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Lavender foal syndrome in Egyptian Arabian horses: molecular and pathological studies

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Abstract

Lavender foal syndrome is one of fatal genetic coat color - associated disorders in Arabian horses caused by a recessive gene. Arabian horse harbor heterozygous genes represent a carrier case with normal criteria, while that harbor homozygous genes mostly will had a characteristic lavender coat color and represented an affected case that will die within few days. Egyptian Arabian horses are incriminated to harbor up to 10% of this syndrome recessive gene and hence of great economic important for Arabian horse's industry. In this study we trace the historical appearance of LFS in the records of one Arabian horses farm, apply PCR followed by sequence analysis for 8 suspected cases. On the other side pathological investigation of early dead foal with lavender coat color was carried out. Our results detected the incriminated single base deletion at the molecular level by sequence analysis of hair samples in four out of the eight suspected horses. Histopathological investigation was carried out on liver, kidneys and different regions of the brain of dead foal with lavender coat color. Moreover, immuno- histochemistry technique was done to clarify the possible LFS pathogenesis. Our result reflects the principle role of myosin Va as a cargo molecule in LFS pathogenesis in association with development of endoplasmic reticulum stress effect with end result of multi-systemic effects.

Keywords: Lavender, foal syndrome, Arabian horse, Myosin Va, Endoplasmic reticulum, pathology.

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Introduction

Lavender foal syndrome is one of coat color genetic disorders that had been identified in many organisms with different synonyms; in human Griscelli syndrome with characteristic silver-gray hair color (Griscelli et al., 1978), in mice dilute-lethal coat (Jenkins et al.,1981), dilute opisthotonus in rats (Dekker-Ohno et al.,1996), lavender foal syndrome (LFS) in Arabian horses (Brooks et al., 2010) and even lavender feather in chicken (Vaez et al., 2008). Combinations of different genes had been incriminated in different species but with common feature of incorporation of myosin Va gene (MyoVa) (Vaez et al., 2008). The abnormal colors of these varied syndromes are due to abnormal deposition of melanosomes within the hair shat rather than abnormal pigment product (Au and Huang, 2002).

In Arabian horses LFS is a rare autosomal recessive lethal disorder affecting Arabian foals and characterized by a dilute coat color and severe neurological signs (Bierman et al., 2010). Horses that have one copy of the mutated genes, in combination with one copy of the normal gene, are physically normal but are considered carriers. The characteristic feature of this condition is the dilute or bleached-out hair coat color, hence the name "lavender foal syndrome" (Fanelli, 2005). No specific observation had been detected in microscopic investigation, so when the coat color is overlooked the associated neurological abnormalities will be confused with other disorders (Page et al., 2006). Although this condition was recorded in other breeding groups, some authors assume this condition to Egyptian Arabian horse strains (Fanelli, 2005 and Brooks et al., 2010). In this study we try to get approach to the status of LFS in our Egyptian Arabian horses through detection of LFS-carrier horses that carry one copy of the LFS gene, among some Egyptian Arabian horses which had a history of foaling with diluted or lavender coat color, neurological symptoms and/ or history of early foals' death. Investigation the possible effect of mutated myosin Va molecules in LFS development was carried out.

Material and Methods

Carcass of early dead Arabian foal (from Arabian horse farm) with characteristic lavender coat colour had admitted to the pathology department (Animal Health Research Institute, on 2014) for investigation. Reviewing the historical reports of that Arabian horse farm; out of 260 Arabian horse, 8 cases (4 females and 4 males) had history of foals with clinical signs of diluted or lavender coat colour, neurological symptoms and / or history of early foal death, but none of them was investigated as LFS.

Hair samples with intact hair follicles were collected from suspected 8 horses; samples were divided into 2 halves; one half had been sent to University of California (UC) Davis - Veterinary Medicine - Veterinary Genetic Laboratory (VGL) – USA. The other halves of hair samples were subjected for molecular investigation in the Biotechnology unit-VACSERA- Egypt.

Polymerase chain reaction (PCR) with specific LFS primer sets was re-carried followed by sequence analysis to achieve the characteristic molecular picture and clarify the state of the investigated 8 cases; either carrier or free for LFS.

1. PCR & Sequences (Biotechnology unit-VACSERA- Egypt)

1.1. The primer sets:

Forward, 5'-3', AGAATGAGGCTGAAGC CCTC/

Reverse, 3'-5', GTGATCTCATGCTGCAG GCT

1.2. DNA was extracted using ChargeSwitchTM Forensic DNA Purification Kits (Cat. No. CS11200). Extraction carried out according to the manufacturer's instructions.

1.3. PCR (conventional) was carried out using Taq PCR Master Kit (Qiagen, Cat. No.; 201443). PCR reaction was done according to kits' manufacture instructions.

1.4. DNA analysis was carried out on the purified PCR products using a Prism

BigDyev301 Kit (Applied Biosystems, Cat. No.; 4336917) on an ABI 310 DNA automated sequencer (Applied Biosystems).

2. Histopathological investigation

Archived formalin-fixed paraffin embedded liver, kidneys and different regions of the brain were processed for H&E staining protocol used by Suvarna et al., (2012). Another set of sections on positive charged slides were prepared for IHC technique. Primary antibodies of PDI (Protein derivative isoform) (C-2) (Santa cruz Biotech.; SC-74551), calregulin (F-4) (Santa cruz Biotech.; SC-373883), Myosin Va (Santa cruz Biotech.; sc-365986) and Hsp 90 (Heat shock protein 90) (Thermo Fischer) were used for IHC technique according to manufacture kits' instructions.

Results

Four hair samples out of the tested eight cases were LFS- carrier; horses carry one copy of the LFS gene as reported by VGL-UC Davis. Other four cases showed normal genes and were reported negative for LFS by PCR technique (Table 1).

Table 1. Result of Lavender foal syndrome PCR testing as reported by VGL-UC Davis, USA

Code Number of tested hair samples; VGL-UC Davis, USA	**Arabian Genetic Lavender Foal Syndrome PCR Test Result**
1) A(CBA9609)	N/N-Normal: horse does not have the LFS gene
2) B (CBA9610)	N/LFS-Carrier: horse carries one copy of the LFS gene
3) C (CBA9611)	N/LFS-Carrier: horse carries one copy of the LFS gene
4) D (CBA9612)	N/LFS-Carrier: horse carries one copy of the LFS gene
5) E (CBA9613)	N/LFS-Carrier: horse carries one copy of the LFS gene
6) F (CBA9614)	N/N-Normal: horse does not have the LFS gene
7) G (CBA9615)	N/N-Normal: horse does not have the LFS gene
8) H (CBA9616)	N/N-Normal: horse does not have the LFS gene

1. PCR and Sequence analysis (Biotechnology Unit–VACSERA- Egypt)

Our PCR results detected the four positive LFS carrier cases, which had identical matching with the results of VGL- UC Davis, USA. Sequence analyses refer to single base deletion of the myosin Va gene (Fig. 1) in comparison with that had been reported on gene bank.

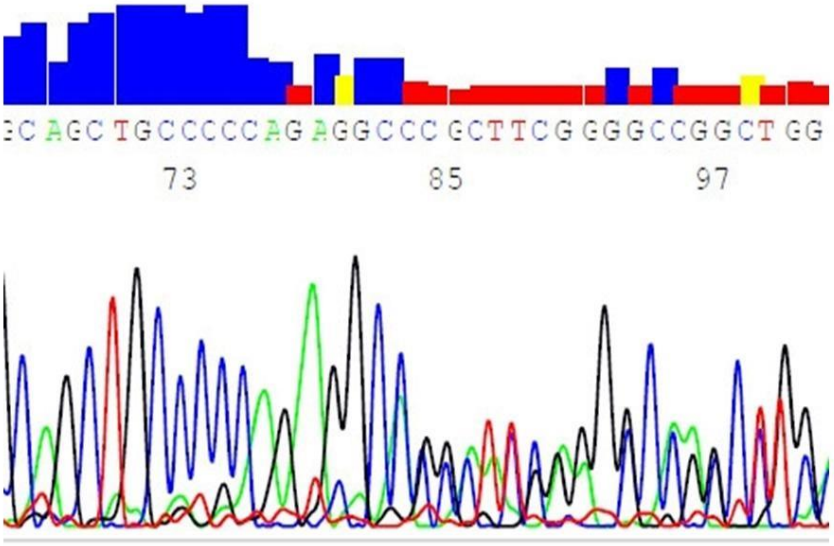

Fig. 1. Sequence analysis of carrier cases of lavender foal syndrome

2 Pathological investigations:

Post mortem investigation to an early dead foal with characteristic lavender coat color was carried out that no gross abnormalities could be detected.

Microscopically, we evaluated the histopathological changes in brain, liver and kidneys. Cerebral cortex revealed features of neuron-degeneration with wide spread of fine vacuolation given spongy appearance; large sized vacuoles could also be detected with disruption of cerebral architecture (Fig. 2a). Some neurons of cerebral cortex underwent neurophagia (Fig. 2b) while others had intra-neuron vacuole (Fig. 2c). White matter showed large sized vacuoles (Fig. 2d) and swollen of oligodendroglia myelin sheaths that give the white matter noticeable spongy appearance with distinct neurofibillary assembly (Fig. 2e). Focal aggregation of microglia (Fig. 2f) was also detected. Peri-vascular plasma cells infiltration was a prominent feature (Fig. 3a). The meningeal vessels contained monocytes (Fig. 3b). Monitoring of endoplasmic reticulum status; indicated to focal localization within the perikaryon (Fig. 3c) and axons (Fig. 3d) using PDI and calregulin primary antibodies respectively. Hepatic changes characterized by predominance of hepatocytes vacuolation, with distinct perinuclear localization. Dilation of hepatic sinusoids with distortion of hepatic cords was a common feature (Fig. 4a).

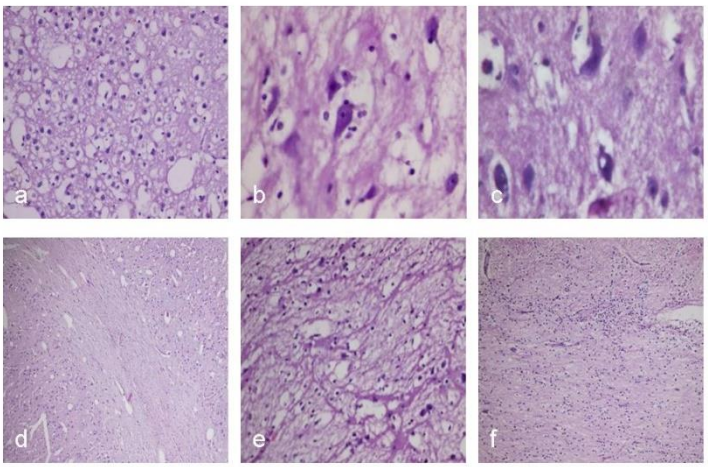

Fig. 2. (a) Cerebral cortex reveals fine neuropil vacuolation and large sized vacuoles associated with disruption of cerebral architecture (H&E X400). (b) Some neurons in the cerebral cortex show feature of neurophagia (H&E X400). (c) Neuron in the cerebral cortex had intra-neural vacuole (H&E X400). (d) White matter shows large sized vacuoles (H&E X200). (e) Swollen of oligodendroglia' myelin sheaths with noticeable spongy appearance and distinct neurofibillary assembly (H&E X400). (f) Focal aggregation of microglia within the white matter (H&E X200). (H&E, a-c and e X400, d and f X200)

Myosin Va molecules with prominent peri-nuclear location was detected (Fig. 4b). Marked reactivity to PDI and calregulin indicate to peri-nuclear vacuolated endoplasmic reticulum of hepatocytes (Fig. 4c).

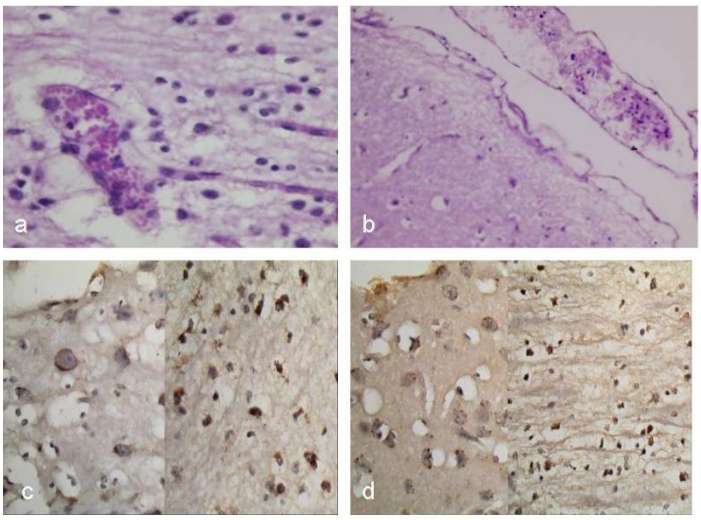

Fig. 3. (a) Peri-vascular plasma cells infiltration (H&E X 400). (b) Meningeal vessels containing monocytes (H&E X400). Endoplasmic reticulum investigation indicates to its perikaryon location (left), while remnants of endoplasmic reticulum are detected in the axons (right); (c) PDI/IHC X400. (d) Calregulin/IHC X400.

Exploring the Hsp90 protein molecule cellular status; indicated to its nuclear translocation (Fig. 4d). Features of hepatic changes chronicity was represented by marked thickening of hepatic portal areas vasculatures (Fig. 5a) and hepatic capsule (Fig. 5b). Renal medulla revealed severe interstitial inflammation with desquamation of lining epithelia (Fig. 6a). Renal cortex showed mild thickening of the glomerular basement membrane, with evidence of mesangiolysis in some glomeruli (Fig. 6b). Some glomeruli contain eosinophilic material, which refer to its amyloid nature when investigated for auto-fluorescent property (Fig. 6c).

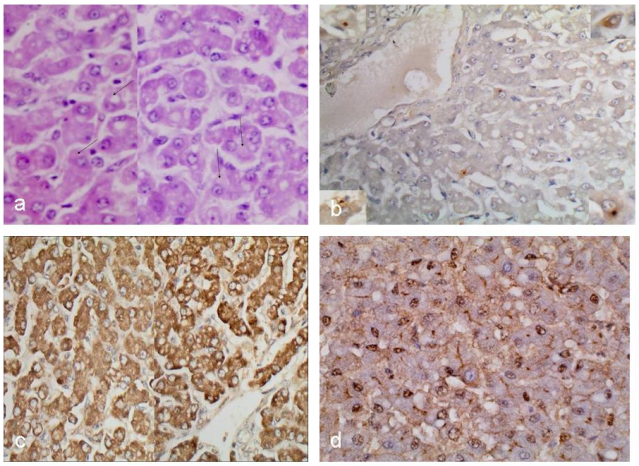

Fig. 4. (a) Hepatocytes with prominent peri-nuclear vacuoles and marked distortion of hepatic cords. (H&E X1000). (b) Hepatocytes harbor myosin Va molecules with prominent peri-nuclear localization. Myosin Va/IHC X400 (In setX600). (c) Hepatocytes reveal peri-nuclear swollen endoplasmic reticulum. (PDI/left &Cal/ right) (IHCX400). (d) Marked reactivity of hepatocytes' nuclei to Hsp90 (Hsp90/IHCX 600).

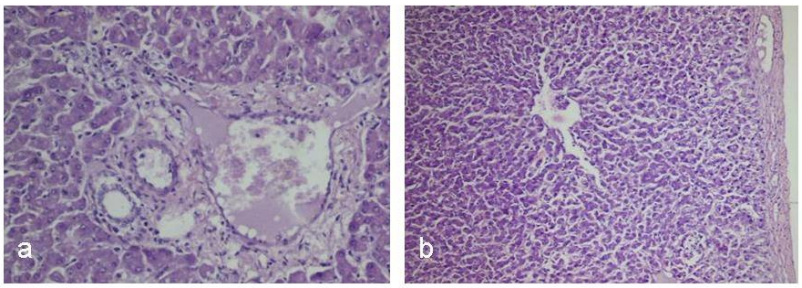

Fig. 5. (a) Marked thickening of hepatic portal areas vasculatures (H&E X400). (b) Thickening of hepatic capsule (H&E X200).

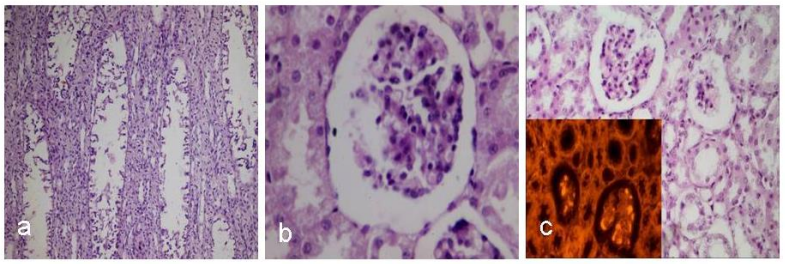

Fig. 6. (a) Renal medulla reveals severe interstitial inflammation (H&E X400). (b) Evidence of mesangiolysis (H&E X400). (c) Renal glomeruli show feature of glomerulo- lysis and contain eosinophilic material that reflect amyloid nature when investigated for auto-fluorescent property (H&E X200, In set FMX 200)

Discussion

Lavender foal syndrome as a genetic disorder is attributed to inheritance of homozygous recessive genes. A single base deletion had been associated with LFS. The responsible gene was myosin Va gene. In our study comparison of the nucleotide sequences between affected and normal horses revealed one base deletion in the fragment amplified by the used primer set, that match the results of Bierman et al. (2010). The deletion produces a truncated protein with insertion of a premature stop codon. The substituted amino acid is Arginine and the resulting truncation of almost half the protein tail is the causative mutation for the disorder (Bierman et al., 2010).

Restriction of LFS to inheritance of homozygous alleles was attributed to the insufficiency of hybrid protein to compete normal protein (Jones et al., 2000). Myosin V is an unconventional motor protein play a critical role in intracellular trafficking phenomena, there are 3 subtypes (a, b & c) each subtype is expressed in certain tissues. Mutations in responsible genes lead to pathological conditions which vary according to the differential expression and the alternative splice variants (Rudolf et al., 2011). Myosin V and its associated cargoes are thus the key components of anterograde transport pathways that move and/or anchor cargo near the cell periphery to deposit organelles at their proper destination (Kathleen, 2008). Myosin V molecules provide continuous transport of organelles, membranous cargo, secretory vesicles, mRNA, lipids and proteins vesicles on actin tracks (Reck-Peterson et al., 2000) with different tissue specificities (Rodriguez et al., 2002), thus mutation of myosin V either lead to failure in cargo complex formation and/or release of anchored molecules in unsuitable amounts and/or time points (Huang et al., 1998).

Clinically affected foal born with diluted or lavender coat color associated with nervous manifestations mostly in form inability to stand, seizer excitation and abnormal eye movements (Page et al., 2006). This characteristic color was explained by loss of biological function of normal myosin Va motor molecules in binding melanosome granules and transferring them to keratinocytes (Fukuda and Kurode, 2004), so the dilute color observed is not because of abnormal pigment production but an abnormal dispersal of melanosomes within the hair shafts (Au and Huang, 2002).

The associated muscular tremor could be attributed to abnormal-regulation of Rab8A, a molecule responsible for insulin signaling mobilization vesicles to the muscle cell (Sun et al., 2014). One the other side motor locomotion deficient could be attributes to the delay in myelination occurs during the development of nervous system as a result of malfunction myosin Va molecules (Röder et al., (2008), hypomyelination in the neurons of the brain and spinal cord had been demonstrated by Sloane and Vartanian (2007).

Another explanation depend on the fact that myosin Va is highly expressed in the hippocampus (Zhao et al., 1996), a brain region that is necessary for the formation of episodic and spatial memory and also in the cerebellum (Espreafico et al., 1992) which control voluntary muscle movement; so abnormalities of these brain structures could explain the severe mental retardation and locomotion defects observed in lavender foals with mutated myosin Va (Rudolf et al., 2011), in addition to abolishing of cerebellar long-term depression (LTD), which is a cellular basis of motor learning lead to locomotor disability (Miyata et al., 2011) that previously documented in human with Griscelli syndrome (Sanal et al., 2000) and foal with lavender syndrome (Page et al., 2006).

Regarding to nervous manifestations, dendritic spines which are small actin-rich protrusions on neuronal dendrites and serve as sites of excitatory synaptic input (Hammer et al., 2011) and synaptic plasticity is one of the most important mechanisms of nerve cells to respond to alterations in neuronal input hence absence of functional myosin Va, could lead to the release of hormones and neuropeptides at incorrect locations, quantities and time (Rudolf et al., 2011). Marked vacuolation and even cavitations in the neuropil of our investigated case could be attributed to accumulation of low density complex vesicles (LDCVS) in the axonal and dendrites spine, with failure in its release, as myosin Va be involved in maturation, transport, and exocytosis of LDCVs. Neuronal LDCVs are transported into axons and dendrites; mutation in neurological myosin Va tail fragment significantly impairs the movement of LDCVs in both dendrites and axons (Bittins et al., 2010), in addition to failure in transport and even accumulation of mRNA in the dendritic spines (Takagishi et al., 2007), which could explain our findings of neuropil vacuolation and cavitations, that could represent swollen dendrites and axons endings with incrimination of endoplasmic reticulum damage. The present study referred to marked axonal swelling with severe damage of endoplasmic reticulum within the axons (as indicated by PDI and calregulin results).

In the present study we could detect macrophage infiltration within the neuropil and active migration of plasma cells from blood vasculature into brain parenchyma, which in similar with findings in some brain of Griscelli disease patients showed feature

of uncontrolled lymphocyte/macrophage activation syndrome (Hurvitz et al., 1993). Hepatic peri-nuclear vacuolation in our investigated case represent hepatic cells death through parapotosis pathway and the peri-nuclear vacuoles represent endoplasmic reticulum engorged with misfolded protein as explained by Ram and Ramakrishna (2014) and demonstrated in the present study through marked reactivity to PDI and calregulin antibodies. These findings in association with detection of myosin Va molecules characteristic peri-nuclear location encourage the suggestion that abnormal myosin Va molecules, and hence associated cargo, lead to endoplasmic reticulum stress with initiation of paraptosis and LFS pathogenesis (with variable mechanisms in different tissues). In the present study rare reactivity of myosin Va molecules confirm the poor immune-reactivity of myosin Va to be localized (Varadi, et al., 2005).

Investigation of cellular status of Hsp90 molecules referred its nuclear translocation, that indicate to state of immune-suppression (Pratt et al., 2004). Renal features in our study revealed to some diabetic nephropathic effect manifested in form of amyloid deposition within the glomerular tufts and mesangiolysis. Although no significant change in blood glucose level of LFS foals (Page et al., 2006) but genetics and biochemistry studies have shown that myosin-Va is targeted to and involved in the transport of insulin secretory granules in pancreatic β-cells (Waselle et al., 2003). In pancreatic β-cells; myosin Va-associated cargo vesicles of Golgi derived secretory granules get accumulated and hindered from release of endocrine cells in case of myosin Va mutation (Desnos et al., 2007).

These data, together with previous evidence for the involvement of myosin-Va in the secretion of insulin (Varadi et al., 2005) and catecholamine (Watanabe et al., 2005) could support our suggestion of the generalized role for myosin-Va in the mechanisms of exocytosis of dense-core granules; in addition to the characteristic neurodegenerative changes in diabetic sensory and autonomic neuropathy.

Conclusion

Regarding to the recorded pathological changes we could conclude that mutation in that cargo molecule will lead to multi-systemic dysfunction with most predominant neuro-muscular appearance (with clear evidence for diabetes development), but we could not ignore the possible disorders result from each gene sporadically or accompanied with other interacting genes mutations due to the variable associated pathological conditions.

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References

Au JSY, Huang JD (2002). A tissue-specific exon of Myosin Va is responsible for selective cargo binding in melanocytes. Cell Motility and the Cytoskeleton, 53: 89–102.

Bierman A, Guthrie A J, Harper CK (2010). Lavender foal syndrome in Arabian horses is caused by a single-base deletion in the MYO5A gene. Animal Genetics, 41(2): 199–201.

Bittins CM, Eichler TW, Hammer JA 3rd, Gerdes HH (2010). Dominant-negative myosin Va impairs retrograde but not anterograde axonal transport of large dense core vesicles. Cellular Molecular of Neurobiology, 30: 369–379.

Brooks SA, Gabreski N, Miller D, Brisbin A, Brown HE, Streeter C, Mezey J, Cook D, Antczak DF (2010). Whole-genome SNP association in the horse: identification of a deletion in myosin Va responsible for Lavender Foal Syndrome. PLoS Genetic 6, e1000909.

Dekker-Ohno K, Hayasaka S, Takagishi Y, Oda S, Wakasugi N, Mikoshiba K, Inouye M, Yamamura H (1996). Endoplasmic reticulum is missing in dendritic spines of Purkinje cells of the ataxic mutant rat. Brain Research, 714: 226–230.

Desnos C, Huet S, Darchen F (2007). 'Should I stay, or should I go?' myosin V functions in organelle trafficking. Biology of Cell, 99: 411-423.

Espreafico EM, Cheney RE, Matteoli M, Nascimento AA, De Camilli PV, Larson RE, Mooseker MS (1992). Primary structure and cellular localization of chicken brain myosin-V an unconventional myosin with calmodulin light chains. Journal of Cell Biology, 119: 1541–1557.

Fanelli HH (2005). Coat colour dilution lethal ('lavender foal syndrome'): a tetany syndrome of Arabian foals. Equine Veterinary Education, 17: 260–263.

Fukuda M, Kuroda TS (2004). Missense mutations in the globular tail of myosin-Va in dilute mice partially impair binding of Slac2-a/melanophilin. Journal of Cell Science, 117(4): 583-591.

Griscelli C, Durandy A, Guy-Grand D, Daguillard F, Herzog C, Prunieras M (1978). A syndrome associating partial albinism and immunodeficiency. American Journal of Medicine, 65: 691–702.

Hammer JA 3rd, Brenowitz SD, Wagner W (2011). Myosin-Va transports the endoplasmic reticulum into the dendritic spines of purkinje neurons. Nature of Cell Biology, 13(1): 40-48.

Huang JD, Mermall V, Strobel MC, Russell LB, Mooseker MS, Copeland NG, Jenkins NA (1998). Molecular genetic dissection of mouse unconventional myosin-VA: tail region mutations. Genetics, 148(4): 1963-1972.

Hurvitz H, Gillis R, Klaus S, Klar A, Gross-Kieselstein F, Okon E (1993). A kindred with Griscelli disease: spectrum of neurological involvement. European Journal of Pediatric, 152: 402–405.

Jenkins NA, Copeland NG, Taylor BA, Lee BK (1981). Dilute coat colour mutation of DBA/2J mice is associated with the site of integration of an ecotropic MuLV genome. Nature, 293: 370-374.

Jones JM, Huang JD, Mermall V, Hamilton BA, Mooseker, MS, Escayg A, Copeland NG, Jenkins NA, Meisler MH (2000). The mouse neurological mutant flailer expresses a novel hybrid gene derived by exon shuffling between Gnb5 and Myo5a. Human Molecular Genetics, 9: 821–828.

Kathleen M Trybus (2008). Myosin V from head to tail. Cell Molecular Life Science, 65(9): 1378–1389.

Miyata M, Kishimoto Y, Tanaka M, Hashimoto K, Hirashima N, Murata Y, Kano M, Takagishi Y (2011). A role for myosin Va in cerebellar plasticity and motor learning: a possible mechanism underlying neurological disorder in myosin Va disease. Journal of Neuroscience, 31(16): 6067-6078.

Page P, Parker R, Harper C, Guthrie A, Neser J (2006). Clinical, clinicopathologic, postmortem examination findings and familial history of 3 Arabians with lavender foal syndrome. Journal of Veterinary Internal Medicine, 20: 1491–1494.

Pratt WB, Galigniana MD, Morishima Y, Murphy PJ (2004). Role of molecular chaperones in steroid receptor action. Essays Biochemical, 40: 41-58.

Ram BM, Ramakrishna G (2014). Endoplasmic reticulum vacuolation and unfolded protein response leading to paraptosis like cell death in cyclosporine A treated cancer cervix cells is mediated by cyclophilin B inhibition. Biochemical Biophysical Acta, 1843(11): 2497-2512.

Reck-Peterson SL, Provance DW Jr, Mooseker MS, Mercer JA (2000). Class V myosins. Biochemical Biophysical Acta, 1496: 36–51.

Röder IV, Petersen Y, Choi KR, Witzemann V, Hammer J A 3rd, Rudolf R (2008). Role of Myosin Va in the plasticity of the vertebrate neuromuscular junction in vivo. PLoS One, 3 (12): e3871.

Rodriguez OC, Cheney RE (2002). Human myosin-Vc is a novel class V myosin expressed in epithelial cells. Journal of Cell Sciences, 115: 991–1004.

Rudolf R, Bittins CM, Gerdes H-H (2011). The role of myosin V in exocytosis and synaptic plasticity. Journal of Neurochemistry, 116: 177-199.

Sanal O, Yel L, Kucukali T, Gilbert-Barnes E, Tardieu M, Texcan I, Ersoy F, Metin A, de Saint Basile G (2000). An allelic variant of Griscelli disease presentation with severe hypotonia, mental-motor retardation, and hypopigmentation consistent with Elejalde syndrome (neuroectodermal melanolysosomal disorder). Journal of Neurology, 247: 570–572.

Sloane JA, Vartanian TK (2007). Myosin Va controls oligodendrocyte morphogenesis and myelination. Journal of Neuroscience, 27: 11366–11375.

Sun Y, Chiu TT, Foley KP, Bilan PJ, Klip A (2014). Myosin Va mediates Rab8A-regulated GLUT4 vesicle exocytosis in insulin-stimulated muscle cells. Molecular Biology of Cell, 25(7): 1159–1170.

Suvarna KS, Layton Ch, Bancroft JD (2012). Bancroft's Theory and Practice of Histological Techniques: Expert Consult: Online and Print, 7e 7th Edition. Hardcover ISBN: 9780702042263. Imprint: Churchill Livingstone.

Takagishi Y, Hashimoto K, Kayahara T, Watanabe M, Otsuka H, Mizoguchi A, Kano M, Murata Y (2007). Diminished climbing fiber innervation of Purkinje cells in the cerebellum of myosin Va mutant mice and rats. Developmental Neurobiology, 67: 909–923.

Vaez M, Follett S A, Bed'hom B, Gourichon D, Tixier-Boichard M, Burke T (2008). A single point-mutation within the melanophilin gene causes the lavender plumage colour dilution phenotype in the chicken. BMC Genetics, 15: 9–7.

Varadi A, Tsuboi T, Rutter GA (2005). Myosin-Va transports dense core secretory vesicles in pancreatic MIN6 beta-cells. Molecular Biology of Cell, 16:2670–2680.

Waselle L, Coppola T, Fukuda M, Iezzi M, El-Amraoui A, Petit C, Regazzi R (2003). Involvement of the Rab27 binding protein Slac2c/MyRIP in

insulin exocytosis. Molecular Biology of Cell, 14(10): 4103–4113.

Watanabe M, Nomura K, Ohyama A, Ishikawa R, Komiya Y, Hosaka K, Yamauchi E, Taniguchi H, Sasakawa N, Kumakura K, Ushiki T, Sato O, Ikebe M, Igarashi M (2005). Myosin-Va regulates exocytosis through the submicromolar Ca^{+2} dependent binding of syntaxin-1A. Molecular Biology of Cell, 16: 4519–4530.

Zhao LP, Koslovsky JS, Reinhard J, Bahler M, Witt AE, Provance DW Jr, Mercer JA (1996). Cloning and characterization of myr 6, an unconventional myosin of the dilute/myosin-V family. Proceeding of National Academic of Science, USA 93: 10826–10831.