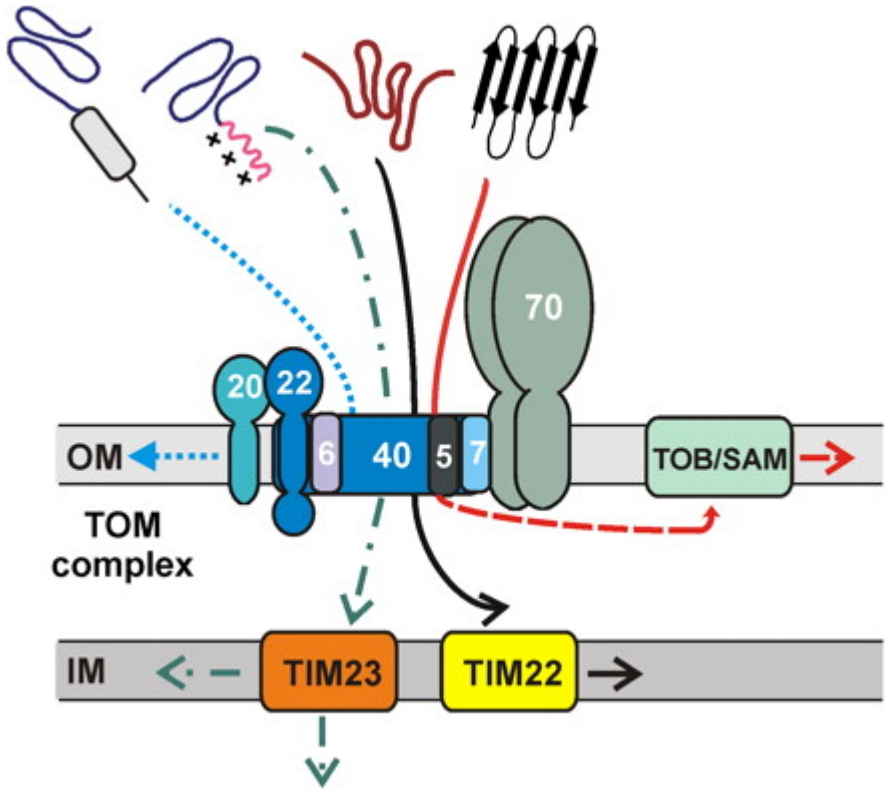
Tom40 and the mt(UPR)

Sonal Gagrani

Gottschalk Lab

TOMM40

- encodes for the channel-forming subunit of a translocase in the outer mitochondrial membrane (Tom) needed for protein transport into mitochondria
- has S (short), L (long) and VL (very long) alleles depending on the length of a poly-T polymorphisms in intron 6
- mean expression of Tom40 due to VL allele is higher than S allele



An Inherited Variable Poly-T Repeat Genotype in *TOMM40* in Alzheimer Disease

Allen D. Roses, MD

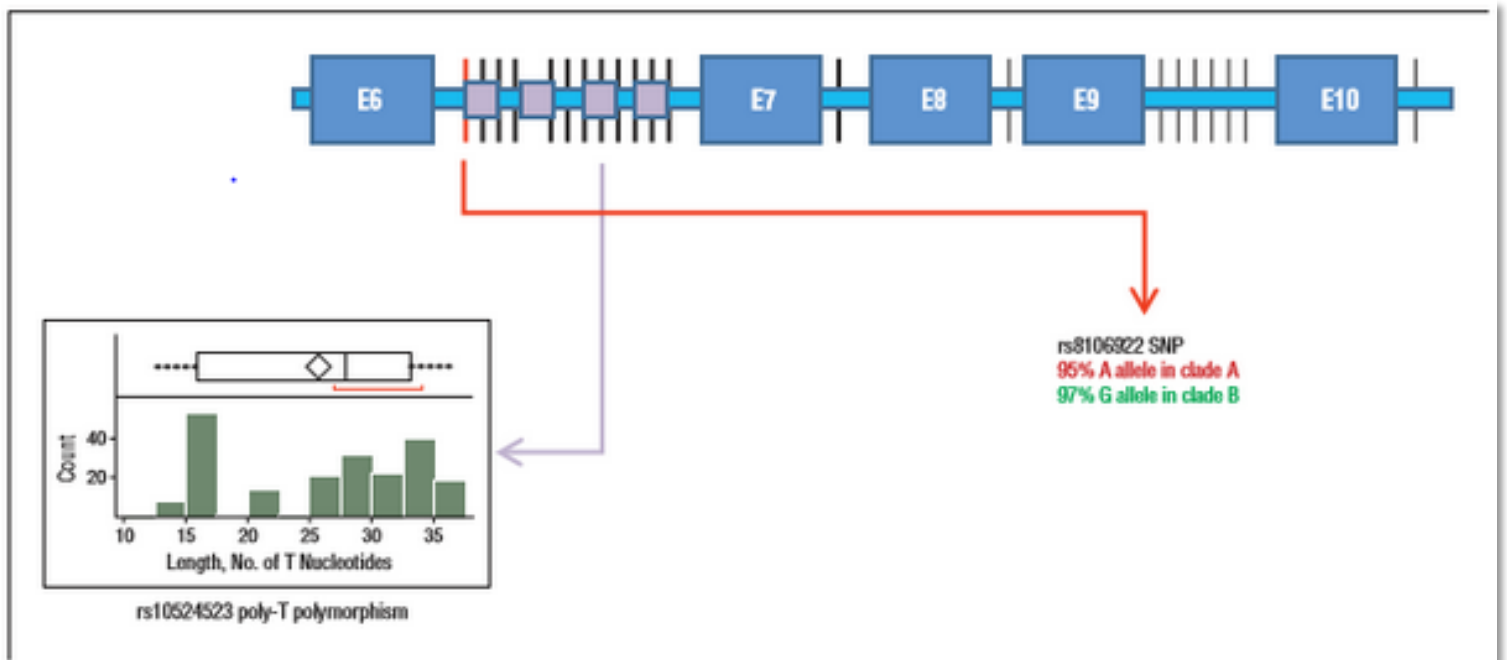


Figure 1. The 10-kilobase region of *TOMM40* included in the phylogenetic analysis. Shown are exons 6 through 10 (E6-E10) of *TOMM40* and intervening introns. Vertical lines indicate single nucleotide polymorphisms (SNPs), and small boxes indicate poly-T variants.

A *TOMM40* variable-length polymorphism predicts the age of late-onset Alzheimer's disease

AD Roses[1,2], MW Lutz[1,2],
H Amrine-Madsen[3],
AM Saunders[1,2], DG Crenshaw[1,2],
SS Sundseth[1,2], MJ Huentelman[4],
KA Welsh-Bohmer[1,5] and
EM Reiman[4,6,7]

The ε4 allele of the apolipoprotein E (*APOE*) gene is currently the strongest and most highly replicated genetic factor for risk and age of onset of late-onset Alzheimer's disease (LOAD). Using phylogenetic analysis, we have identified a polymorphic poly-T variant, rs10524523, in the translocase of outer mitochondrial membrane 40 homolog (*TOMM40*) gene that provides greatly increased precision in the estimation of age of LOAD onset for *APOE* ε3 carriers. In two independent clinical cohorts, longer lengths of rs10524523 are associated with a higher risk for LOAD. For *APOE* ε3/4 patients who developed LOAD after 60 years of age, individuals with long poly-T repeats linked to *APOE* ε3 develop LOAD on an average of 7 years earlier than individuals with shorter poly-T repeats linked to *APOE* ε3 (70.5 ± 1.2 years versus 77.6 ± 2.1 years, $P = 0.02$, $n = 34$). Independent mutation events at rs10524523 that occurred during Caucasian evolution have given rise to multiple categories of poly-T length variants at this locus. On replication, these results will have clinical utility for predictive risk estimates for LOAD and for enabling clinical disease prevention studies. In addition, these results show the effective use of a phylogenetic approach for analysis of haplotypes of polymorphisms, including structural polymorphisms, which contribute to complex diseases.

The *cis*-regulatory effect of an Alzheimer's disease-associated poly-T locus on expression of *TOMM40* and apolipoprotein E genes

Colton Linnertz[a], Lauren Anderson[a], William Gottschalk[a,b], Donna Crenshaw[a,b], Michael W. Lutz[a,b], Jawara Allen[d], Sunita Saith[d], Mirta Mihovilovic[a], James R. Burke[a,b], Kathleen A. Welsh-Bohmer[b], Allen D. Roses[a,b,c], Ornit Chiba-Falek[a,b,d,*]

Results: The expression of both genes was significantly increased with disease. Mean expression of *APOE* and *TOMM40* mRNA levels were higher in VL homozygotes compared with S homozygotes in the temporal and occipital cortexes from normal and LOAD cases. Results of a luciferase reporter system were consistent with the human brain mRNA analysis; the 523 VL poly-T resulted in significantly higher expression than the S poly-T. Although the effect of poly-T length on reporter expression was the same in HepG2 hepatoma and SH-SY5Y neuroblastoma cells, the magnitude of the effect was greater in the neuroblastoma than in the hepatoma cells, which implies tissue-specific modulation of the 523 poly-T.

mt(UPR)

- a stress response of the mitochondria that activates transcription of nuclear encoded mitochondrial chaperone and protease genes
- to maintain protein homeostasis and avoid autophagy or apoptosis

REVIEW

The mitochondrial unfolded protein response, a conserved stress response pathway with implications in health and disease

Virginija Jovaisaite*, Laurent Mouchiroud* and Johan Auwerx‡

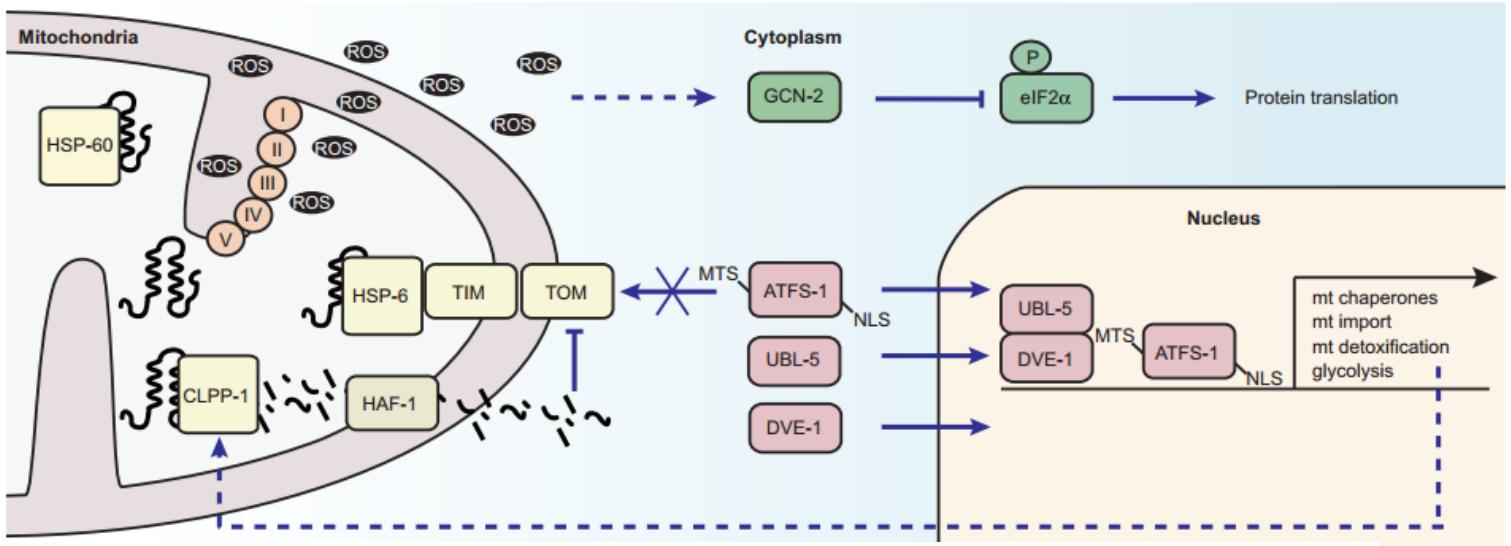


Fig. 1. Overview of the mitochondrial unfolded protein response (UPR^{mt}) signaling in *Caenorhabditis elegans*.

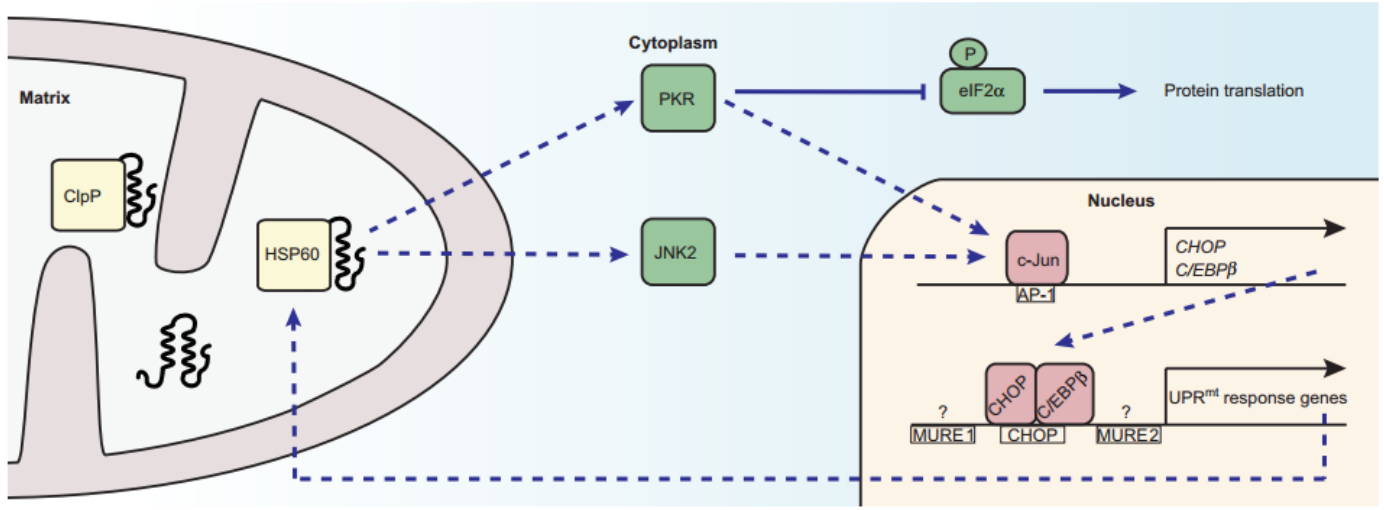


Fig. 2. Overview of the mitochondrial matrix UPR^{mt} in mammals. Upon mitochondrial stress, c-Jun N-terminal kinase (JNK)2 and dsRNA-activated protein kinase (PKR) contribute to the activation of transcription factor c-Jun, which binds to AP-1 elements and activates transcription of the transcription factors CHOP and C/EBPβ. Dimers of CHOP and C/EBPβ then bind to the CHOP element on the promoter of UPR^{mt} genes that encode mitochondrial protein quality control proteins, such as HSP60 and ClpP, and mitochondrial import components. Two other conserved elements in promoters of UPR^{mt} response genes, MURE1 and MURE2, are presumably bound by factors that have not yet been identified. PKR inhibits cytosolic protein translation by mediating phosphorylation of eIF2α.

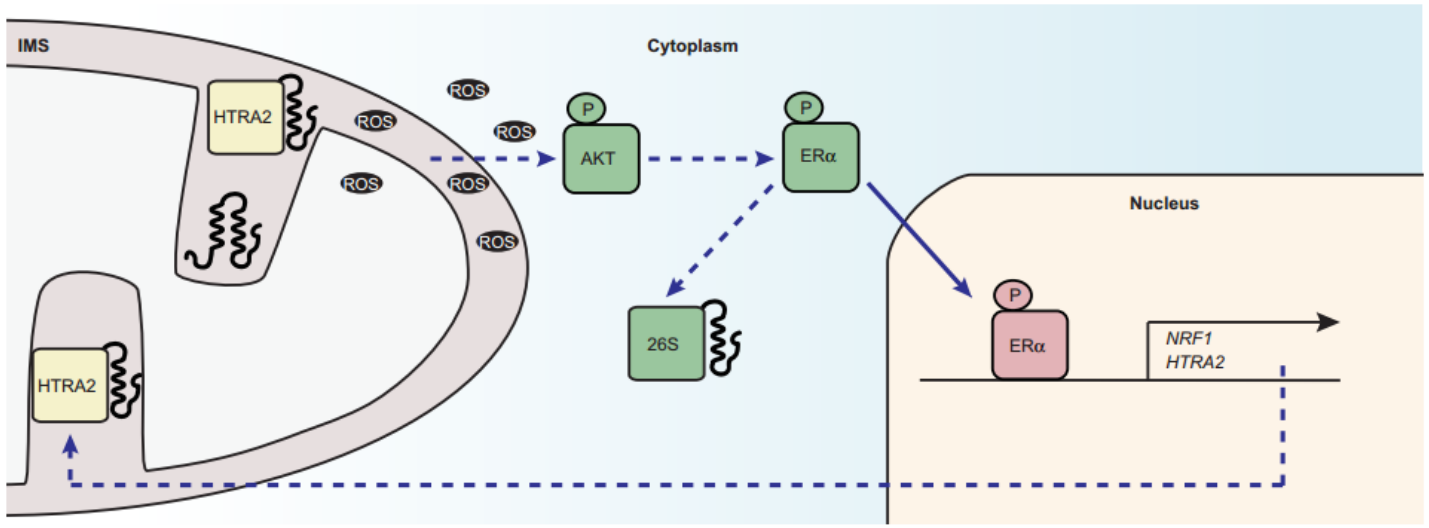


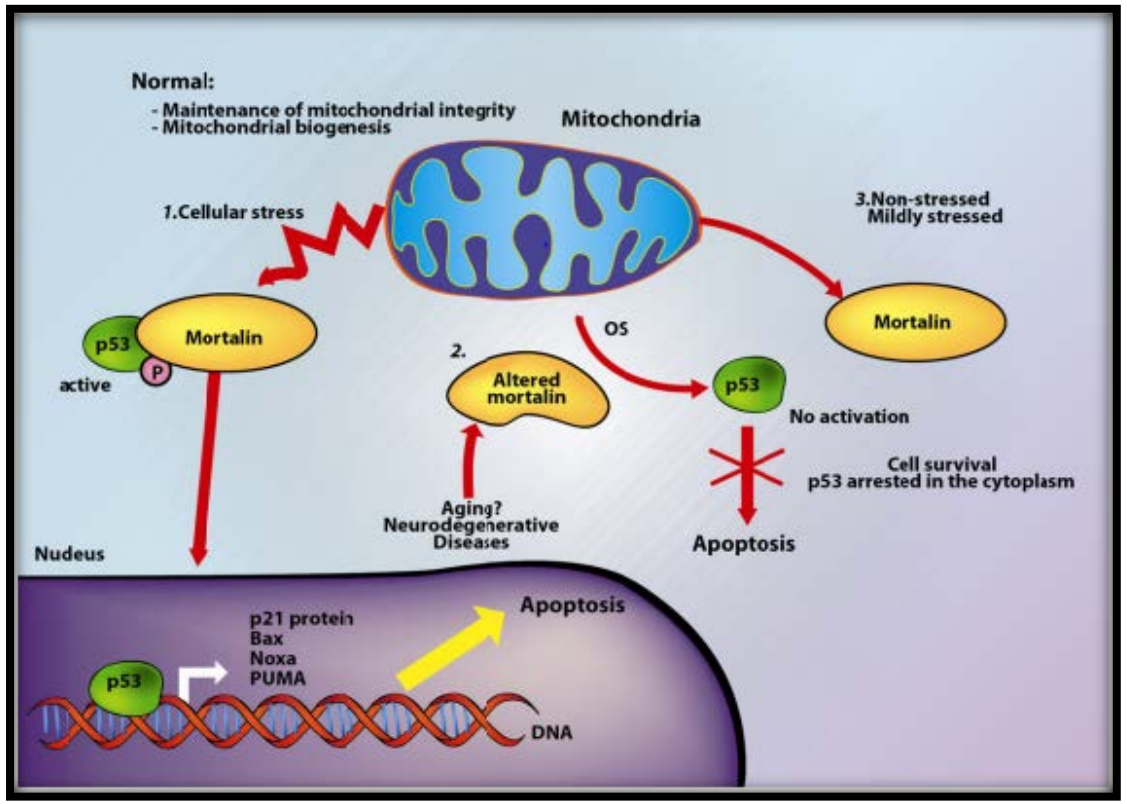
Fig. 3. Overview of the response to unfolded proteins in the intermembrane space (IMS) in mammals. ROS, produced by stressed mitochondria, activate protein kinase B (AKT), which consequently phosphorylates estrogen receptor α (ERα). Activated ERα then induces IMS protein quality control by increasing the activity of proteasome 26S and inducing the transcription of IMS protease *HTRA2* and mitochondrial regulator *NRF1*.

Proteins

- **GRP75** – part of the Hsp70 family of chaperones, involved in mt(UPR)

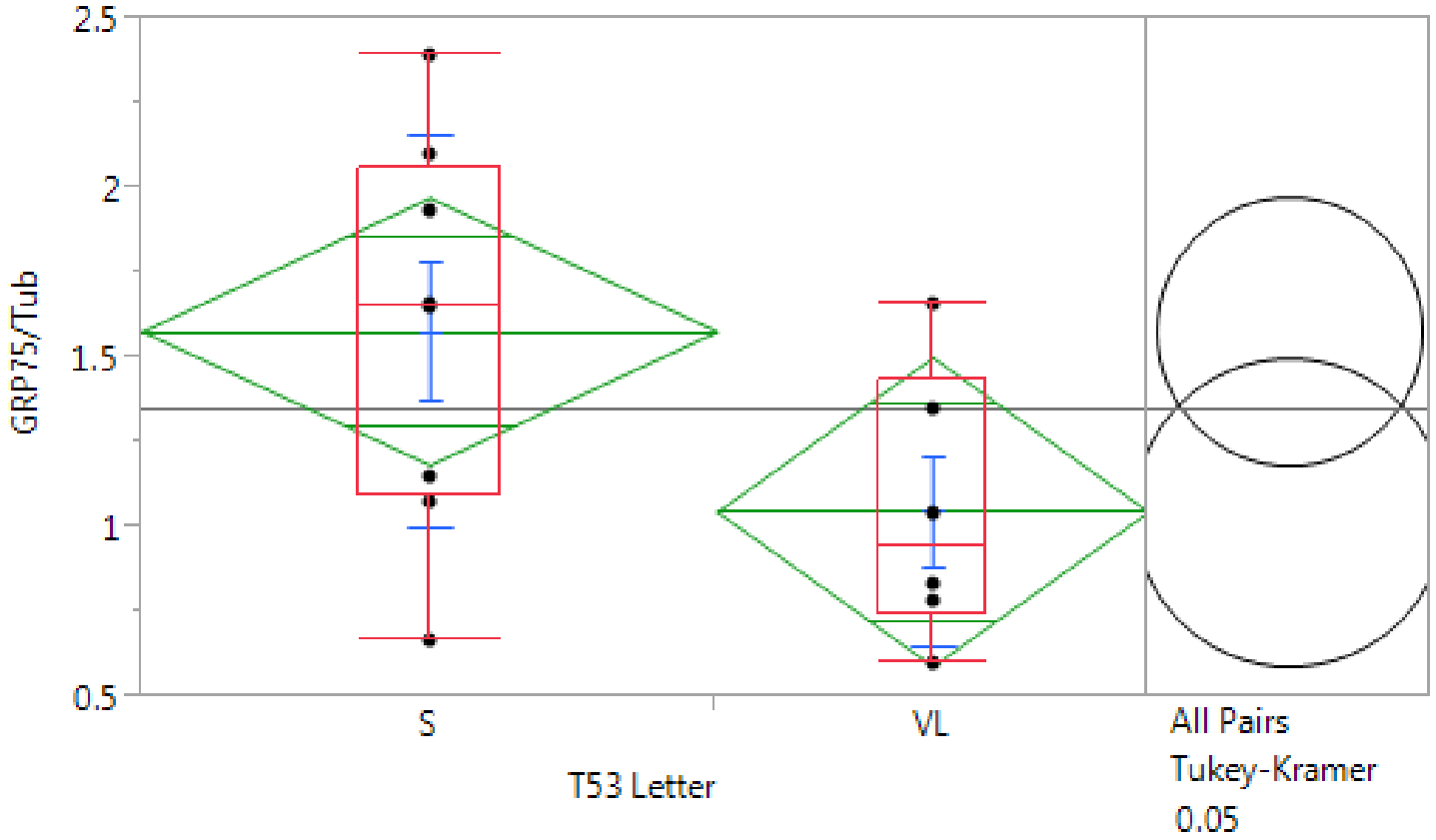
Mitochondrial proteolytic stress induced by loss of mortalin function is rescued by Parkin and PINK1

LF Burbulla[1,2,12], JC Fitzgerald[1,2,12], K Stegen[1,2,3], J Westermeier[1,2,3], A-K Thost[4], H Kato[5], D Mokranjac[6], J Sauerwald[1,2], LM Martins[7], D Woitalla[8], D Rapaport[5], O Riess[9], T Proikas-Cezanne[4], TM Rasse*[,10] and R Krüger*[,1,2,11]



or impaired mitochondrial proteins.[15,16] Mortalin is a key player in mitochondrial stress response, aging and programmed cell death.[17–19] Overexpression of mortalin extends lifespan in human cells and the nematode *C. elegans*, indicating a protective function *in vitro* and *in vivo*.[11,20,21] In contrast, partial loss of mortalin results in alterations in mitochondrial morphology, impaired MMP and increased cellular levels of reactive oxygen species (ROS).[11] Analysis of three PD-associated variants in the *mortalin* gene revealed a loss of protective mortalin function in human cells.[11] Notably, mortalin was linked to neurodegeneration in PD based on substantially reduced levels of the protein in brain samples of patients.[19,22] It was further observed that the

GRP75 in normal frontal samples APOE 3/3

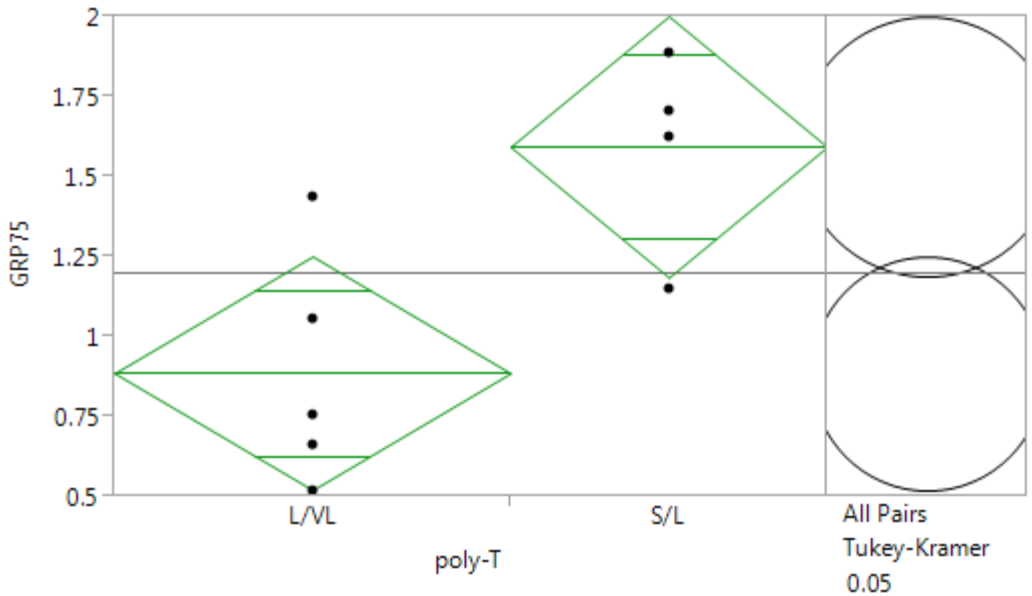


Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
S	VL	0.5320280	0.2760890	-0.069520	1.133576	0.0780

p = .0780

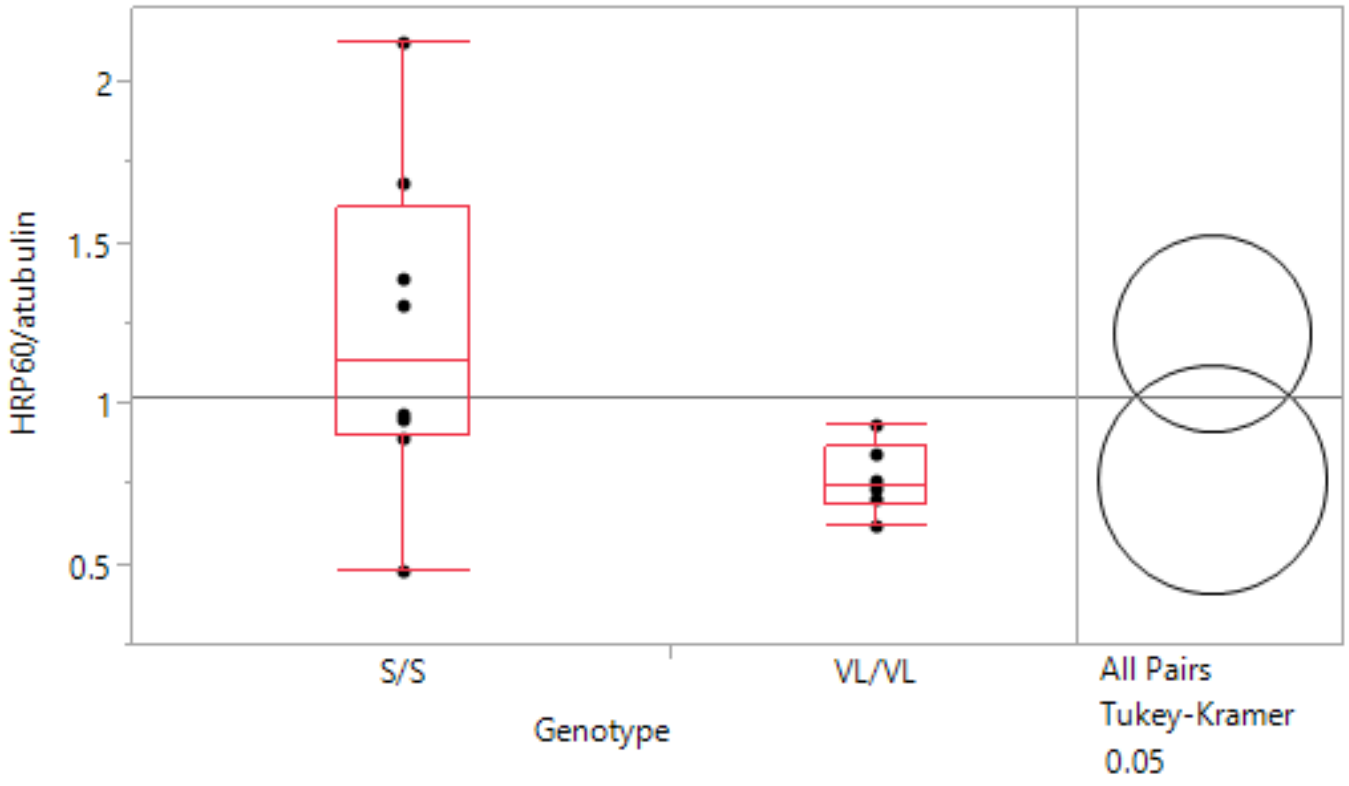
GRP75 in AD occipital samples APOE 3/4



Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
S/L	L/VL	0.7071323	0.2315968	0.1595201	1.254744	0.0185*

HRP60 in normal frontal samples APOE 3/3

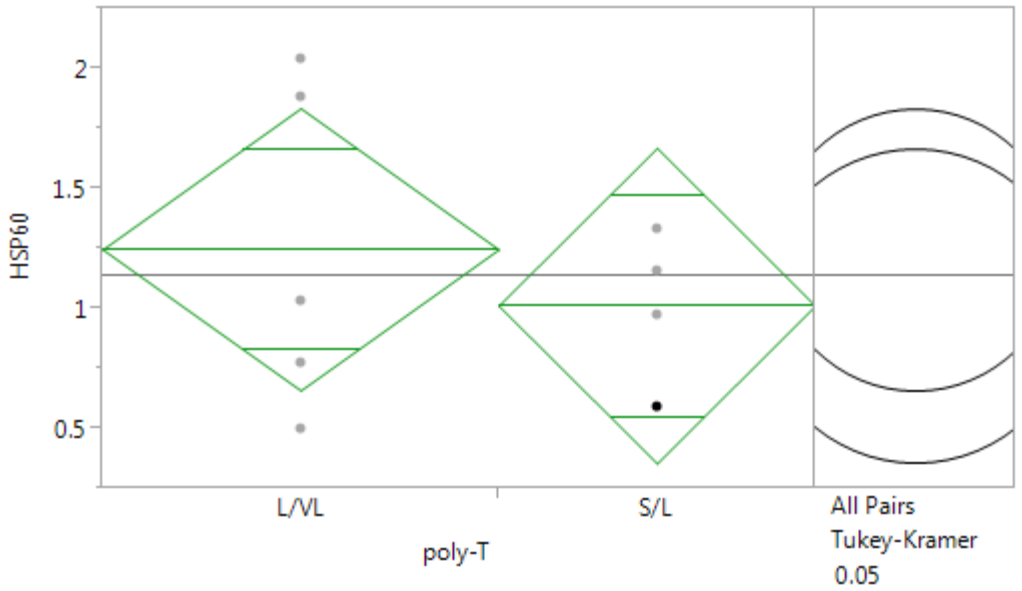


p=.0561

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
S/S	VL/VL	0.4568985	0.2160546	-0.013846	0.9276427	0.0561

HSP60 in AD occipital samples APOE 3/4

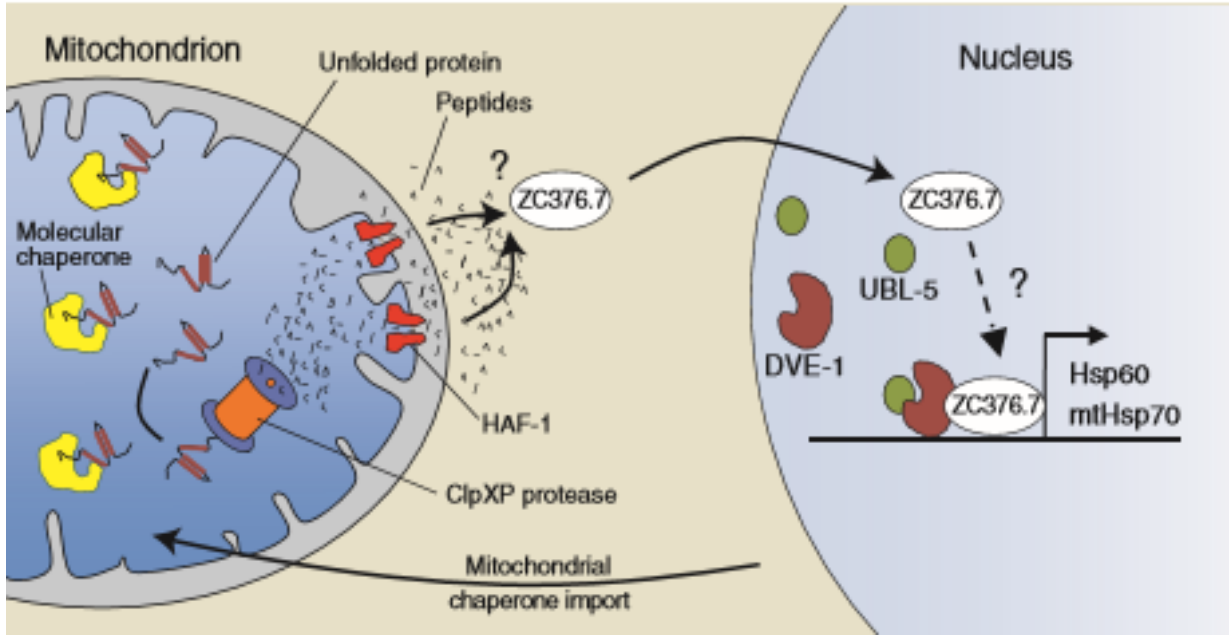


Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
L/VL	S/L	0.2345029	0.3727893	-0.646960	1.115966	0.5493

Proteins

- **ABCB10** - ATP binding cassette, transporter in mito inner membrane, involves transport of cargo on the matrix
- close analogue in C. elegans is haf-1; when haf-1 is repressed, the mitochondrial UPR is not activated



Mitochondrial ABC transporters function: the role of ABCB10 (ABC-me) as a novel player in cellular handling of reactive oxygen species

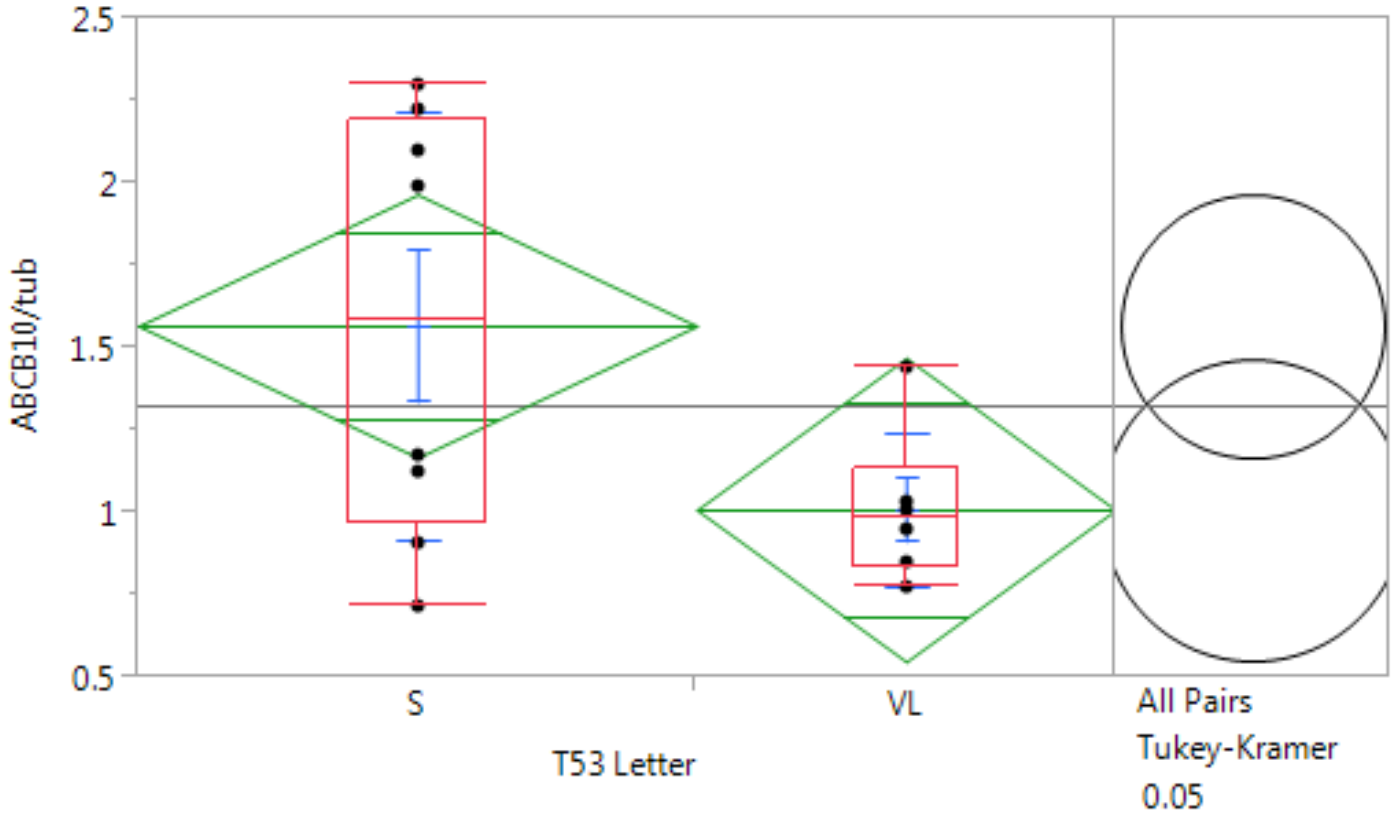
Marc Liesa, **Wei Qiu**, and **Orian S. Shirihai***

Therefore, haf-1 function will be discussed in this review, as its description might provide valuable information in order to understand ABCB10 function, despite not being the closest ABCB10 *C. elegans* orthologue (ABCB10 shares a 40% of identity with haf-1, covering 77% of its sequence). This similarity is lower when compared to haf-3 but still significant (see above).

The matrix peptide exporter HAF-1 signals a mitochondrial unfolded protein response by activating the transcription factor ZC376.7 in C. elegans

Cole M. Haynes[1,5,6], **Yun Yang**[1], **Steven P. Blais**[1], **Thomas A. Neubert**[1,4], and **David Ron**[1,2,3,5]

ABCB10 in normal frontal samples APOE 3/3

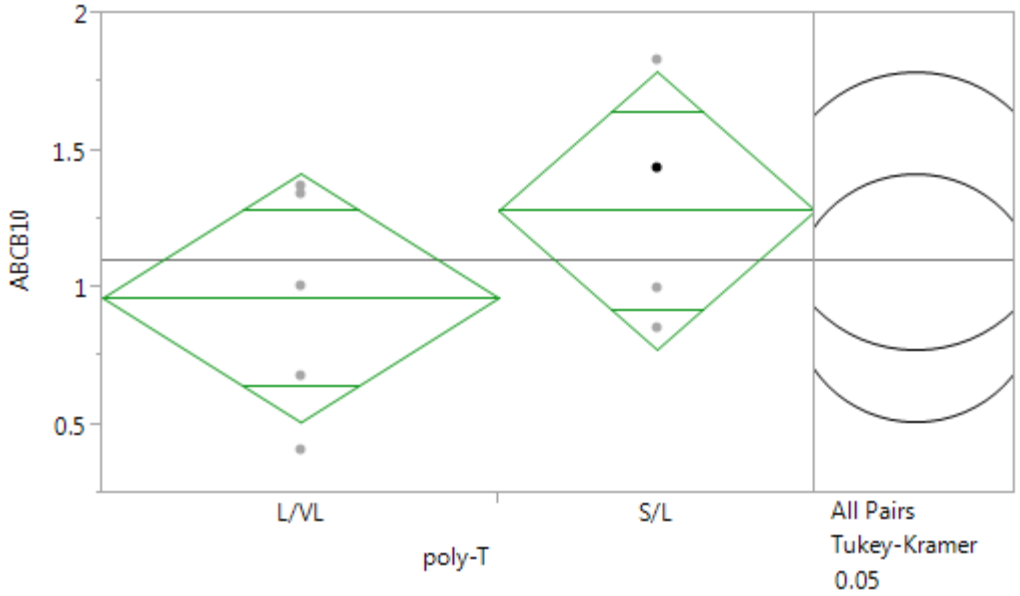


Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
S	VL	0.5582496	0.2797991	-0.051382	1.167882	0.0692

p = .0692

ABCB10 in AD occipital samples APOE 3/4



Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
S/L	L/VL	0.3180819	0.2876500	-0.362069	0.9982324	0.3054

Proteins

- **ACO2** - found in mitochondrial matrix
- Seen to be chopped up early by Lon in oxidative modification or stress response

Oxidative damage during aging targets mitochondrial aconitase

LIANG-JUN YAN*, RODNEY L. LEVINE†, AND RAJINDAR S. SOHAL*‡

ABSTRACT **The mechanisms that cause aging are not well understood. The oxidative stress hypothesis proposes that the changes associated with aging are a consequence of random oxidative damage to biomolecules. We hypothesized that oxidation of specific proteins is critical in controlling the rate of the aging process. Utilizing an immunochemical probe for oxidatively modified proteins, we show that mitochondrial aconitase, an enzyme in the citric acid cycle, is a specific target during aging of the housefly. The oxidative damage detected immunochemically was paralleled by a loss of catalytic activity of aconitase, an enzyme activity that is critical in energy metabolism. Experimental manipulations which decrease aconitase activity should therefore cause a decrease in life-span. This expected decrease was observed when flies were exposed to hyperoxia, which oxidizes aconitase, and when they were given fluoroacetate, an inhibitor of aconitase. The identification of a specific target of oxidative damage during aging allows for the assessment of the physiological age of a specific individual and provides a method for the evaluation of treatments designed to affect the aging process.**

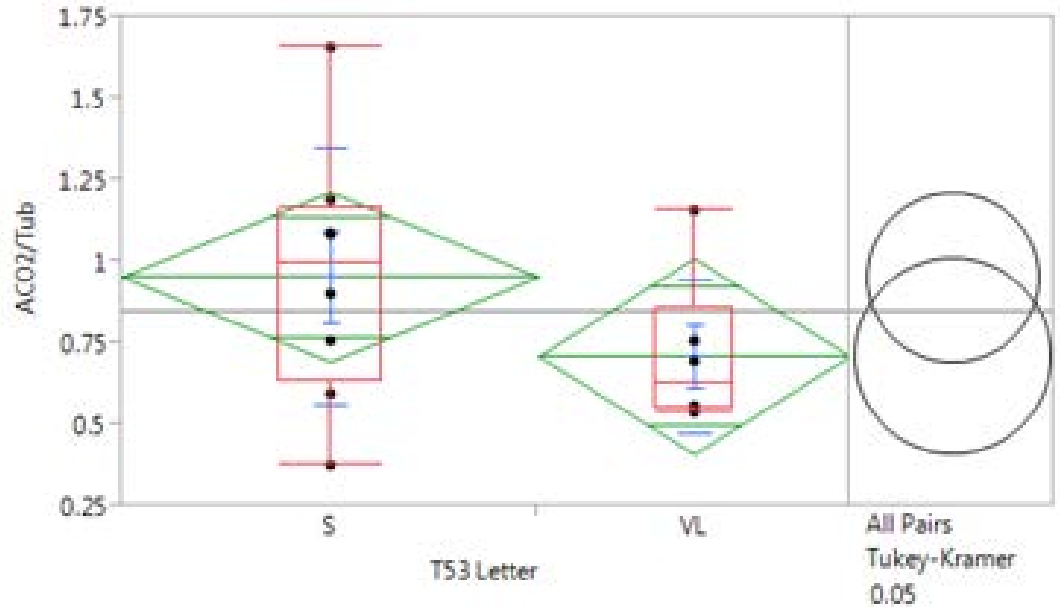
MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS: CAUSE AND CONSEQUENCE OF EPILEPTIC SEIZURES

MANISHA PATEL

Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, CO 80262, USA

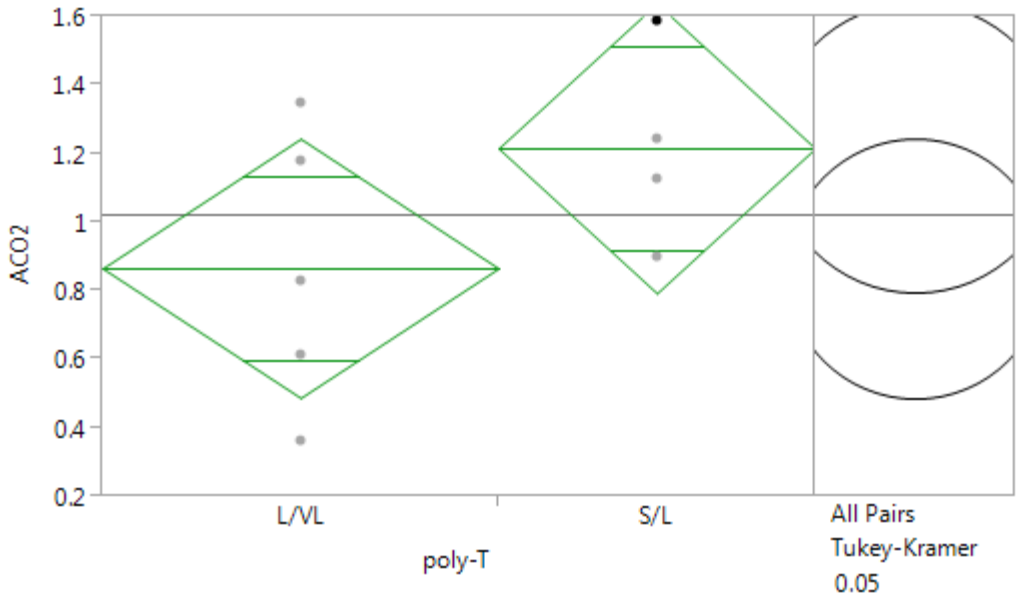
Abstract—Mitochondrial dysfunction has been implicated as a contributing factor in diverse acute and chronic neurological disorders. However, its role in the epilepsies has only recently emerged. Animal studies show that epileptic seizures result in free radical production and oxidative damage to cellular proteins, lipids, and DNA. Mitochondria contribute to the majority of seizure-induced free radical production. Seizure-induced mitochondrial superoxide production, consequent inactivation of susceptible iron–sulfur enzymes, e.g., aconitase, and resultant iron-mediated toxicity may mediate seizure-induced neuronal death. Epileptic seizures are a common feature of mitochondrial dysfunction associated with mitochondrial encephalopathies. Recent work suggests that chronic mitochondrial oxidative stress and resultant dysfunction can render the brain more susceptible to epileptic seizures. This review focuses on the emerging role of oxidative stress and mitochondrial dysfunction both as a consequence and as a cause of epileptic seizures.

ACO2 in normal frontal samples APOE 3/3



Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
S	VL	0.2434175	0.1818857	-0.152879	0.6397137	0.2056

ACO2 in AD occipital samples APOE 3/4

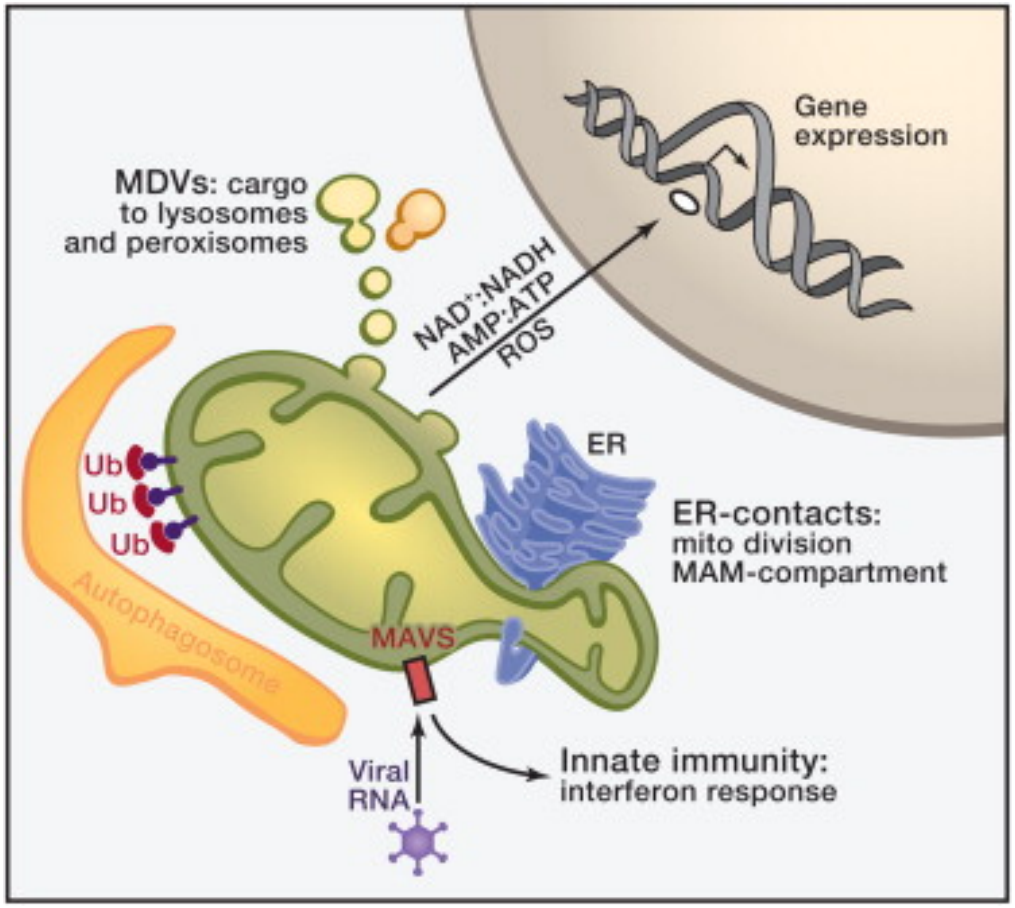


Ordered Differences Report

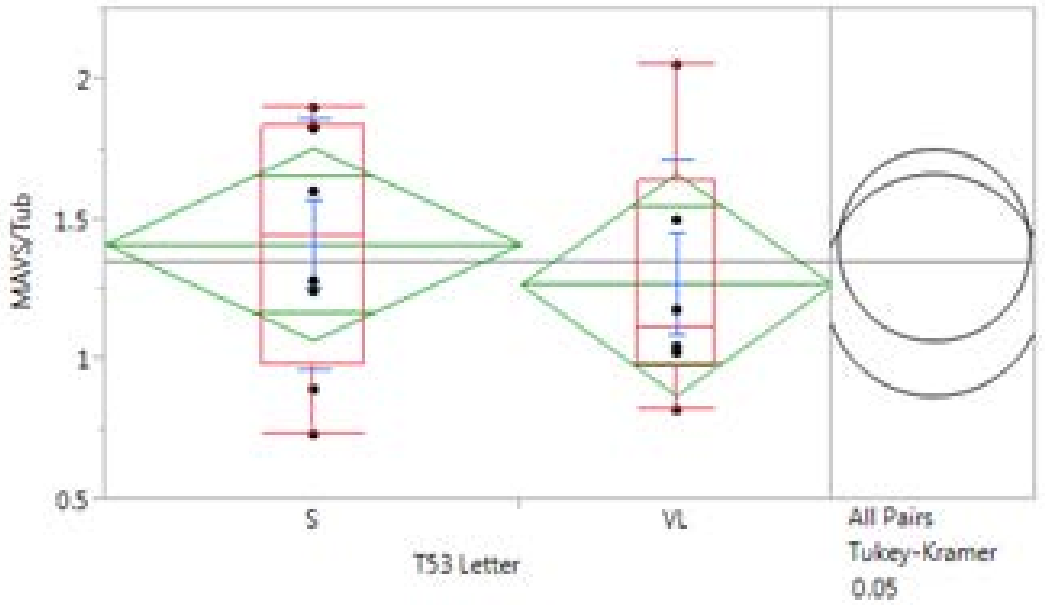
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
S/L	L/VL	0.3498575	0.2397022	-0.216920	0.9166349	0.1878

Proteins

- **MAVS -** Anti viral signaling protein, so also involved in stress responses, but not specifically UPR(mt)



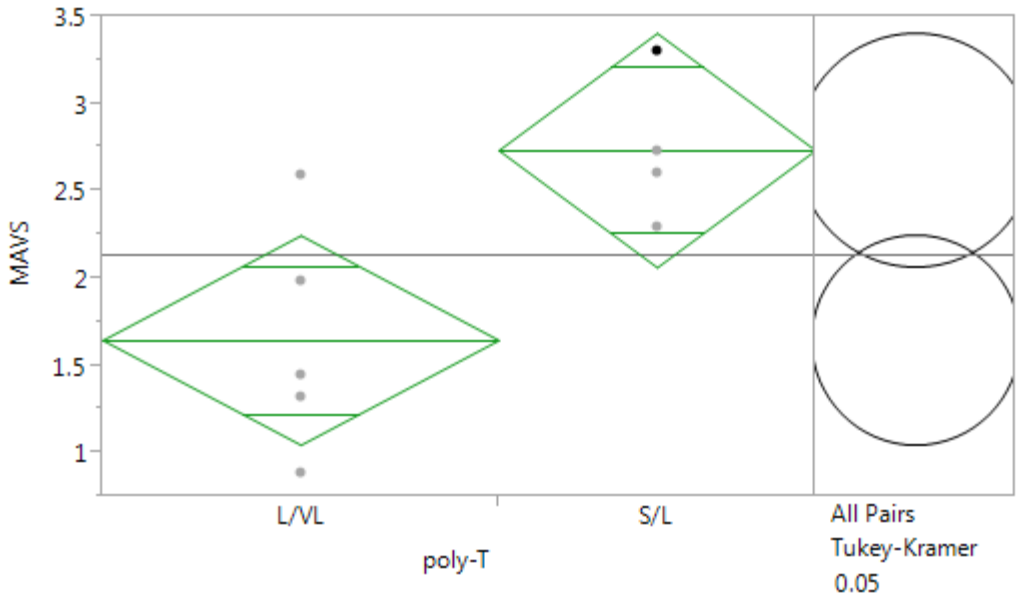
MAVS in normal frontal samples APOE 3/3



Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
S	VL	0.1436736	0.2407162	-0.380804	0.6681511	0.5617

MAVS in AD occipital cortex samples APOE 3/4



Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
S/L	L/VL	1.088083	0.3808075	0.1876608	1.988505	0.0244*

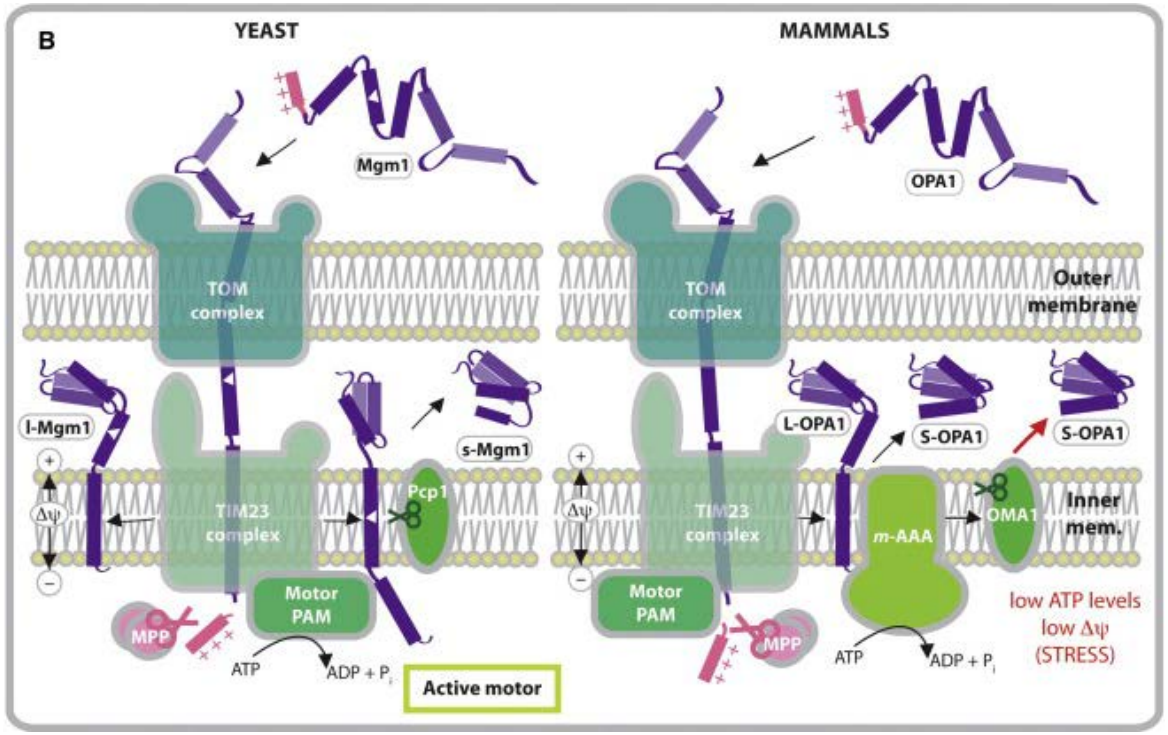
Proteins

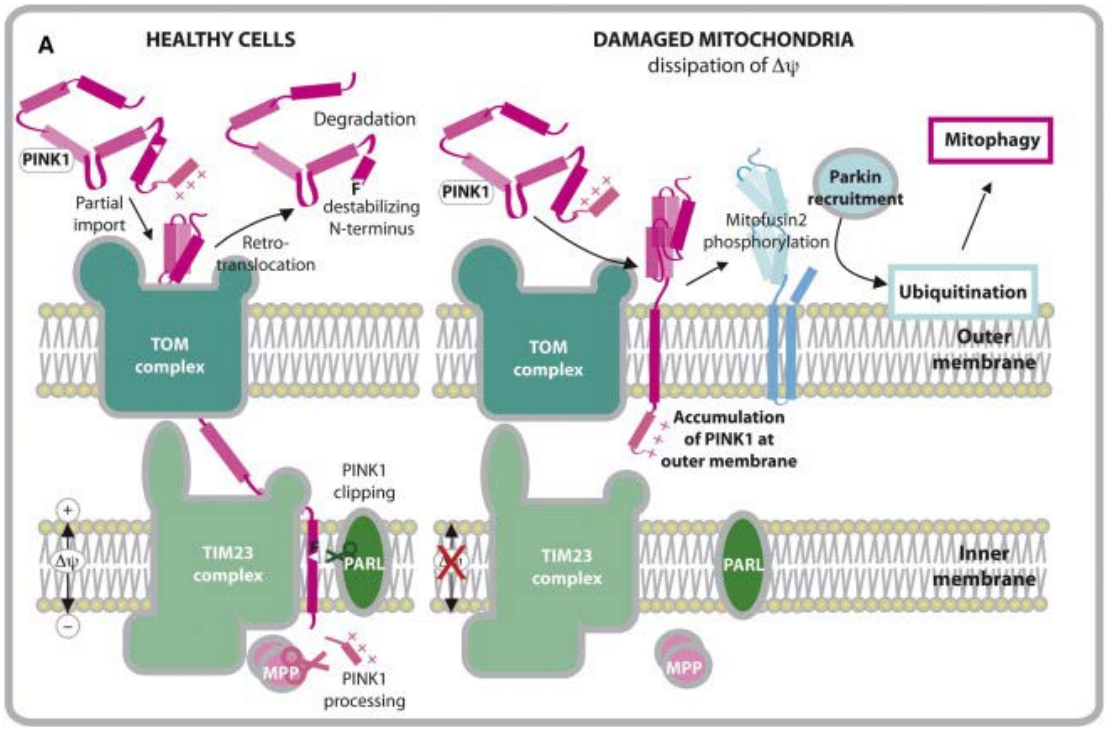
- **OMA1 -** Mitochondrial protease in inner membrane
- Cuts long proteins into short pieces especially under stress conditions

The Protein Import Machinery of Mitochondria—A Regulatory Hub in Metabolism, Stress, and Disease

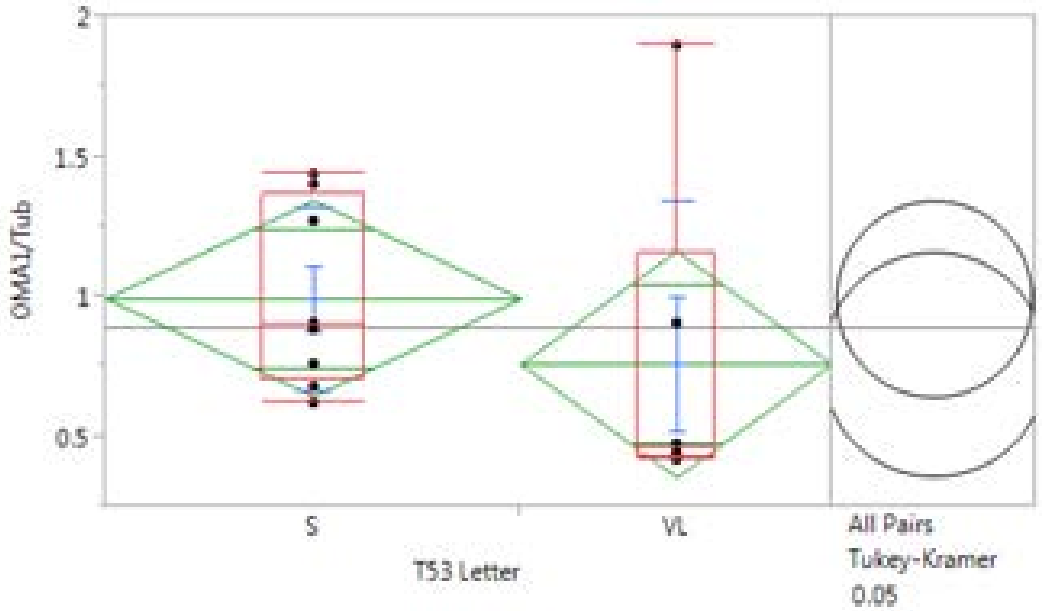
Angelika B. Harbauer,[1,2,3,4] René P. Zahedi,[5] Albert Sickmann,[5,6] Nikolaus Pfanner,[1,4,*] and Chris Meisinger[1,4,*]

cessing. However, an additional inner-membrane-bound protease OMA1, which functions in an ATP-independent manner, can convert long OPA1 isoforms into short isoforms, in particular under stress conditions like low ATP or reduced membrane





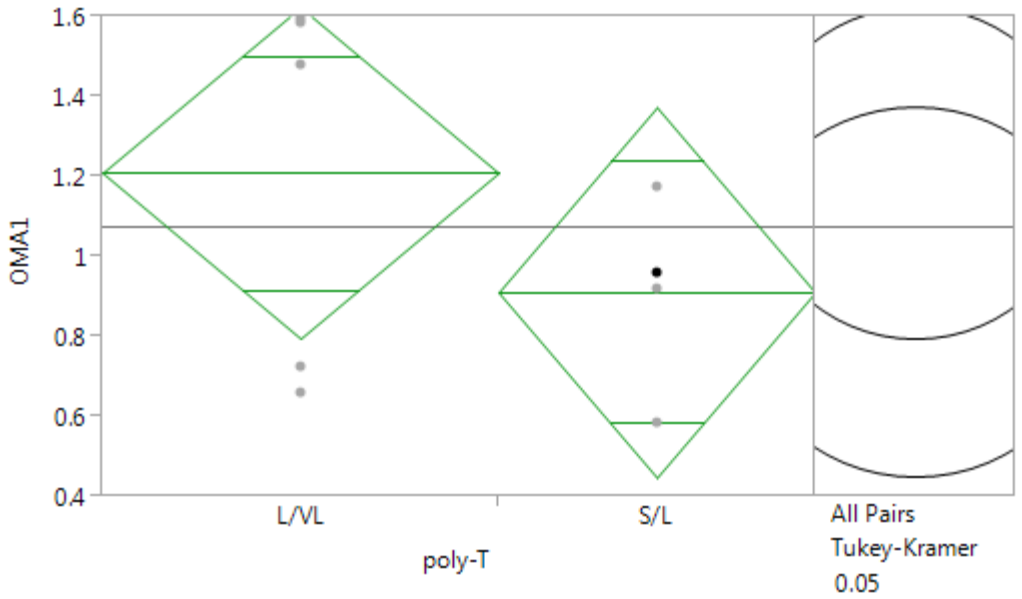
OMA1 in normal frontal samples APOE 3/3



Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
S	VL	0.2345605	0.2451213	-0.299515	0.7686357	0.3575

OMA1 in AD occipital samples APOE 3/4



Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
L/VL	S/L	0.2985555	0.2625841	-0.322326	0.9194374	0.2930

Conclusions Thus Far

- Wide variation, which tended to be larger for “S” samples than for “VL” – could just represent sampling
- No significant differences in normal frontal cortex samples
- Some proteins did reach statistical significance in occipital samples, but only in APOE 3/4 samples

NEXT: mt(UPR) may be associated with longevity

Beneficial miscommunication

Natural variations in the rate of protein translation in cellular organelles called mitochondria have been found to correlate with lifespan, suggesting a unified mechanism for the effects of metabolic alterations on longevity. SEE ARTICLE P.451

SUZANNE WOLFF & ANDREW DILLIN

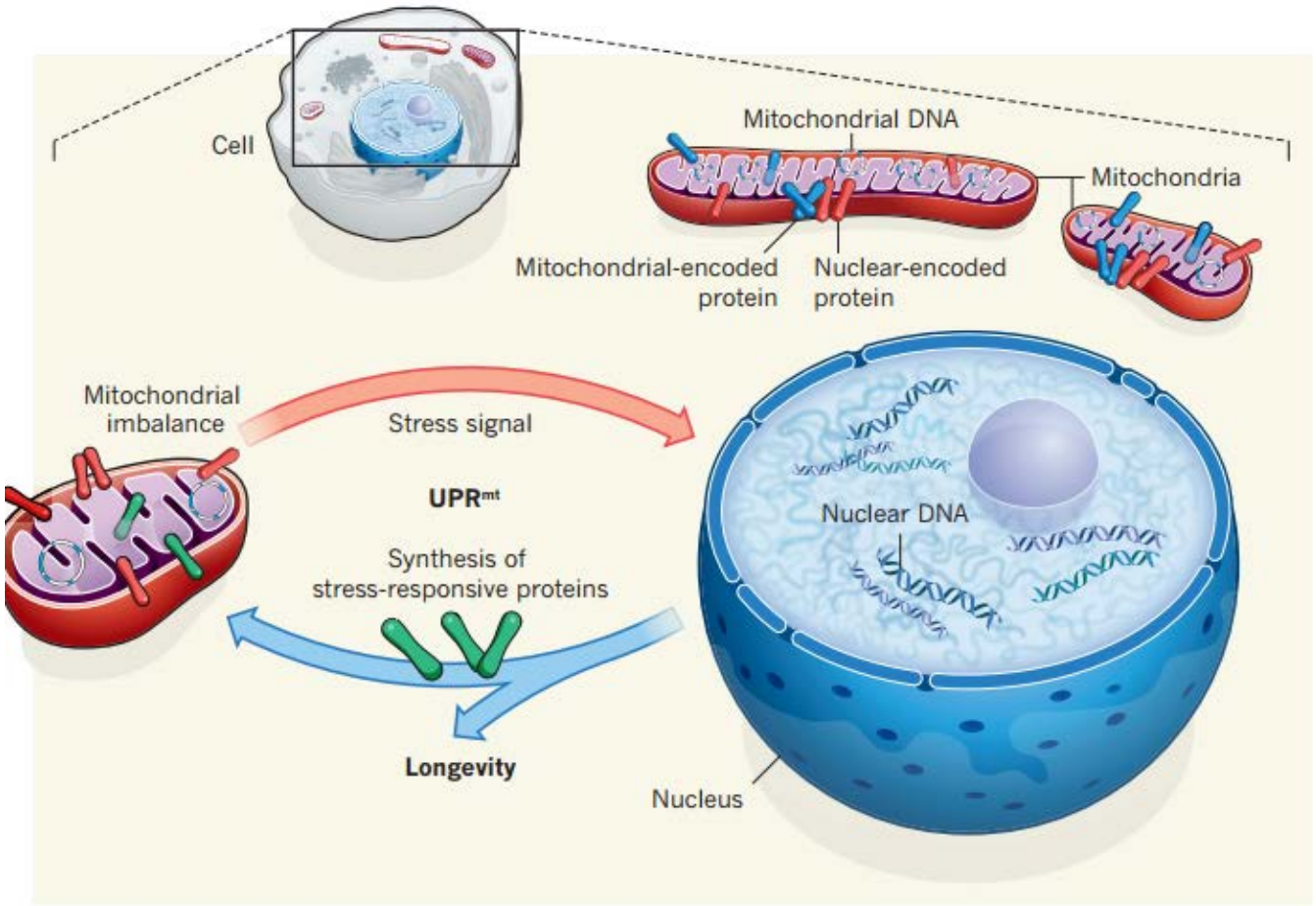


Figure 1 | Consequences of a cellular imbalance. Mitochondria carry a full complement of nuclear-encoded and mitochondrial-encoded proteins. An imbalance between mitochondrial and nuclear proteins triggers the mitochondrial unfolded protein response (UPR^{mt}), whereby mitochondria send a signal to the nucleus to induce the production of stress-related proteins, which restores the mitochondrial balance. Houtkooper *et al.*[2] find that increased UPR^{mt} is correlated with longer lifespan in mice and nematode worms.

The authors hypothesized that a deficiency in the function of MRPs might cause an imbalance in the relative levels of mitochondrial- and nuclear-encoded components of the electron transport chain, the mitochondrion's energy factory. This imbalance may secondarily activate the UPR^{mt}. Importantly, this effect seemed reciprocal: addition of rapamycin or resveratrol (pharmacological agents associated with attenuated cytoplasmic, rather than mitochondrial, translation, but which alter the metabolic state of the cell through the regulation of mitochondrial biogenesis) was sufficient to both upregulate the UPR^{mt} and extend lifespan.

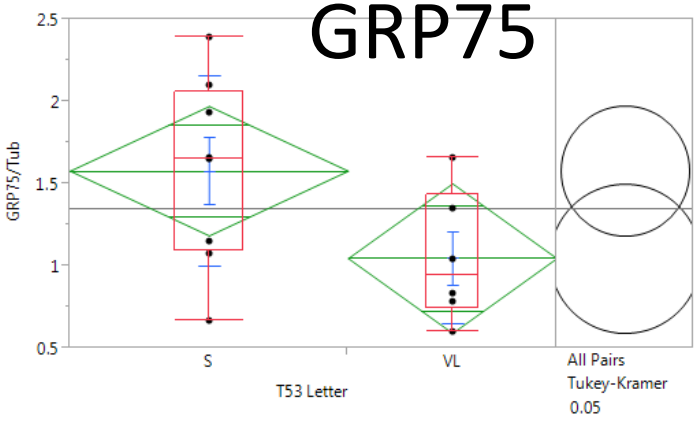
Tom40 and mt(UPR)

- To determine whether TOMM40 poly-T 523 has an effect on this, we will look at nuclear vs. mitochondrial encoded mitochondrial proteins
- Measure levels of MTCO1 (cytochrome oxidase subunit, mitochondrial encoded) and ATP5 (ATP synthase subunit, nuclear encoded) in human tissue
- Use doxycycline to induce mito-nuclear imbalance in 2 models of cultured cells
  - HeLa cells that express different levels of Tom40 (HeLa C3 vs TomMix)
  - SH-SY5Y (S/S) vs IMR32 (L/VL)
- Continue looking at larger tissue sample set as well as more proteins involved in mt(UPR)

SPARE SLIDES

Normal Frontal Cortex (APOE 3/3)

GRP75

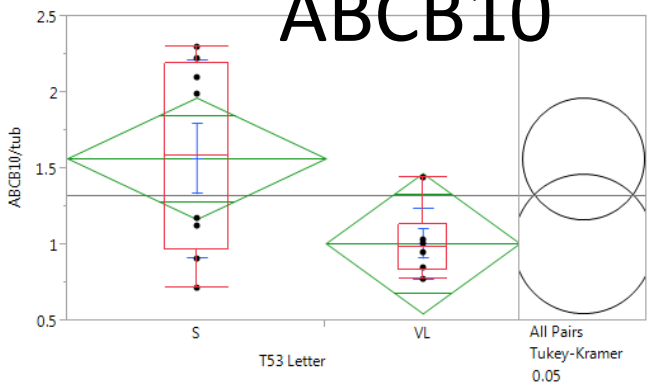


Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
S	VL	0.5320280	0.2760890	-0.069520	1.133576	0.0780

p = .0780

ABCB10



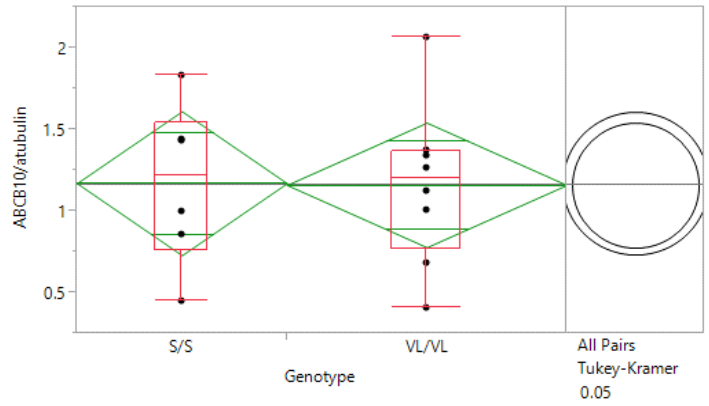
Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
S	VL	0.5582496	0.2797991	-0.051382	1.167882	0.0692

p = .0692

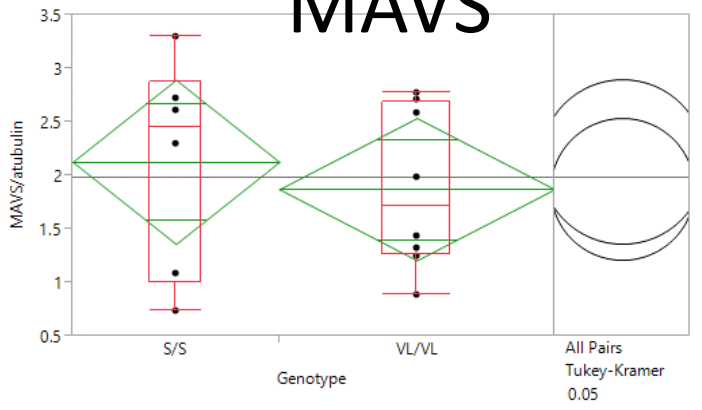
AD Occipital Cortex (APOE 3/3 and 3/4)

ABCB10



Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
S/S	VL/VL	0.0104793	0.2679995	-0.573443	0.5944020	0.9695

MAVS

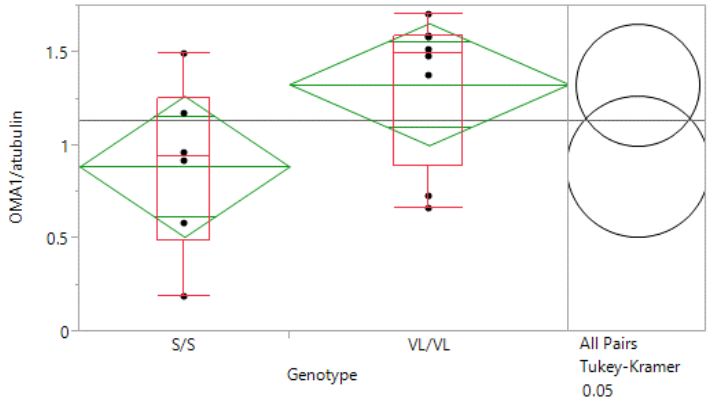


Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
S/S	VL/VL	0.2554454	0.4658991	-0.759665	1.270556	0.5935

- VL/VL and VL/L samples are grouped together as are S/S and S/L
- All samples used are not preferred by quality, need to increase sample size

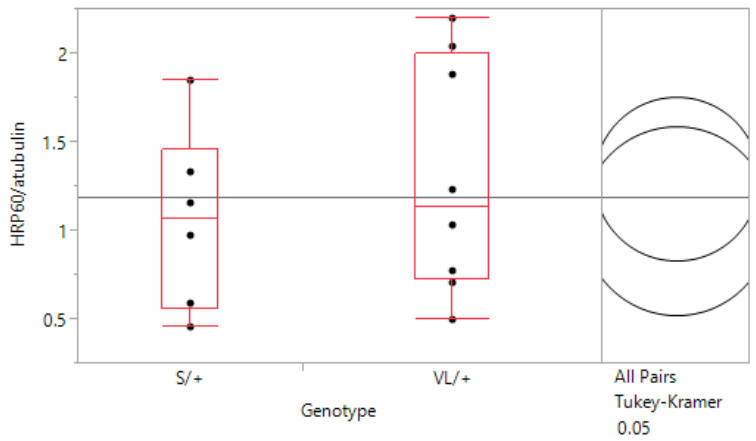
OMA1

p = .0787



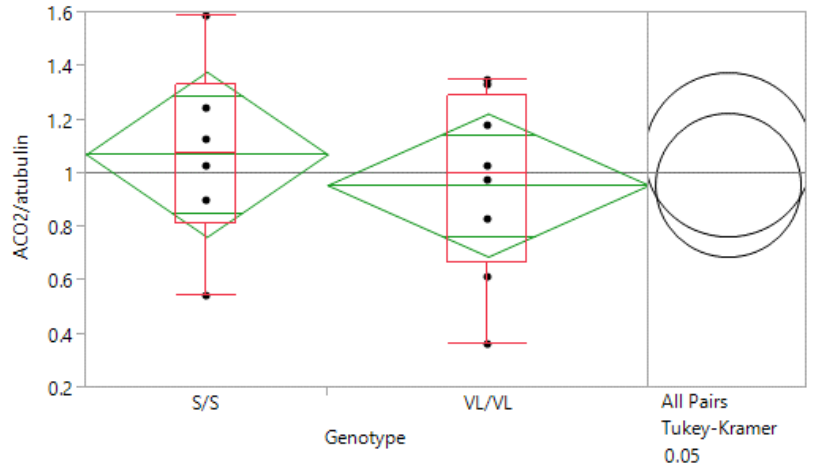
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
VL/VL	S/S	0.4419682	0.2300052	-0.059172	0.9431083	0.0787

AD Occipital Cortex (APOE 3/3 and 3/4)



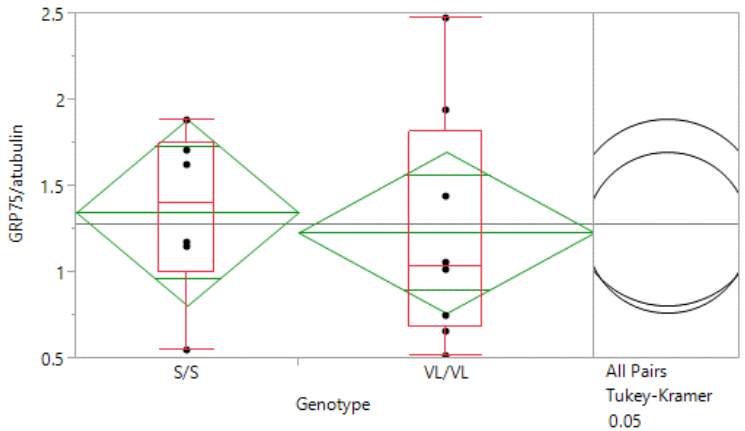
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
VL/+	S/+	0.2381419	0.3249448	-0.469854	0.9461382	0.4777

HSP60



ACO2

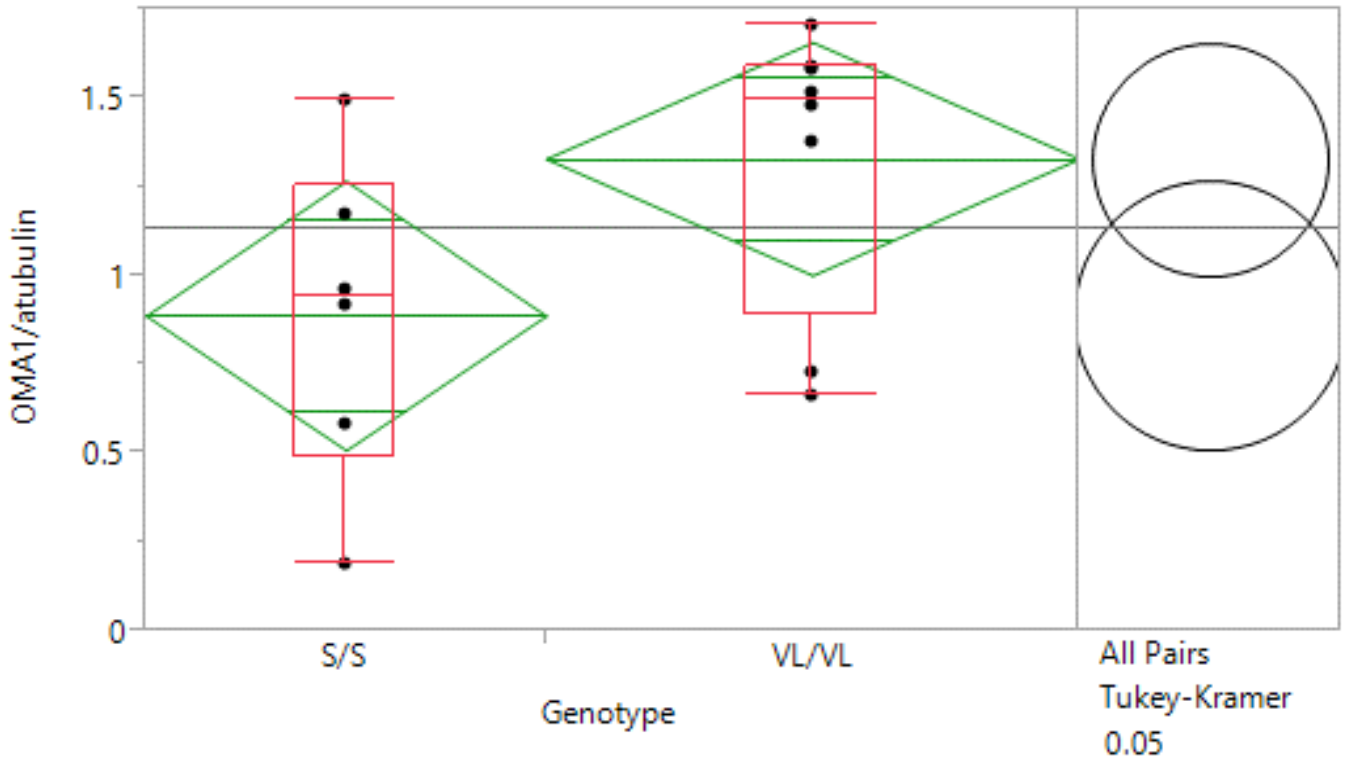
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
S/S	VL/VL	0.1150055	0.1871875	-0.292842	0.5228534	0.5504



GRP75

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
S/S	VL/VL	0.1166556	0.3280738	-0.598158	0.8314694	0.7283

OMA1 in AD occipital samples (APOE 3/3 and 3/4)



Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
VL/VL	S/S	0.4419682	0.2300052	-0.059172	0.9431083	0.0787

p = .0787