes of seizures included septicemia, hypoglycemia, and electrolyte disturbances. Intracranial causes such as hypoxic-ischemic encephalopathy, meningoencephalitis secondary to septicemia, and congenital anomalies excluded foals from the study. Genetic testing for LFS, CA, and OAAM1 was performed at the Veterinary Genetics Laboratory at the University of California at Davis, and genetic testing for SCID was performed at the Veterinary Genetic Services at the University of Michigan.

## Results

Ten Egyptian Arabian foals (5 females and 5 males) were phenotyped. These foals were part of a retrospective study of 22 foals with JIE.[1] DNA samples from the remaining 12 foals from the study were not available. Archived DNA samples investigated for the various mutations belong to foals from Egyptian lineage with an onset of seizures at a mean and median age of 18 days of age (standard deviation [SD], 7.6; range, 7–30 days old). Seizure-free recorded age had a mean and median of 5 months (SD, 1; range, 4–7 months old). Results of genetic testing identified 1 horse as being a carrier of CA, and none of the horses was a carrier for the LFS, SCID, and OAAM1 mutations.

## Discussion

Based on our results, JIE and LFS appear to be independent syndromes because none of the horses with JIE carried the *MYO5A* gene mutation. However, because of the low number of foals with JIE, a carrier state cannot be entirely ruled out. Furthermore, a report of 2 foals with periodic seizures born from parents producing foals with LFS warrants investigation.[21] These foals were apparently normal between seizures, similar to the confirmed foals with JIE from a previous study.[1,21] Another possibility for the observed periodic seizures in the 2 reported foals is that the seizures were clinical manifestations or variants displayed by foals that are carriers of the LFS mutation but not having JIE. This possibility also warrants further investigation. It is estimated that approximately 10.3% of Egyptian Arabians and 1.8% of non-Egyptian Arabian horses are carriers of the *MYO5A* mutation.[20] The mutation affects myosin Va which is part of a complex of proteins involved in the trafficking of melanosomes to keratinocytes, and transportation of secretory granules, glutamate receptors, and mRNA in neurons.[31–33] Mutation of the *MYO5A* gene explains the observed dilute color and various neurologic deficits in diseased foals.[21,27] Phenotypically, JIE and LFS are different. Foals with JIE are born normal and appear healthy after birth.[1] This epileptic syndrome has an early onset of signs from a few days to 6 months of age and is a self-limiting disorder with clinical manifestations resolving over a period of months.[1,2] Foals also appear normal on physical and neurologic examination between seizures.[1] In terms of survival, JIE has a good prognosis provided complications such as head trauma and aspiration pneumonia are prevented or treated properly.[1] Prognosis for athletic performance is also good. A coat color predilection such as that reported in foals with LFS has not been noted in foals with JIE.[1,19] In contrast, foals with LFS are born with profound neurologic dysfunction resulting in euthanasia within a few days of life.[19,21,27] The most commonly reported abnormalities include lateral recumbency with inability to rise or assume sternal recumbency, opisthotonus, intermittent paddling, and extensor rigidity.[19,21] Other abnormalities include intermittent horizontal nystagmus and ventral strabismus.[19] Most foals have a strong suckle reflex, and the remainder of the neurologic examination is normal.[19,21] Coat colors in foals with LFS include dilute pale chestnut and lavender.[19–21,27] A sex predisposition has not been noted for either disorder.[1,19,21]

Investigation of co-mutations in pure-bred horses is important to avoid breeding practices that might result in perpetuating disease or a carrier state in breeding animals.[28] As an example of strategic breeding, the American Quarter Horse Association is requiring genetic testing for 5 diseases in all registered breeding stallions.[34] These diseases include glycogen branching enzyme deficiency (GBED), hereditary equine regional dermal asthenia (HERDA), hyperkalemic periodic paralysis (HYPP), malignant hyperthermia (MH), and polysaccharide storage myopathy type 1 (PSSM1).[34] Investigation of known genetic mutations also will prevent producing horses with more severe clinical phenotypes of disease when co-mutations occur. Such is the case of Quarter Horses with both PSSM type 1 and MH which results in a more severe myopathic disorder that is more challenging to address than when present alone.[29] From our investigation, we identified 1 foal that carried the genetic mutation responsible for CA.[24] Cerebellar abiotrophy is inherited as an autosomal recessive trait and is associated with a single-nucleotide polymorphism on chromosome 2 (13074277G>A), located in the 4th exon of *TOE1* and in proximity to *MUTYH* on the antisense strand.[35] This mutation results in loss of Purkinje neurons with secondary loss of the granular cell layer of the cerebellum.[25,36] Signs can be recognized at birth or up to a few months of age and include ataxia, hypermetria, intention tremors, and lack of menace response.[36] The prevalence of CA in a population of Arabian horses in South Africa was estimated to be 5.1%.[37] Historically, there have been no reports of horses having produced foals with CA from our database of horses with JIE.

Foals in our study also were negative for the mutation that causes SCID in Arabians. The disorder is caused by a 5-base pair deletion in the gene encoding the catalytic subunit of DNA-dependent protein kinase (*DNA-PK*) and is inherited as an autosomal recessive trait.[38,39] The lack of activity of DNA-PK leads to failure of T and B lymphocytes to cut, rearrange, and anneal genes that encode surface-expressed antigen-specific receptors.[38] Foals with SCID lack mature functional B and T lymphocytes have profound lymphopenia (<1,000/μL) and low immunoglobulin concentrations followed by agammaglobulinemia after maternal antibodies disappear.[40] The frequency of the SCID gene carrier state was estimated to be 8.5% in the Arabian population.[41] Foals with JIE have apparently normal immune function. Furthermore, laboratory results are normal if no concurrent diseases are present.[1]

Recently, a deletion of a 2.7-kb segment located 4.4 kb downstream from the end of *HOXD4* and 8.2 kb upstream from the start of *HOXD3* was identified in 1 Arabian foal with OAAM.[42] The homeobox *HOX* gene cluster is a region highly conserved across species and involved in the development of the axial and appendicular skeleton.[42] Occipitoatlantoaxial malformation is a developmental defect in which the first cervical vertebra (atlas) resembles the base of the skull (occiput), and the second vertebra (axis) resembles the atlas.[43] It is 1 of 6 types of OAAM described in the horse.[44] This disorder is suspected to be transmitted as an autosomal recessive trait.[43] The frequency of a carrier status for the homeobox deletion was determined to be 1.2% from the DNA database in a small population of Arabian horses from the Veterinary Genetics Laboratory at UCD.[42] Affected foals can present with a range of signs from stiffness and abnormal posture to varying grades of ataxia.[43] Foals with JIE lack skeletal abnormalities, and posture is normal.[1] Foals in our study were negative for the deletion in the homeobox *HOX* gene.

In conclusion, there is no apparent association between JIE and LFS, CA, SCID, and OAAM1. However, the presence of a carrier state or co-mutations in foals with JIE is possible because the estimated prevalence of known genetic mutations ranges from 1.2 to 10.3% in the Arabian horse population. Juvenile idiopathic epilepsy and LFS appear to be phenotypically and genetically different disorders that occur in Egyptian Arabian foals. Because a genetic marker has not been identified in foals with JIE, the carrier state of a putative mutation in the Arabian population is unknown. A whole-genome single-nucleotide polymorphism association approach and a case versus control design might be more suitable to identify the molecular basis of JIE in Arabian horses. Strategic breeding by testing for identified genetic mutations in Arabian horses of Egyptian lineage with a family history of epileptic disorders will aid in eliminating disease.

## Acknowledgments

*Conflict of Interests Declaration*: Authors declare no conflict of interest.

*Off-label Antimicrobial Declaration*: Authors declare no off-label use of antimicrobials.

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