Mechanisms of Vitamin E Deficient Neurodegeneration in Small and Large Animal Models

Carrie J. Finno, DVM, PhD, Diplomate ACVIM

Assistant Professor, UC Davis School of Veterinary Medicine

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September 1, 2017

NIH Council of Councils

Overview

1. Vitamin E : Role in neurologic disease
2. Animal models of Ataxia with VitE Deficiency (AVED)
   - Equine neuroaxonal dystrophy (eNAD)
   - *Ttpa*$^{-/-}$ mouse
3. Transcriptional profiling of CNS tissue
4. Functional electrophysiological evaluation
5. Proposed mechanisms

Vitamin E (VitE)

- Major lipid soluble antioxidant
- 8 forms

Tocopherol (saturated)	Tocotrienol (unsaturated)
α-tocopherol	α-tocotrienol
β-tocopherol	β-tocotrienol
γ-tocopherol	γ-tocotrienol
δ-tocopherol	δ-tocotrienol

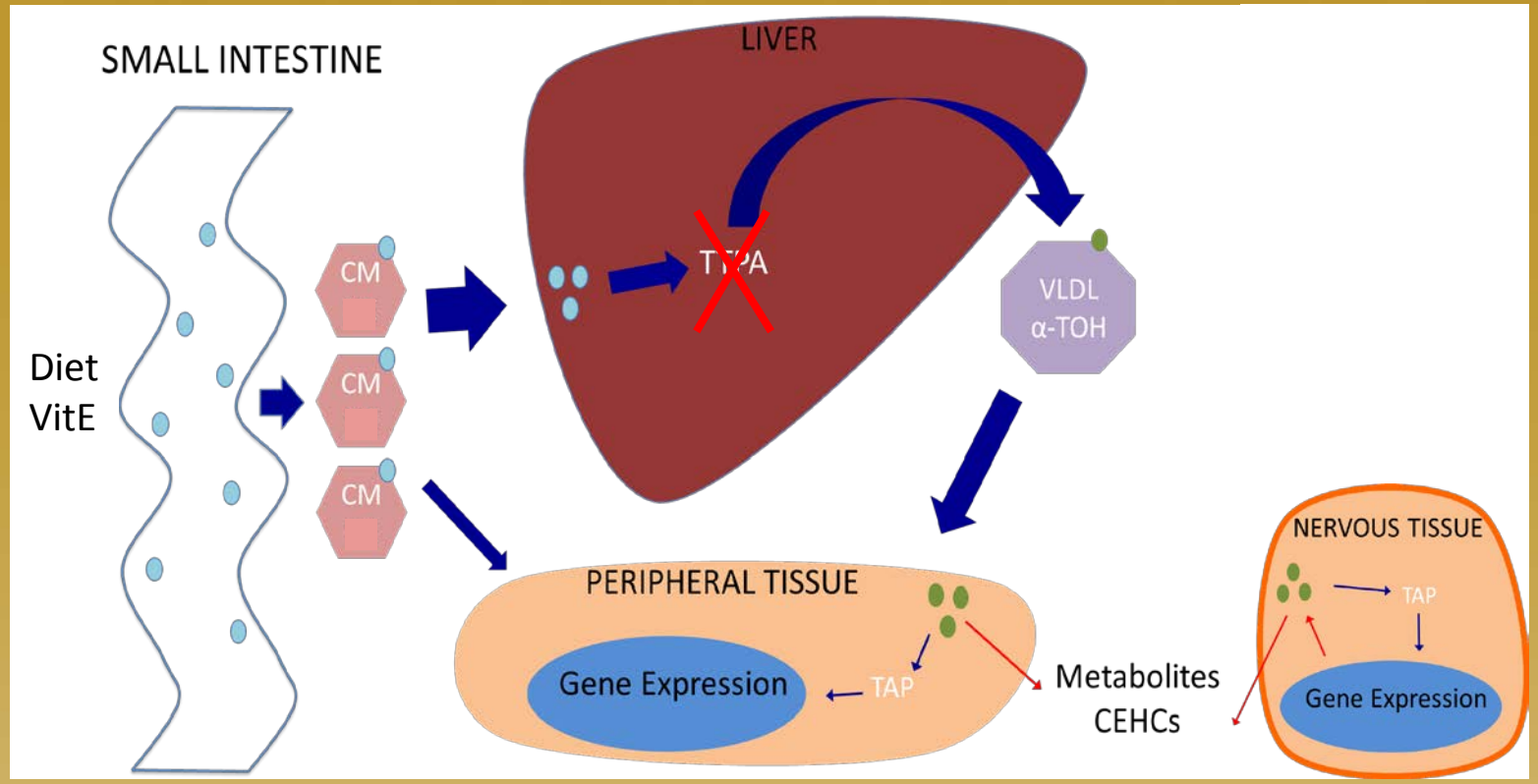
- Common absorption and delivery pathways as cholesterol
- Critical in maintaining neurological health?

VitE Deficiency

- CNS + PNS + retina + skeletal muscle
  - Areflexia
  - Cerebellar ataxia
  - Distal loss of proprioception*
  - Pigmentary retinopathy
  - Generalized muscle weakness

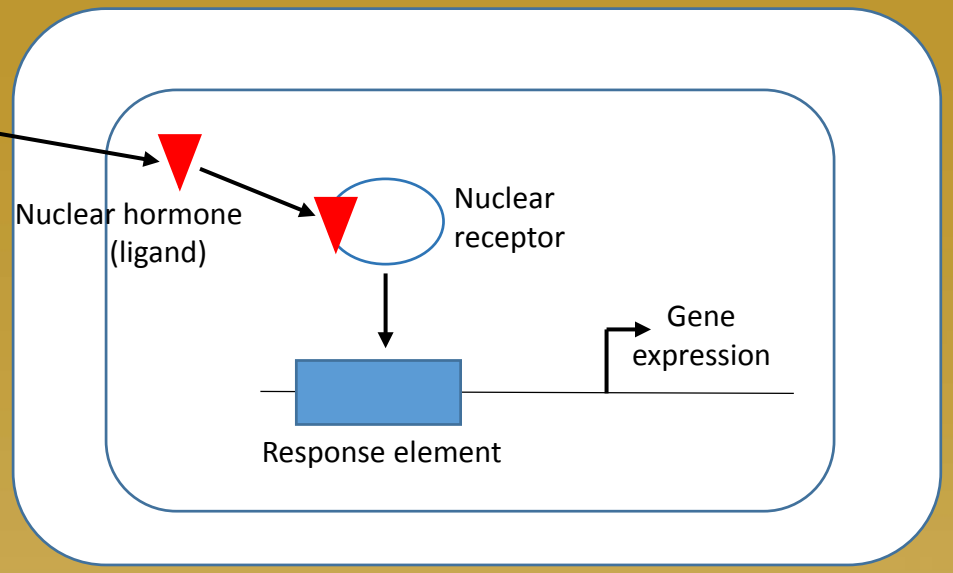
VitE Deficiency

1. Abetalipoproteinemia
2. Other fat malabsorptive conditions
3. Ataxia with VitE Deficiency (AVED)
   - Low serum, CSF, tissue [α-TOH]
   - Rescued with vitE supplementation



GAPS in knowledge

1. What is the origin of the lesion?
2. Is vitE acting as an antioxidant OR modifying gene transcription?
3. How does vitE deficiency lead to sensory deprivation?



Equine Neuroaxonal Dystrophy (eNAD)

Normal Horse

Equine AVED

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eNAD

J Vet Intern Med 2011;25:1439–1446

Equine Degenerative Myeloencephalopathy in Lusitano Horses

C.J. Finno, R.J. Higgins, M. Aleman, R. Ofri, S.R. Hollingsworth, D.L. Bannasch, C.M. Reilly, and J.E. Madigan

J Vet Intern Med 2013;27:177–185

Pedigree Analysis and Exclusion of Alpha-Tocopherol Transfer Protein (*TTPA*) as a Candidate Gene for Neuroaxonal Dystrophy in the American Quarter Horse

C.J. Finno, T. Famula, M. Aleman, R.J. Higgins, J.E. Madigan, and D.L. Bannasch

J Vet Intern Med 2015;29:1667–1675

Blood and Cerebrospinal Fluid α-Tocopherol and Selenium Concentrations in Neonatal Foals with Neuroaxonal Dystrophy

C.J. Finno, K.E. Estell, S. Katzman, L. Winfield, A. Rendahl, J. Textor, D.L. Bannasch, and B. Puschner

Veterinary Ophthalmology (2012) **15**, Supplement 2, 3–7 DOI:10.1111/j.1463-5224.2012.00997.x

BRIEF COMMUNICATION

Electrophysiological studies in American Quarter horses with neuroaxonal dystrophy

Carrie J. Finno,* Monica Aleman,† Ron Ofri,‡ Steven R. Hollingsworth,§ John E. Madigan,** Laramie Winfield,† Danika L. Bannasch,*

The Veterinary Journal 202 (2014) 543–549

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Risk of false positive genetic associations in complex traits with underlying population structure: A case study

Carrie J. Finno [a,*], Monica Aleman [b], Robert J. Higgins [c], John E. Madigan [b], Danika L. Bannasch [a]

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eNAD

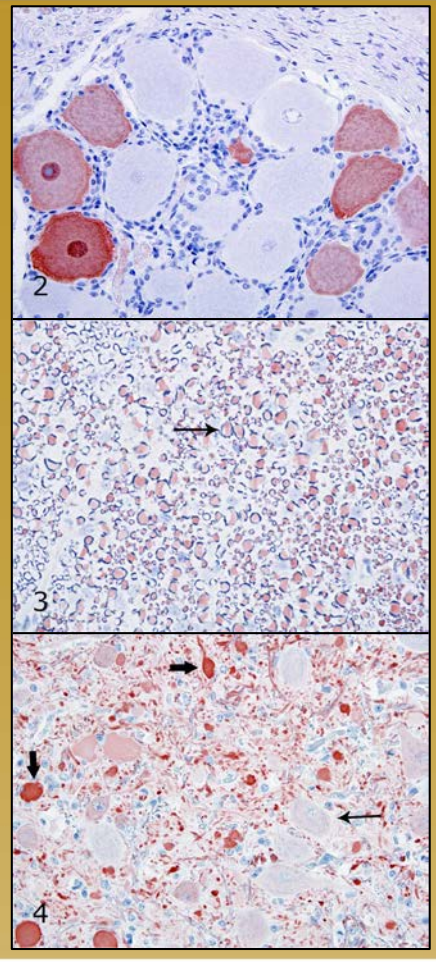
Degenerative and Immune-mediated Disease–Original Article

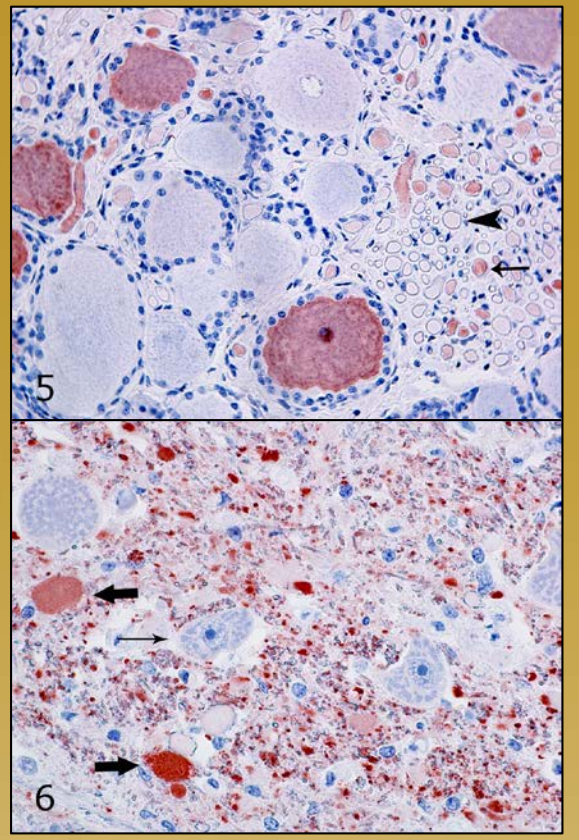
Evidence of the Primary Afferent Tracts Undergoing Neurodegeneration in Horses With Equine Degenerative Myeloencephalopathy Based on Calretinin Immunohistochemical Localization

C. J. Finno[1], S. J. Valberg[1], J. Shivers[2], E. D'Almeida[2], and A. G. Armién[1,2]

Veterinary Pathology
2016, Vol. 53(1) 77-86

DOI: 10.1177/0300985815598787
vet.sagepub.com





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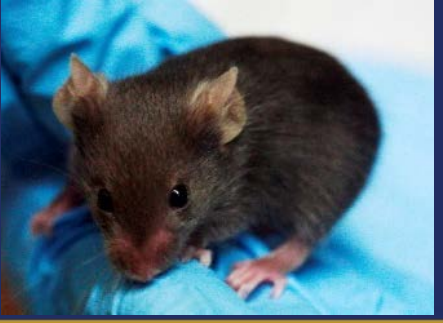
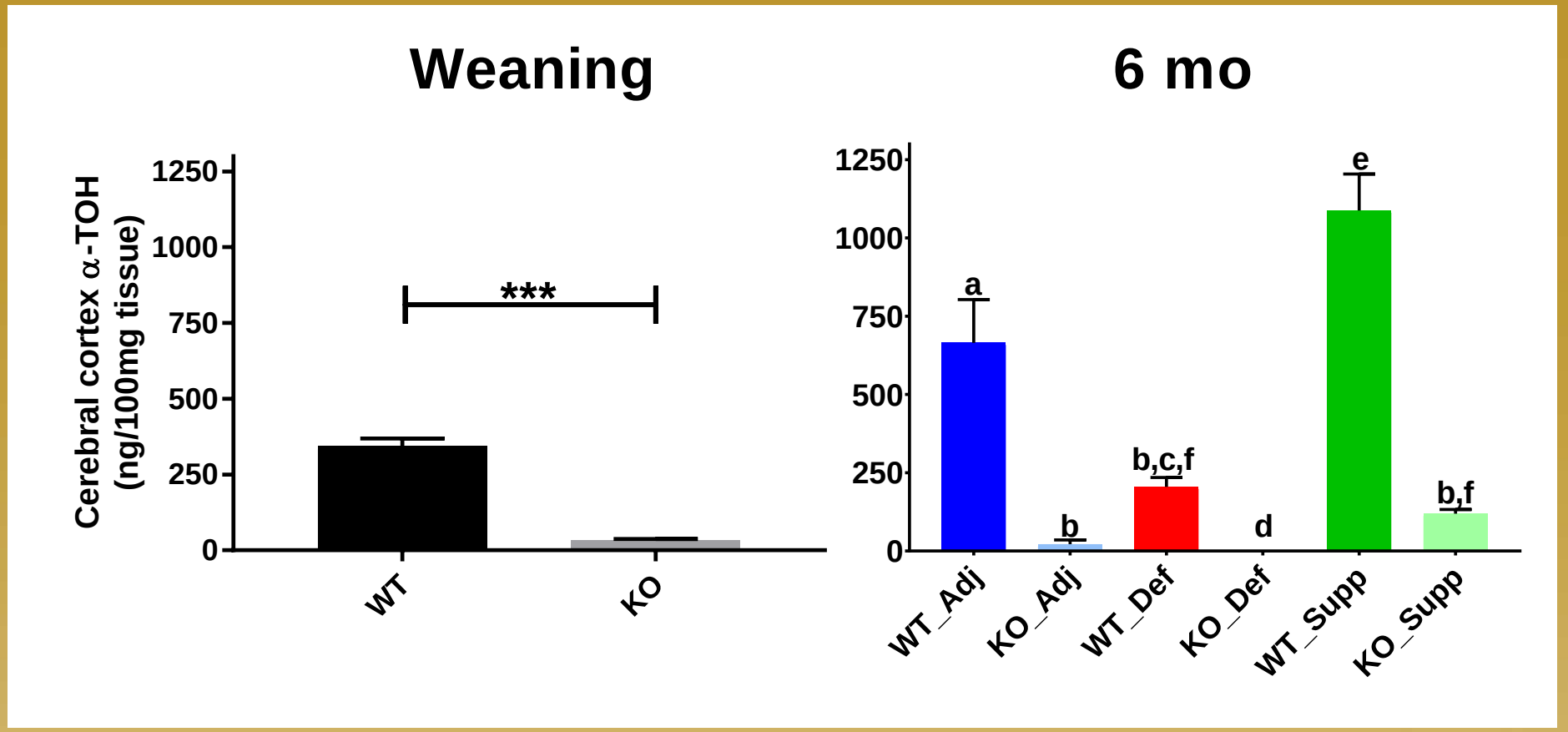


Ttpa$^{-/-}$ mouse model



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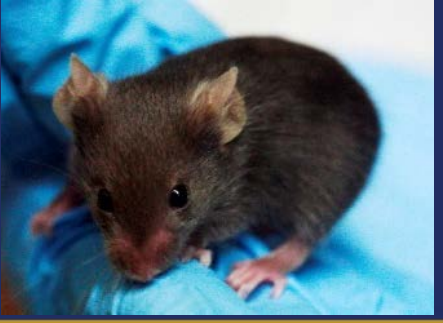
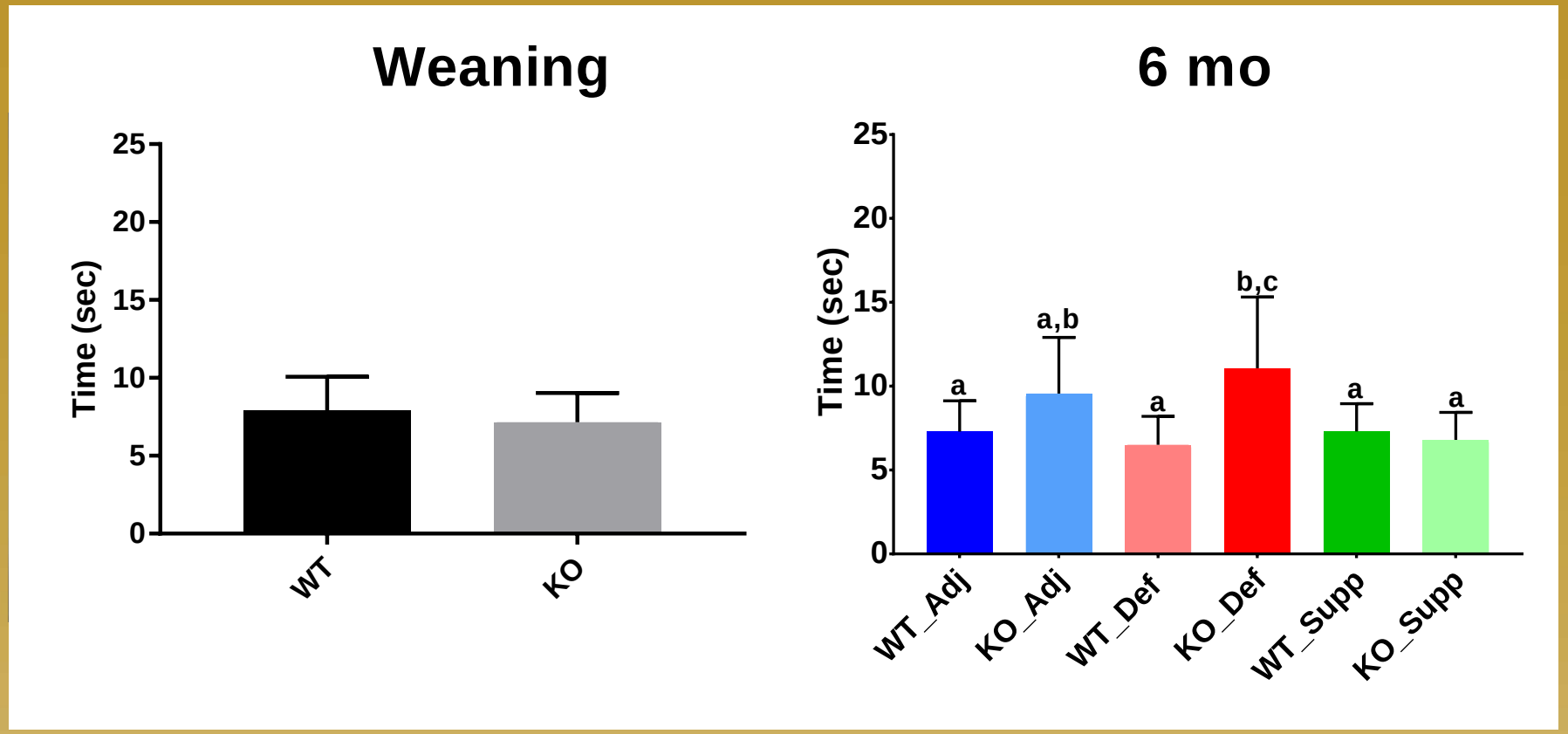


Ttpa$^{-/-}$ mouse model



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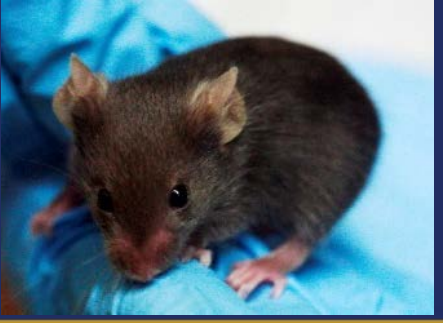
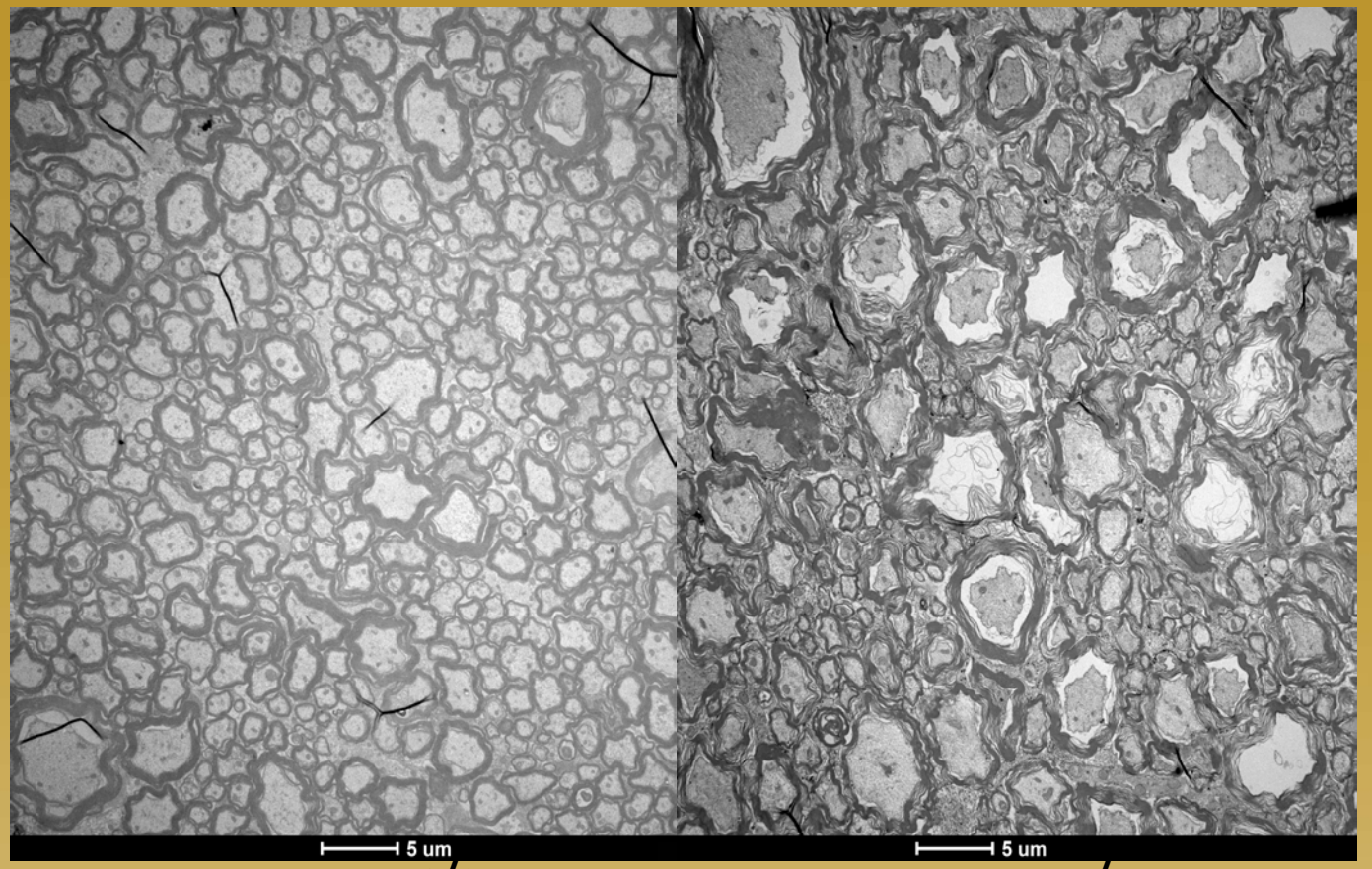


Ttpa$^{-/-}$ mouse model

- Mimics phenotype of AVED
  - 6 months of age
    - Overt proprioceptive deficits
  - 6-12 months: histologic changes
    - Dorsal column degeneration: medulla oblongata and spinal cord
  - 17-20 months
    - Purkinje cell loss
- Rescued with <u>early</u> α-TOH supplementation



***Ttpa*$^{+/+}$** ***Ttpa*$^{-/-}$**

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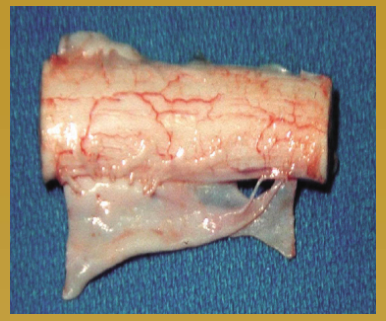
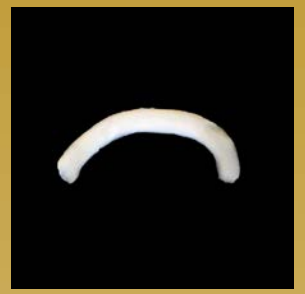
Hypothesis

Transcriptional profiling in eNAD and *Ttpa*$^{-/-}$ mice will demonstrate similar dysregulated pathways associated with α-TOH deficiency

Methods





Identify dysregulated genes and pathways

Test protein function

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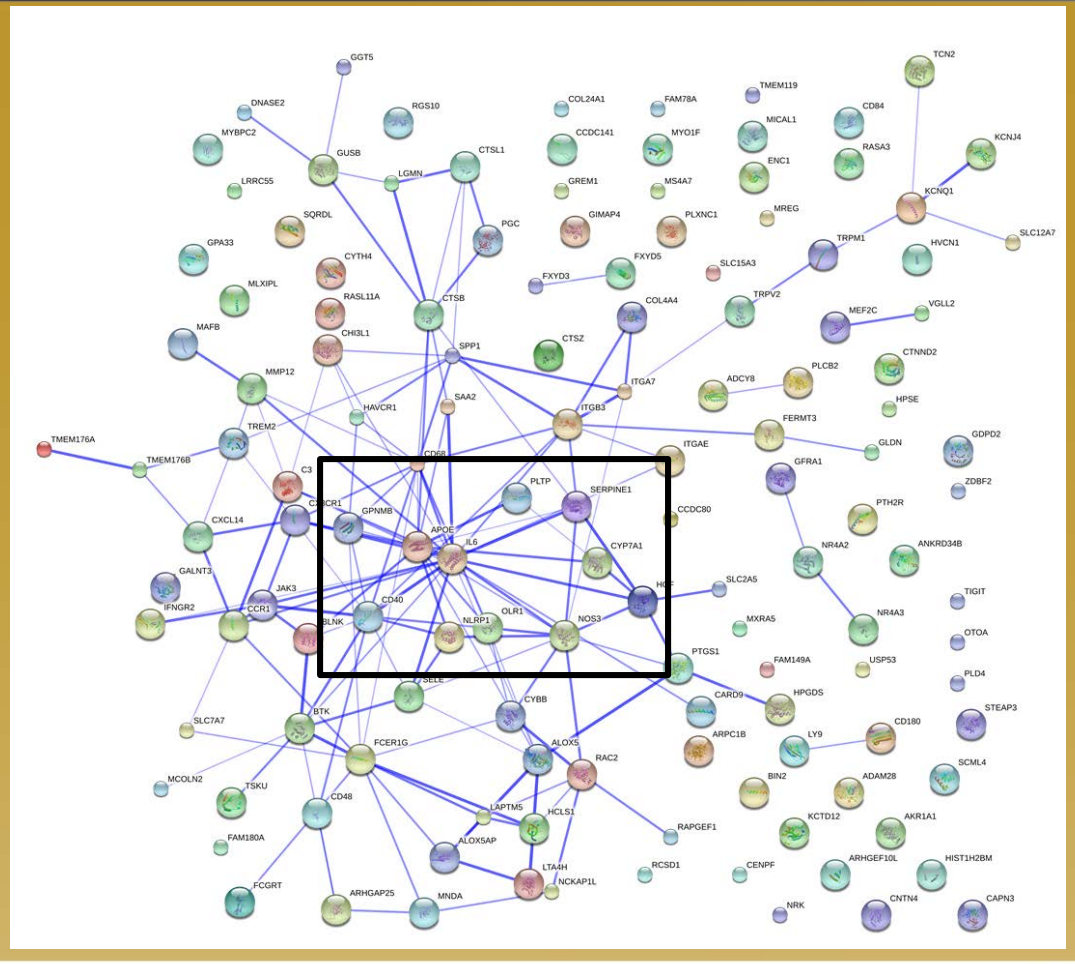
Results: Equine Spinal Cord

- 157 DETs (FDR=0.05)
  - 19 downregulated
  - 138 upregulated



Upregulated DETs

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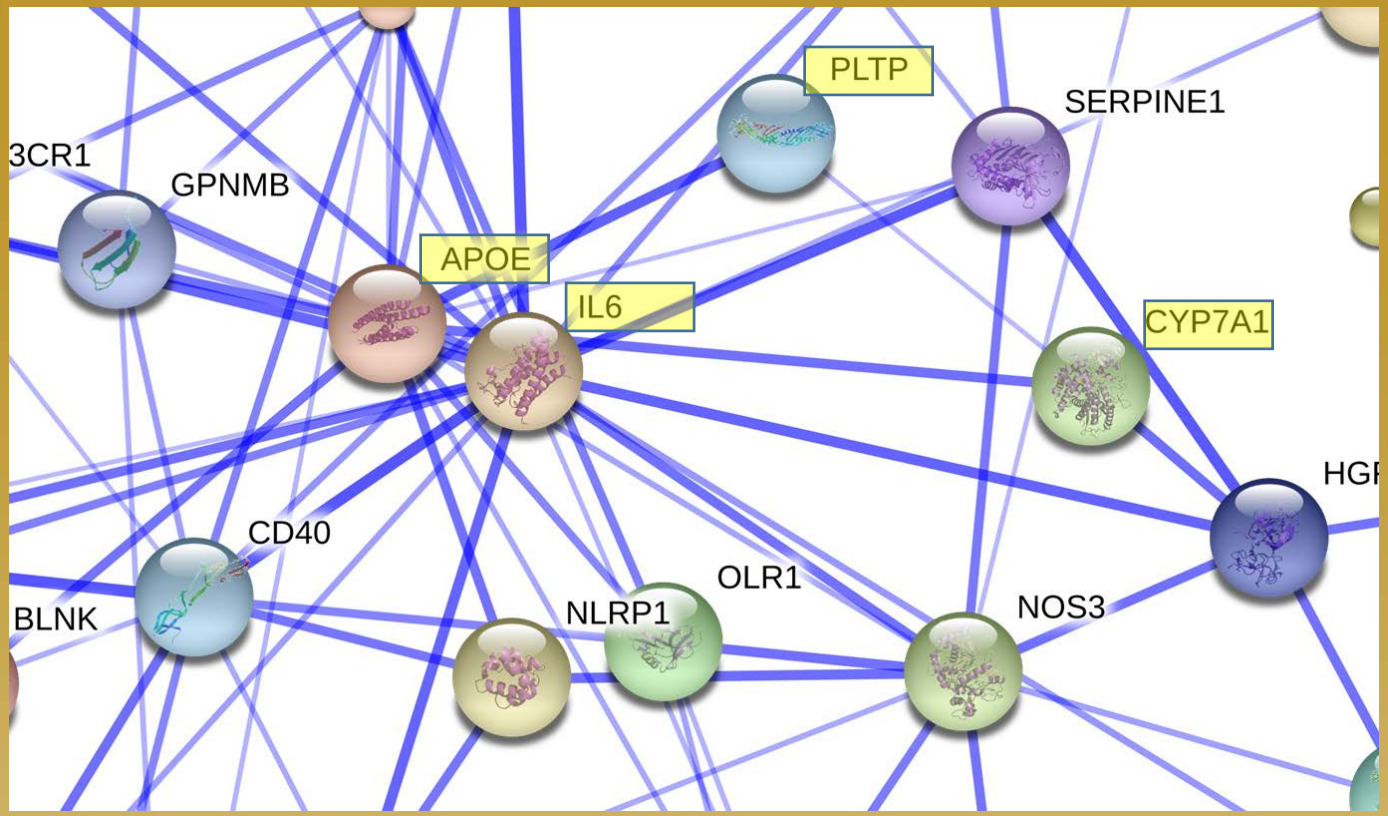
Results: Equine



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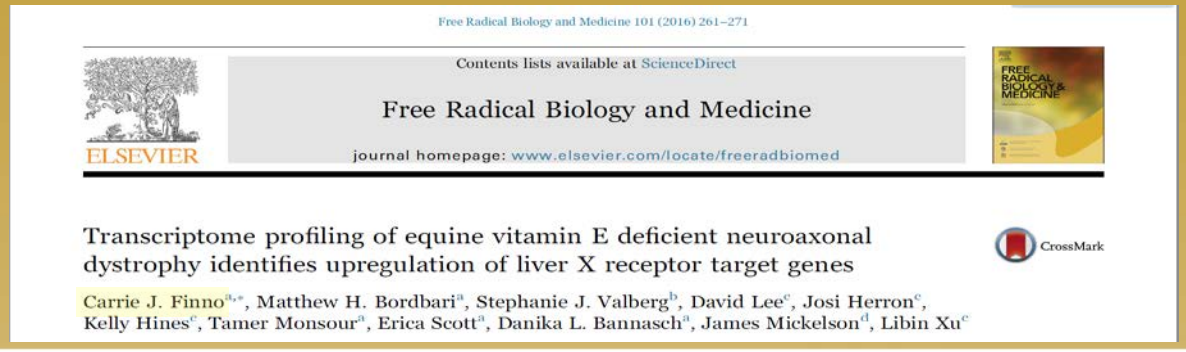
Liver X Receptor Targeted Genes

Liver X Receptor (LXR)

- Nuclear receptor : reverse cholesterol transport
  - Cellular response to oxysterols = LXR activation and decrease cholesterol biosynthesis
  - Upregulated oxidized lipoprotein receptor (*OLR1*)



Free Radical Biology and Medicine 101 (2016) 261–271

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Transcriptome profiling of equine vitamin E deficient neuroaxonal dystrophy identifies upregulation of liver X receptor target genes

Carrie J. Finno[a,*], Matthew H. Bordbari[a], Stephanie J. Valberg[b], David Lee[c], Josi Herron[c], Kelly Hines[c], Tamer Monsour[a], Erica Scott[a], Danika L. Bannasch[a], James Mickelson[d], Libin Xu[c]

Pathway	Up or down-regulated	$P_{Bonferroni}$
Ionotropic glutamate receptor pathway	-	7.7×10^{-5}
Synaptic vesicle trafficking	-	0.002
Metabotropic glutamate receptor group III pathway	-	0.009
Cholesterol biosynthesis	-	0.02

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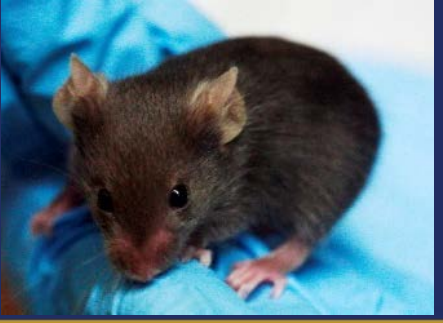
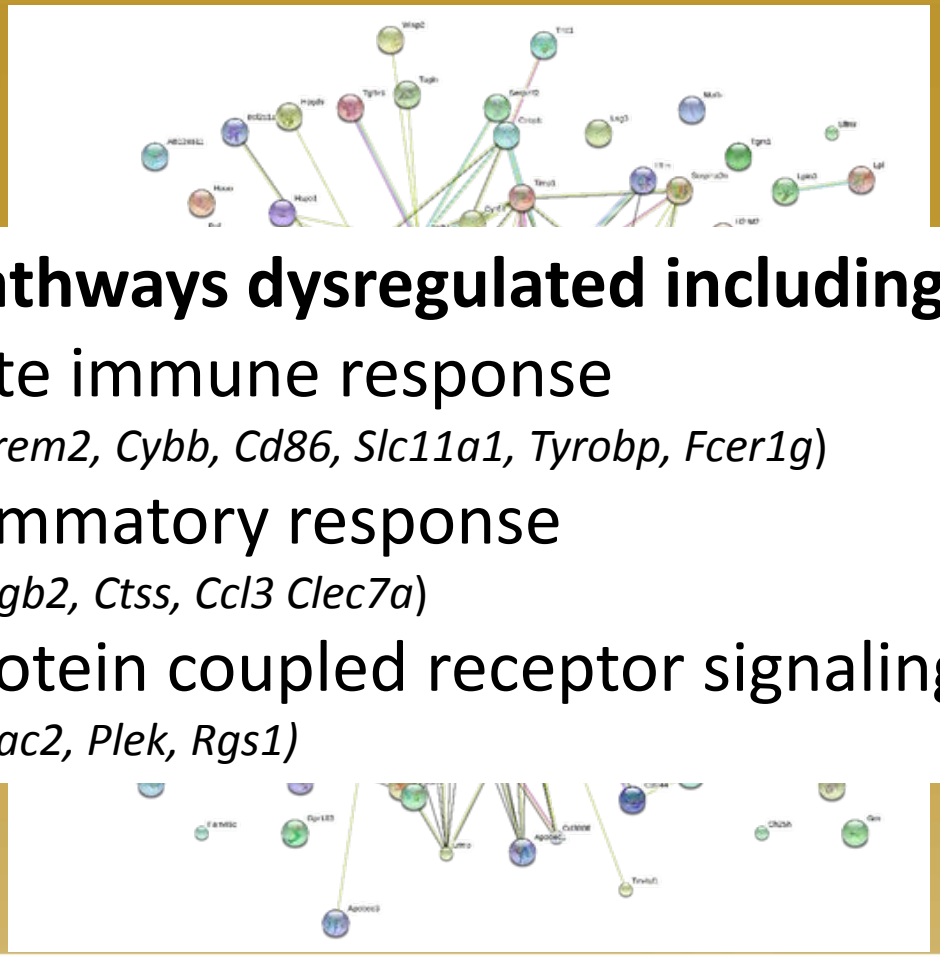
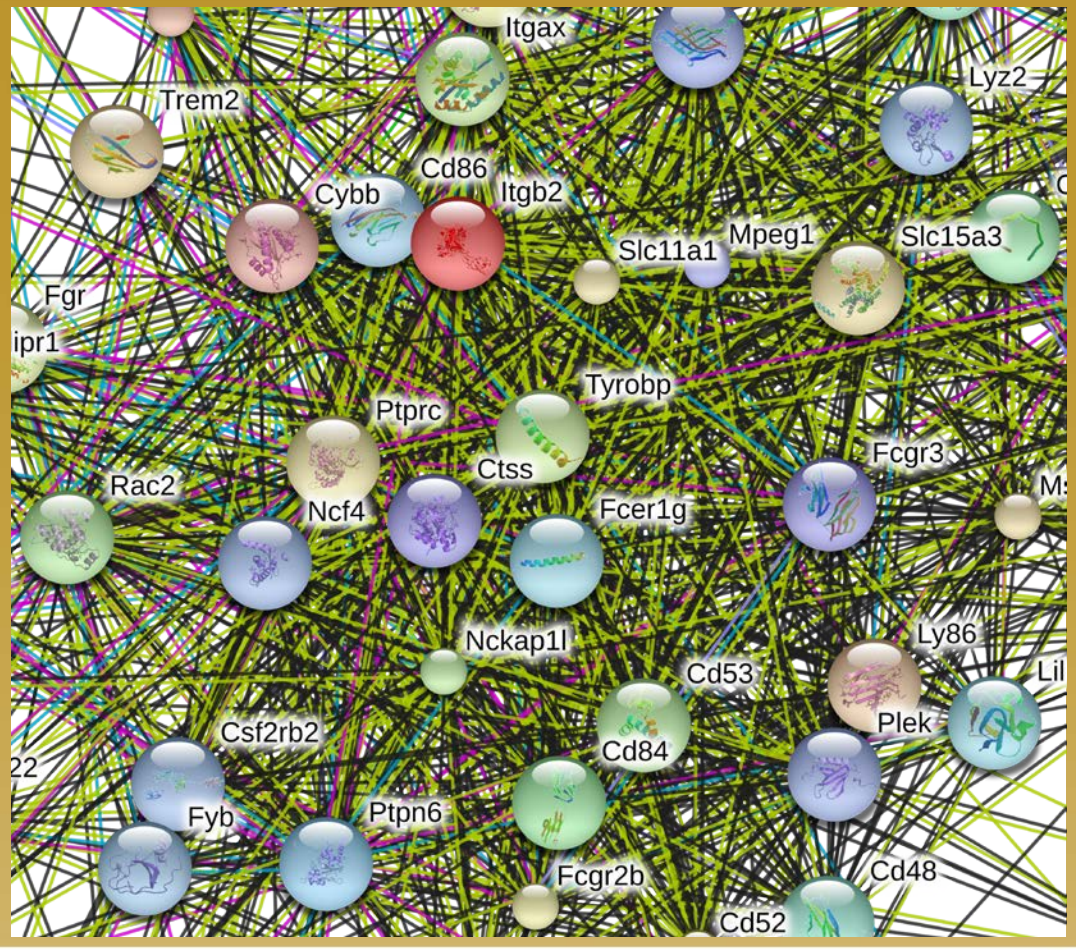


Results: 6 mo VitE- : KO vs WT



21 pathways dysregulated including:

- Innate immune response
  - (*Trem2, Cybb, Cd86, Slc11a1, Tyrobp, Fcer1g*)
- Inflammatory response
  - (*Itgb2, Ctss, Ccl3 Clec7a*)
- G-protein coupled receptor signaling
  - *(Rac2, Plek, Rgs1)*



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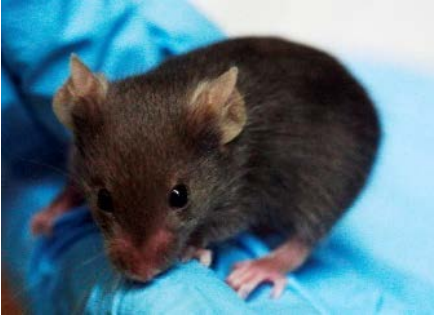
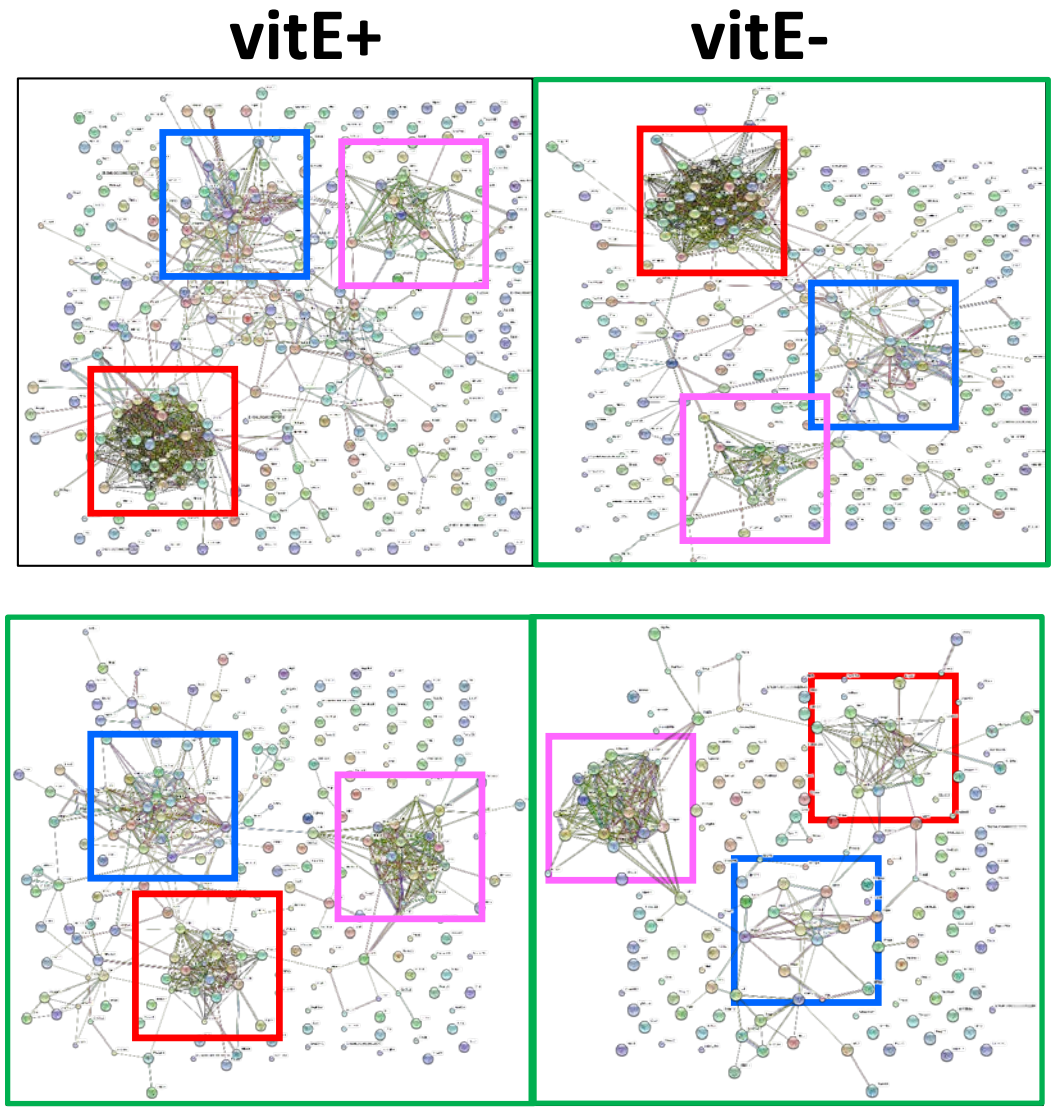


DOWNREGULATED
Weaning → 6 mo

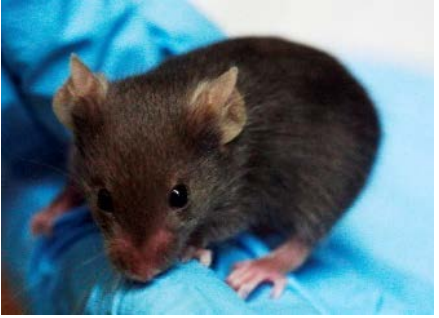


Cell cycle / Mitosis
(Aurkb, Ube2c, Cenpa)

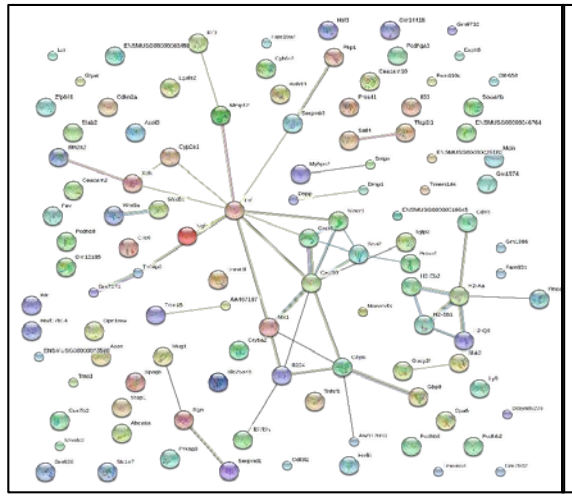
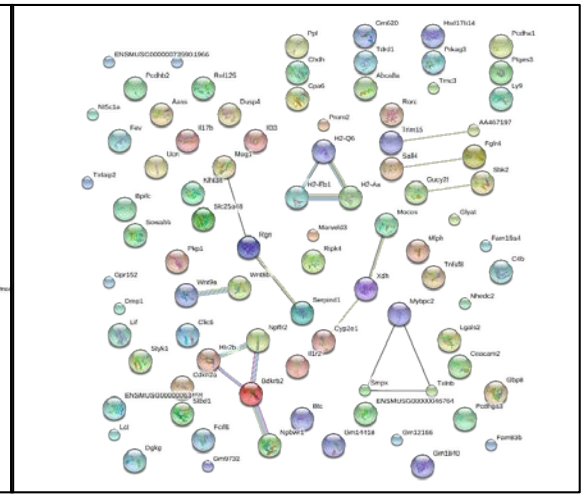
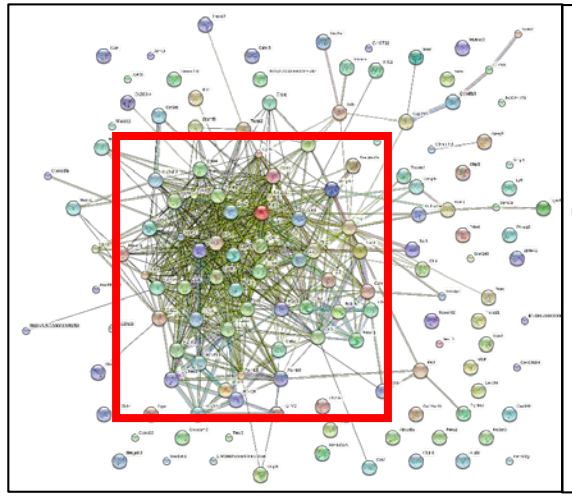
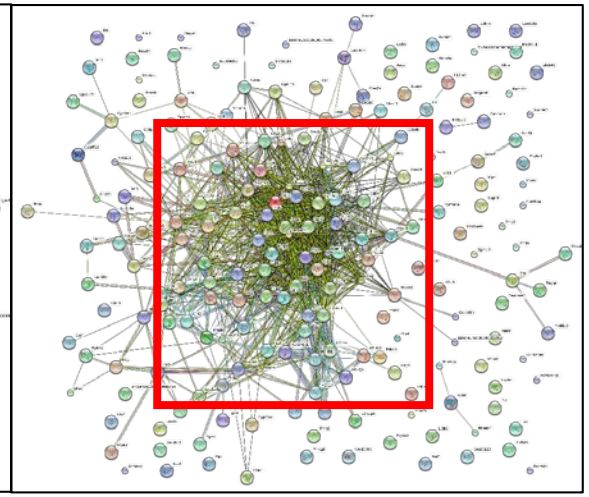
Collagen *(Col3a1, Col1a1, Col9a3)*

Cholesterol Biosynthesis
(Sqle, Dhcr7, Mvk)

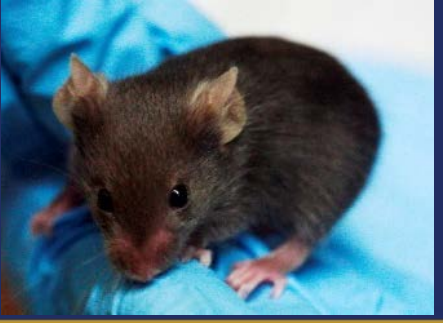
Myelination
(Cntnap1, Mbp, Plp1)



UPREGULATED
Weaning → 6 mo

	vitE+	vitE-
WT		
KO		

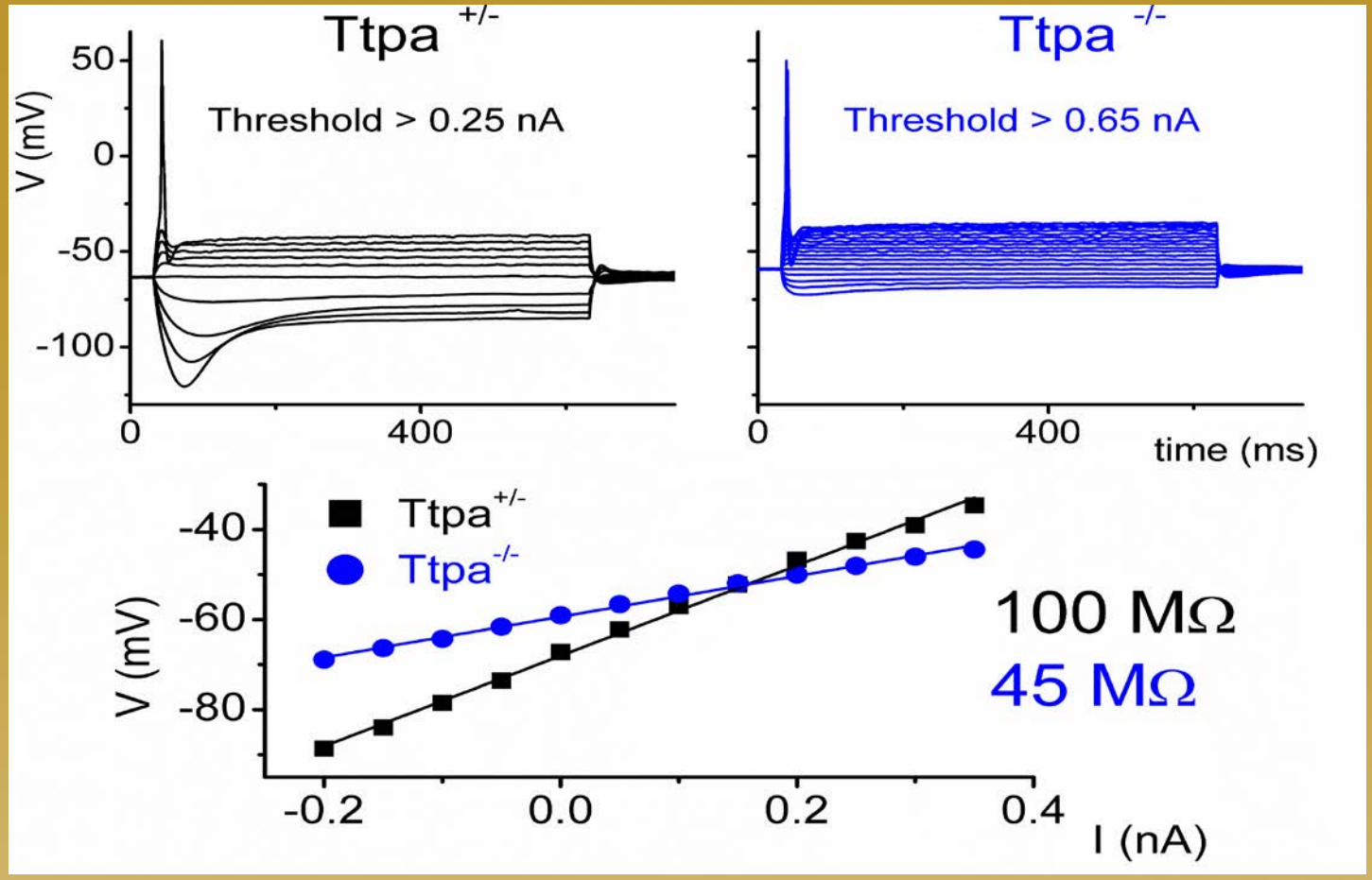
Inflammatory Response
(Itgb2, Ctss, Ccl3 Clec7a)



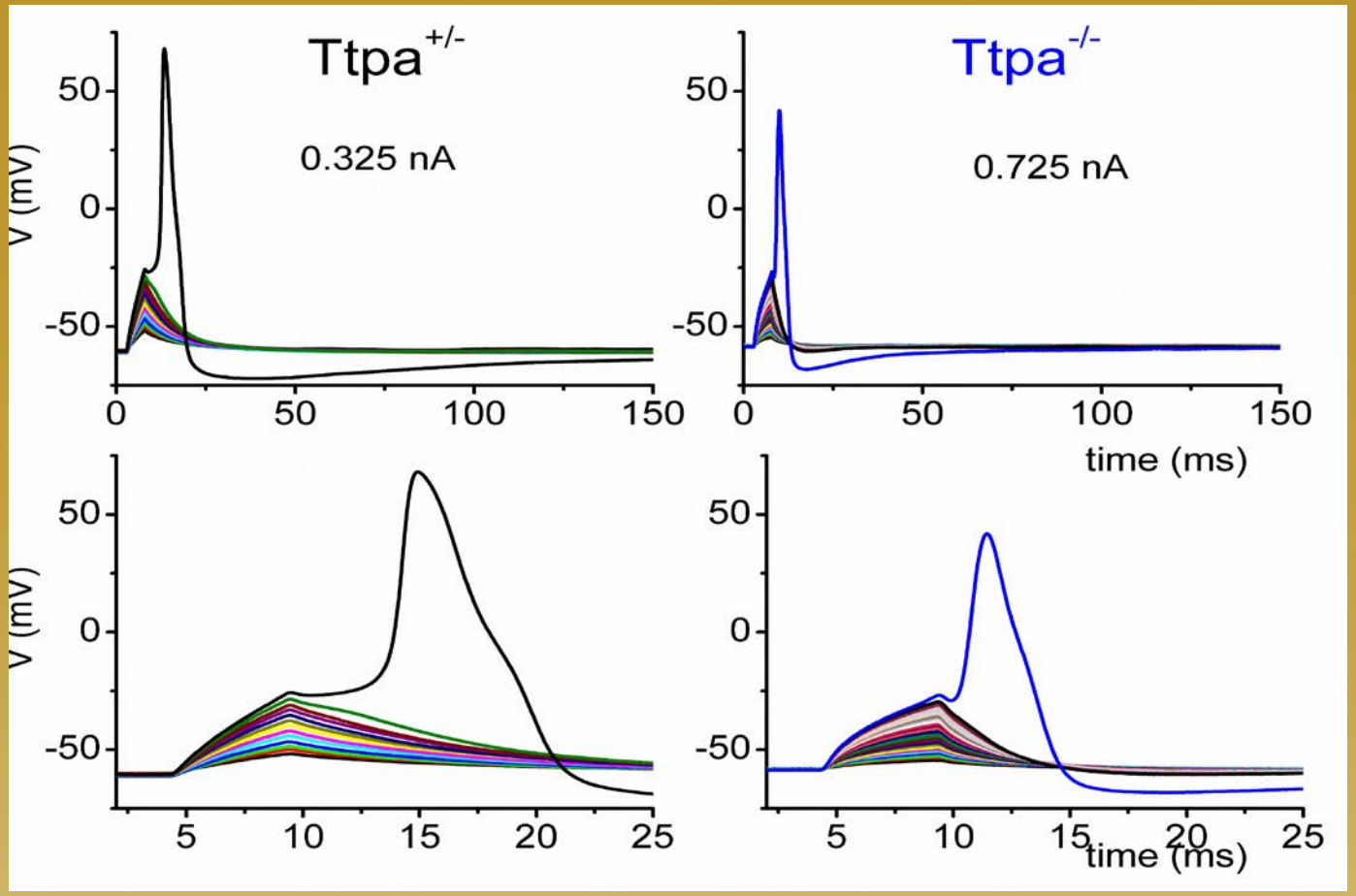
VitE : Nuclear Receptor Activation (Wean → 6 mo)

- Sufficient vitE: Retinoid orphan related receptor (*Rora*) ACTIVATED
  - Ligand= Cholesterol
  - Synaptic maintenance (*ITPR1*)
  - Antioxidant proteins (glutathione peroxidase 1, peroxiredoxin 6)
- Insufficient vitE: Liver X receptor ACTIVATED
  - Ligand= Oxidized cholesterol
  - Lipid homeostasis
  - INACTIVATES *Rora*

Input resistance of Ttpa$^{-/-}$ DRG neurons plummets by ~2 fold



Membrane excitability of Ttpa$^{-/-}$ DRG neurons is reduced



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Membrane excitability of Ttpa$^{-/-}$ DRG neurons is reduced

	Ttpa $^{-/+}$ (n=9)		*Ttpa* $^{-/-}$ (n=9)		
	Mean	SD	Mean	Sd	*P*
RMP	-61.34	3.21	-62.17	3.89	0.75
Threshold	-33.28	2.87	-35.46	3.13	0.34
Amplitude	112.97	6.77	94.59	7.56	0.01
AP_{50}	4.51	0.34	2.45	0.21	0.0001
AHP	13.67	2.14	9.5	1.66	0.02

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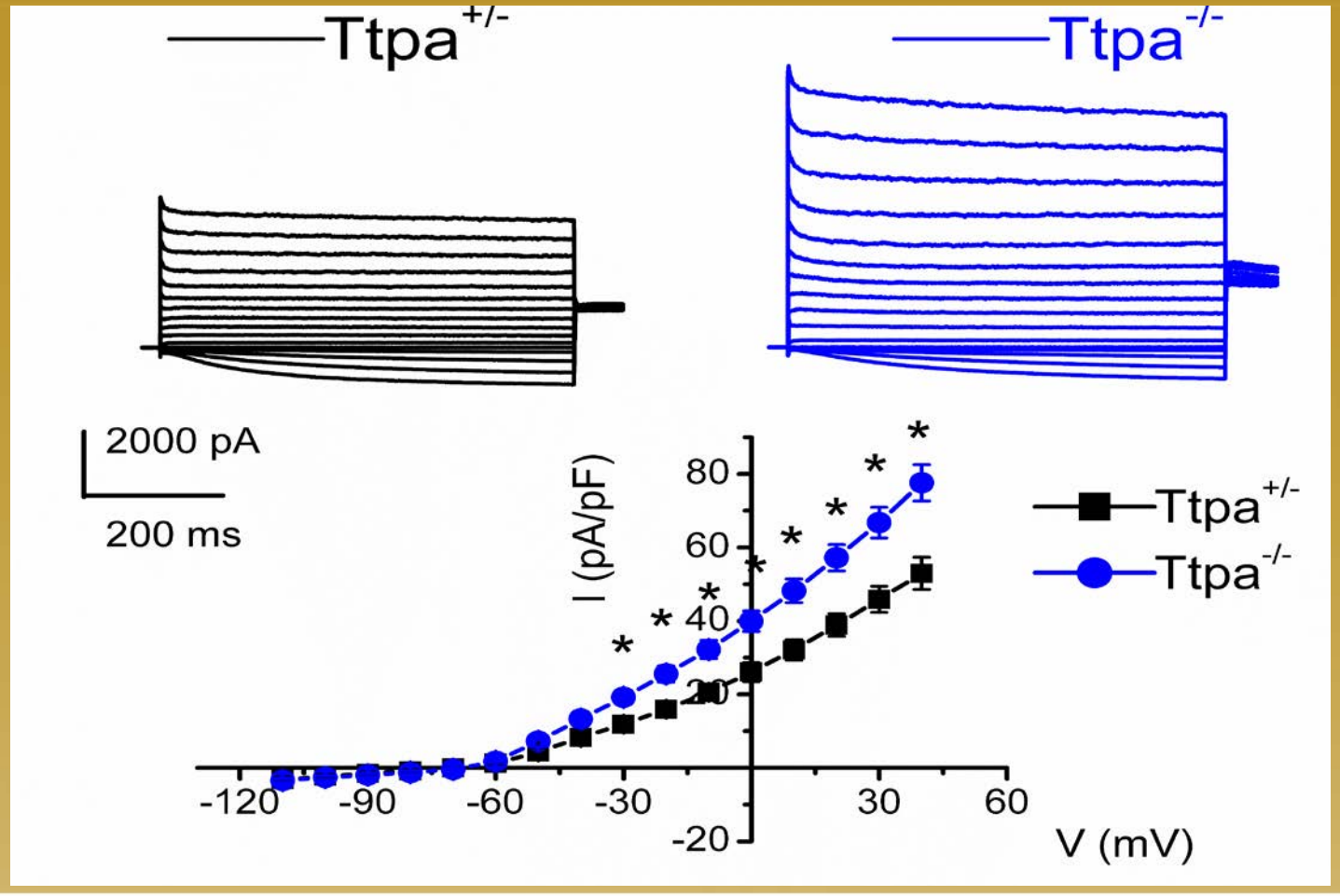
Enhanced outward K^+ currents in *Ttpa*$^{-/-}$ neurons



Future Goals

1. Determine identity of enhanced K^+ channels
2. Determine mechanism of enhancement

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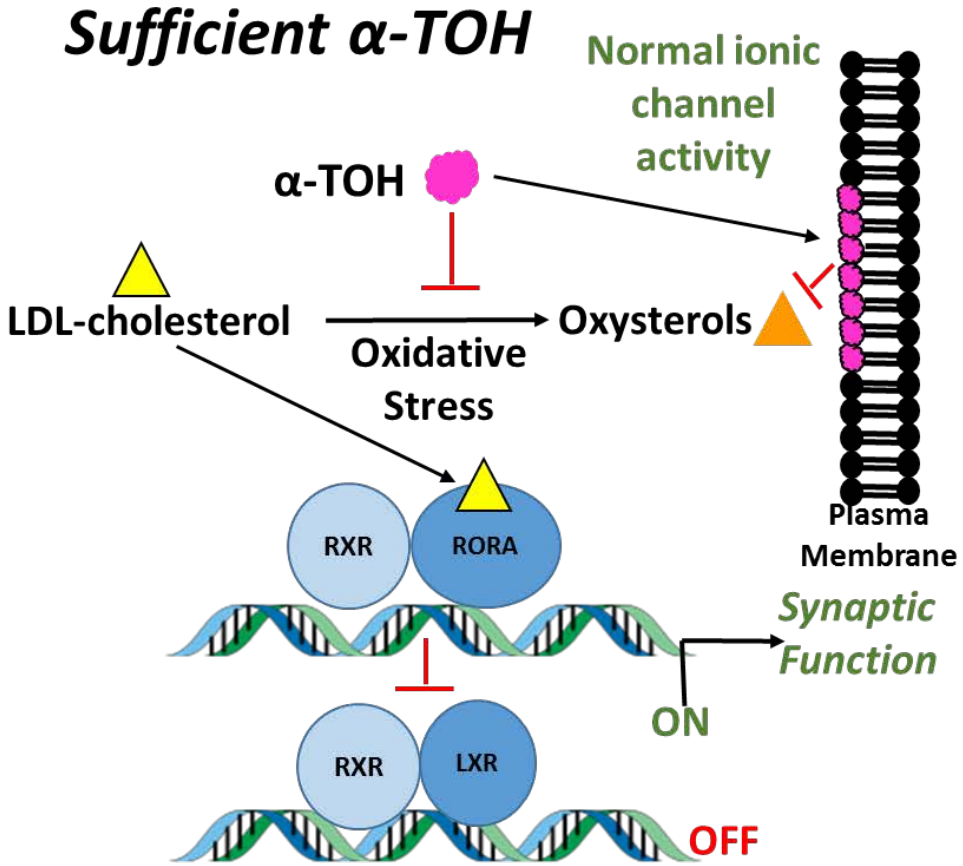
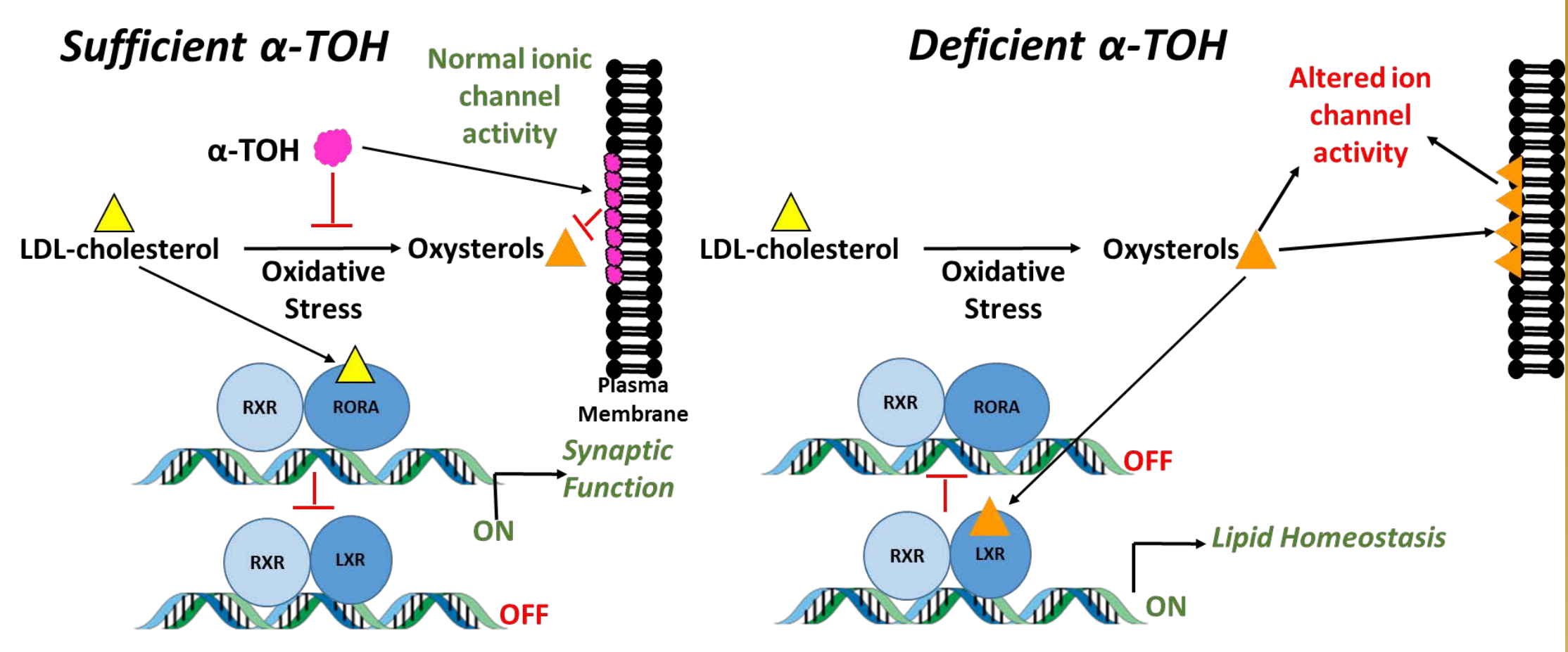
Proposed Molecular Mechanism of VitE



Proposed Molecular Mechanism of VitE



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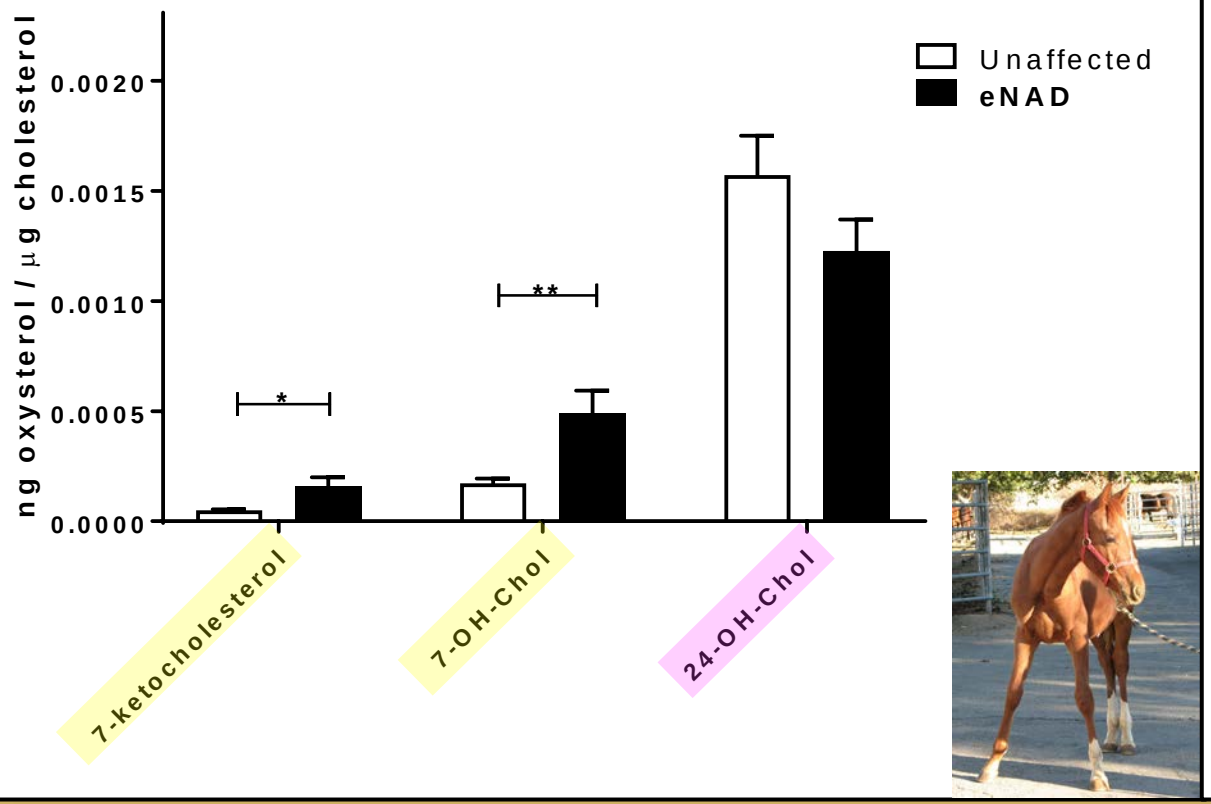
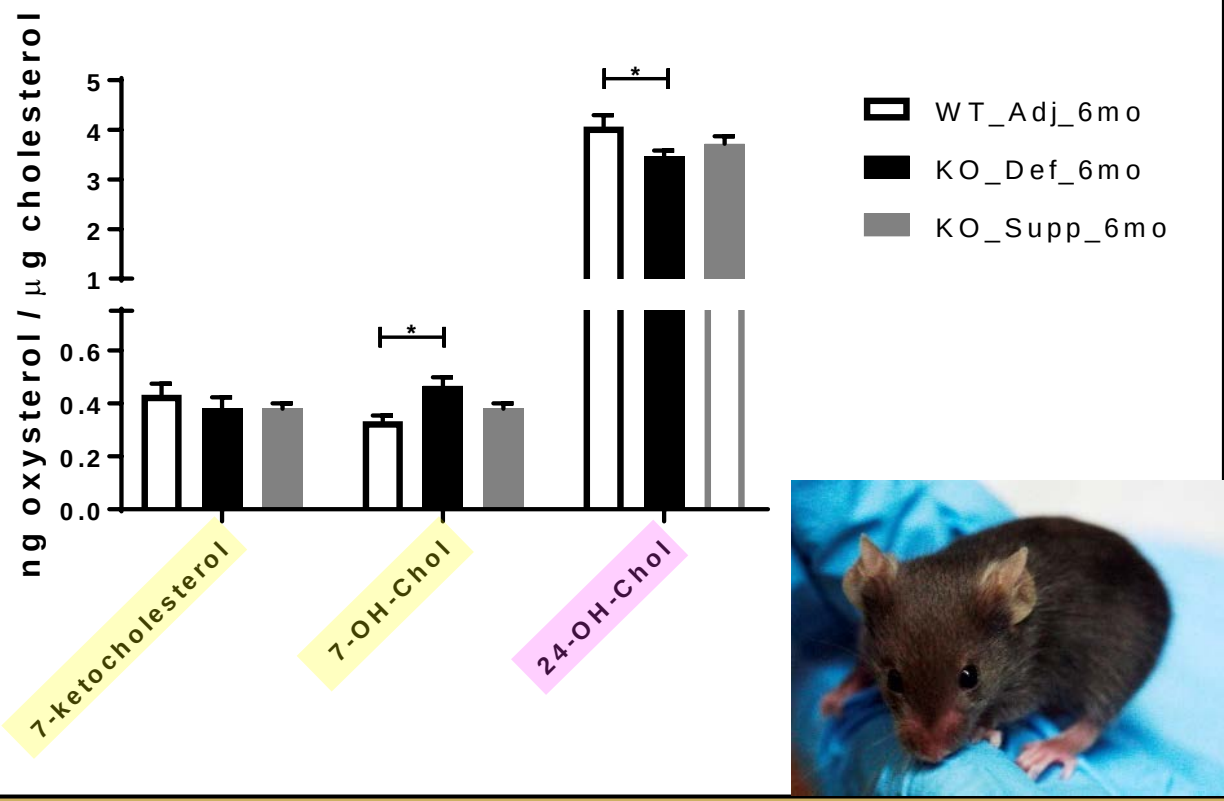
Spinal Cord Oxysterols





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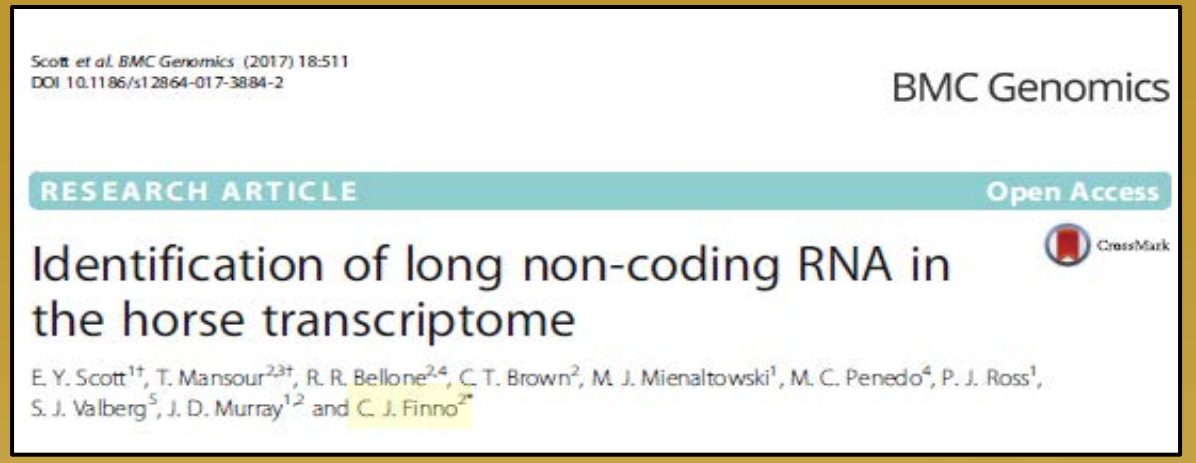
Produced by ROS | **Produced by enzymatic activity (CYP46A1)**

Conclusions

- Postnatal development
  - Role of α-TOH in modulating the inflammatory response
  - Decreased myelination with vitE insufficiency
  - Nuclear receptor activation affected by α-TOH concentrations
    - Sufficient vitE : RORA
    - Insufficient vitE: LXR
  - Reduced DRG membrane activity with vitE deficiency
- Molecular dysregulation may lead to neurologic phenotype

ORIP K01 Support (2013-2018)

- 18 (of 37) publications
- 55 research abstracts
- 20 invited presentations
- Additional grants (as PI)
  - 3 Extramural
  - 6 Intramural
- 4 graduate students trained

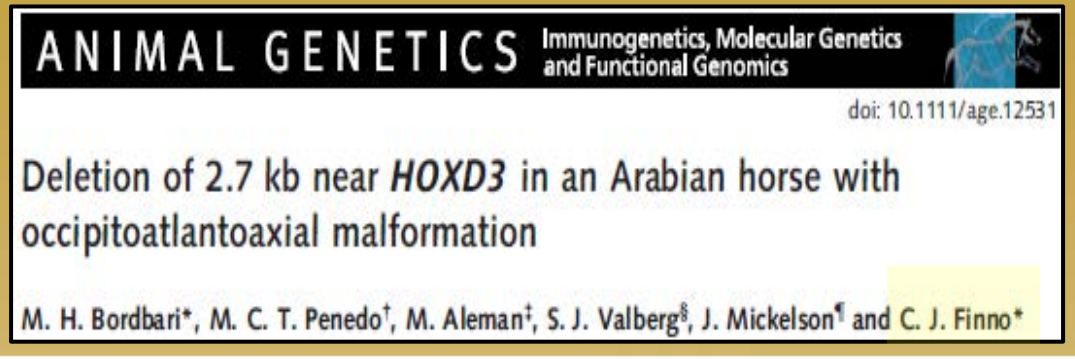

Scott et al. BMC Genomics (2017) 18:511
DOI 10.1186/s12864-017-3884-2

BMC Genomics

RESEARCH ARTICLE Open Access

Identification of long non-coding RNA in the horse transcriptome

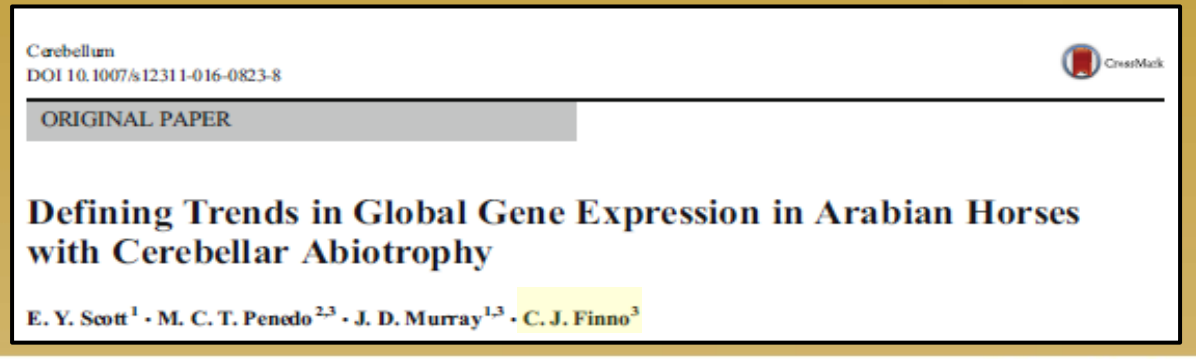
E. Y. Scott[1†], T. Mansour[2,3†], R. R. Bellone[2,4], C. T. Brown[2], M. J. Mienaltowski[1], M. C. Penedo[4], P. J. Ross[1], S. J. Valberg[5], J. D. Murray[1,2] and C. J. Finno[2*]


ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

doi: 10.1111/age.12531

Deletion of 2.7 kb near *HOXD3* in an Arabian horse with occipitoatlantoaxial malformation

M. H. Bordbari*, M. C. T. Penedo†, M. Aleman‡, S. J. Valberg§, J. Mickelson¶ and C. J. Finno*


Cerebellum
DOI 10.1007/s12311-016-0823-8

ORIGINAL PAPER

Defining Trends in Global Gene Expression in Arabian Horses with Cerebellar Abiotrophy

E. Y. Scott[1] · M. C. T. Penedo[2,3] · J. D. Murray[1,3] · C. J. Finno[3]

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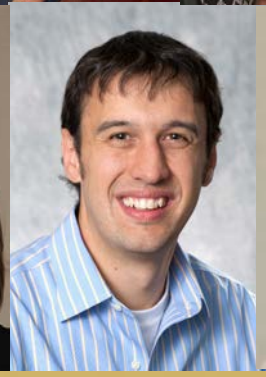
Acknowledgments

- NIH 1K01OD015134
  - Supplemental Award: Office of Dietary Supplements
- NIH LRP L40 TR001136
- Morris Animal Foundation D14EQ-021
- UMN Equine Center
- UCD Center for Equine Health



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