Vitamin D Workshop 2018

VITAMIN D, IN THE PREVENTION OF HEALTH DISPARITIES DURING ADULT LIFE

The clues for new roles of vitamin D and vitamin D receptor in neurodegeneration.

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DEPARTMENT OF MEDICAL BIOLOGY

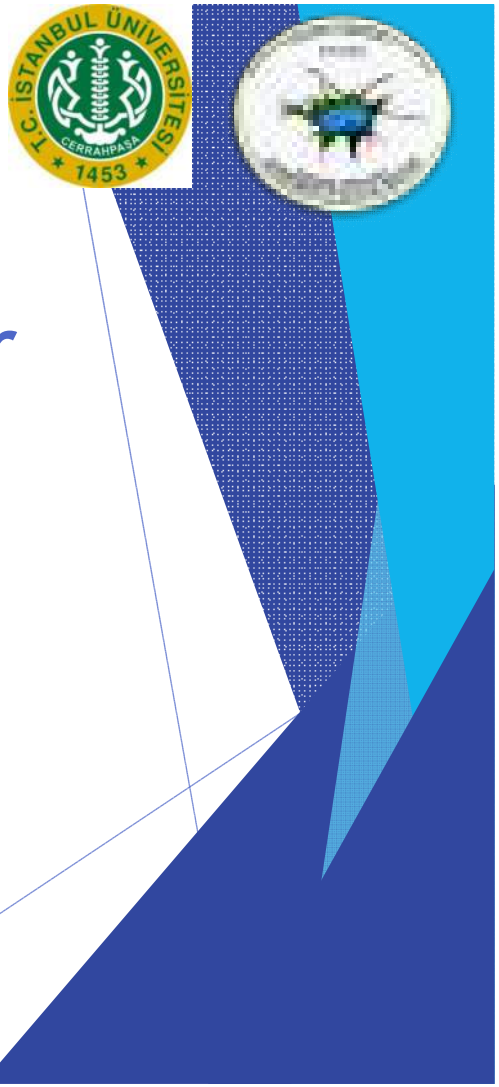
BRAIN AND NEURODEGENERATIVE DISORDERS RESEARCH UNIT

ISTANBUL - TURKEY

THESSALONIKI, GREECE

2018

Nothing to declare



The begining...

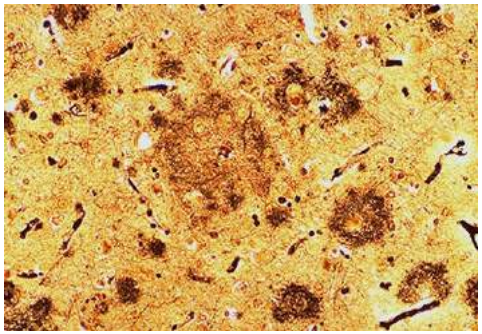
- Last decade gave us the opportunity to investigate the role of vitamin D and its receptor in development and disorders of central nervous system.
- Yet still the debate is going on validaiting the action of vitamin D and vitamin D receptor in brain
- The aim of this talk is
- To draw a picture of

1. **What we have asked?**
2. **What we have proved?**
3. **What we have learned?**
4. **What still remains to be discovered? ☺ a lot...**

- in vitamin D basis of neurodegeneration

Alzheimer's disease

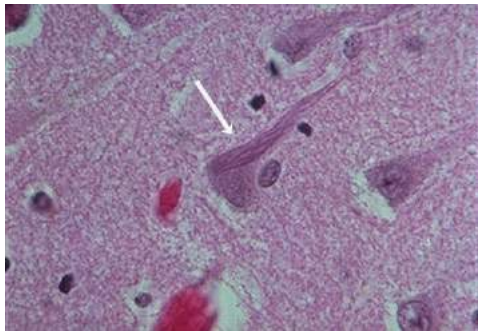
Amyloid Plaques



Senile plaques (Silver Staining):
Amyloid aggeragtions (diffuse or dense) stained dark brown.

(http://www-medlib.med.utah.edu/WebPath/CNSHTML/CNS090.html)

Neurofibrillary Tangles

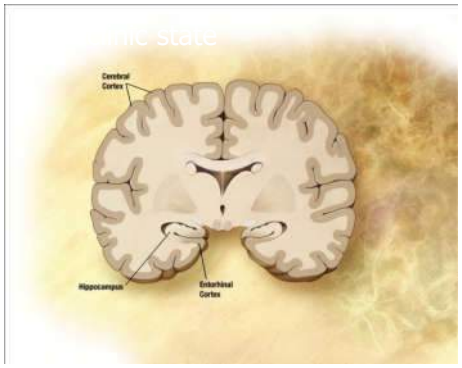


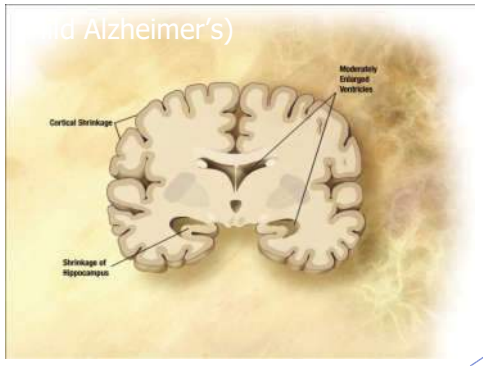
NFT, hematoxylene eosin.
Intracytoplasmic dense fibrillary structures•

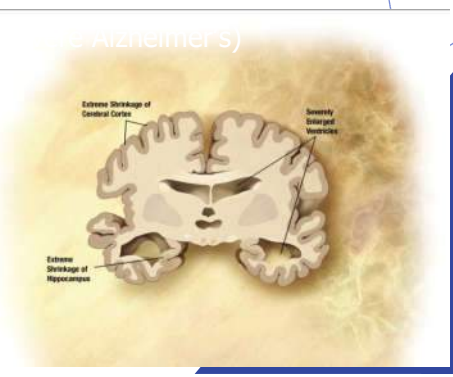
(http://www-medlib.med.utah.edu/WebPath/CNSHTML/CNS094.html)

These pathological structures cause:

- Distruption of axonal transport,
- Distruption of signal transmission between neurons,
- Distruption of neurotrophic factor synthesis
- Distruption of neuronal calcium homeostasis
- Induction of oxidative stress

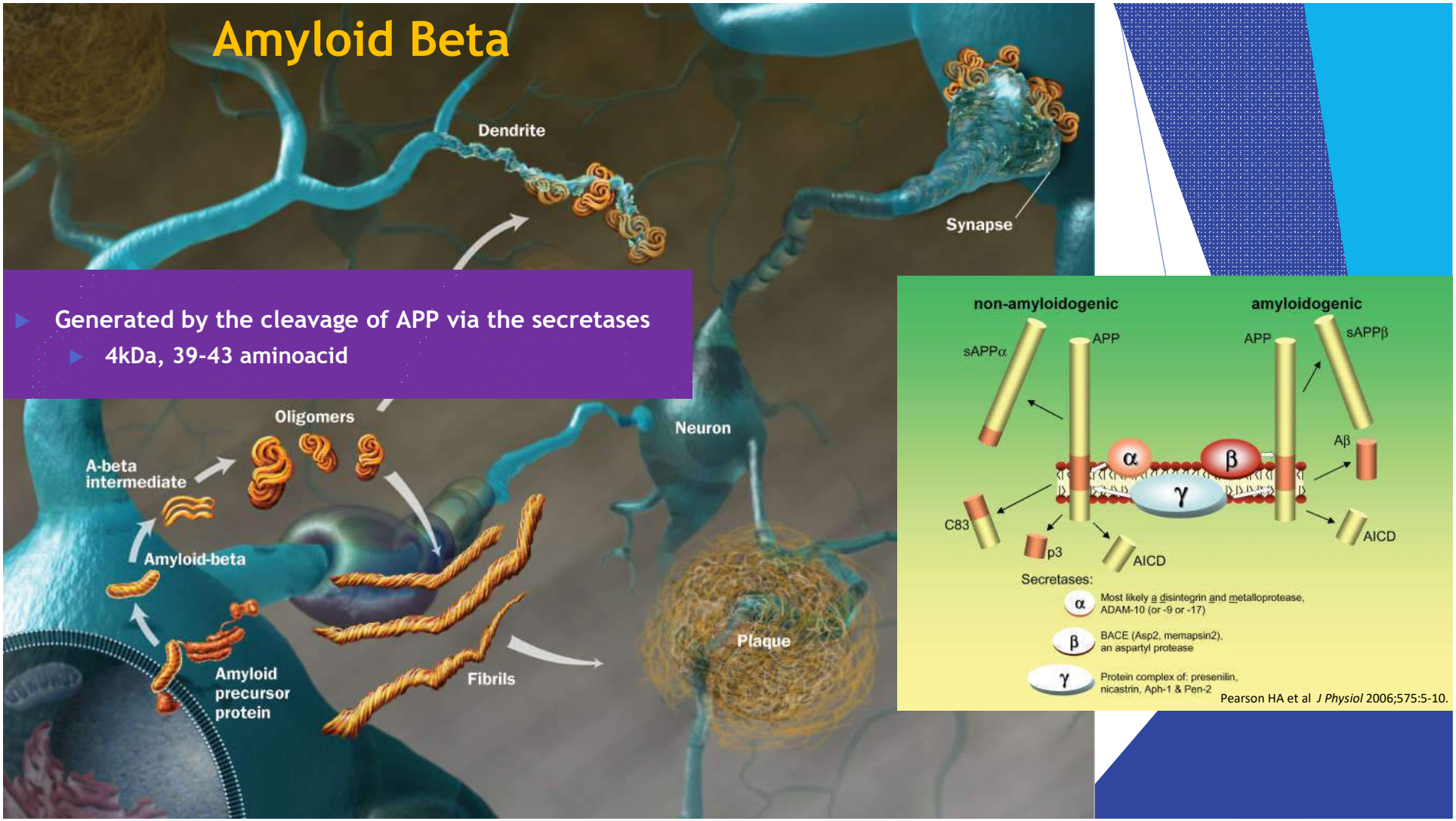






Amyloid Beta

- Generated by the cleavage of APP via the secretases
  - 4kDa, 39-43 aminoacid



Pearson HA et al *J Physiol* 2006;575:5-10.

Does vitamin D act in brain?

Vitamin D receptor gene (VDR)- Alzheimer's disease

- 1992 Sutherland et al.: **The hippocampi of the AD patients have decreased VDR mRNA expression.**
- 2001 Paduslo et al.: linkage study; indicated a **AD related risk locus on chromosome 12q**. No sigificant gene reported but the locus involved VDR in addition to other genes.
- 2006-2011: the first studies indicating **the relation between vitamin D deficiency and cognitive decline**.
- 2007 Gezen-Ak et al.: Certian **VDR polymorphisms increase the risk of developing AD 2.3 times**.
- 2009 Beecham et al.: **GWA study** (including 550.000 SNPs) **reported a AD associated novel locus at chromosome 12q13.** They indicated that among other genes VDR is the most probabale candidate risk gene for AD given the data of Gezen-Ak study.
- 2012 Gezen-Ak et al.: **VDR "TaubF" haplotype** is more frequently seen in AD patients.

Genetic background of VDR-vitamin D pathway in neurodegenerative disorders

- **Association between VDR polymorphisms and Parkinson's disease:**
  - Butler MW, et al. (2011) Vitamin D receptor gene as a candidate gene for Parkinson disease. *Ann Hum Genet* **75**, 201-210
  - Gatto NM et al. Vitamin D receptor gene polymorphisms and Parkinson's disease in a population with high ultraviolet radiation exposure. J Neurol Sci. 2015;352(1-2):88-93
  - Gezen-Ak D, et al (2016) GC and VDR SNPs and Vitamin D Levels in Parkinson's Disease: The Relevance to Clinical Features. Neuromolecular Med.
- **Association between Low density lipoprotein receptor-related protein 2 (LRP2 or megalin) the transporter of vitamin D at the plasma membrane and AD:**
  - Vargas T, et al. (2010) A megalin polymorphism associated with promoter activity and Alzheimer's disease risk. *Am J Med Genet B Neuropsychiatr Genet* **153B**, 895-902.
  - Wang LL, et al. (2011) A single nucleotide polymorphism in LRP2 is associated with susceptibility to Alzheimer's disease in the Chinese population. *Clin Chim Acta* **412**, 268-270
- **Association between LRP2 (megalin) polymorphisms and cognitive decline**
  - Beydoun MA, et al. (2012) Vitamin D receptor and megalin gene polymorphisms and their associations with longitudinal cognitive change in US adults. *Am J Clin Nutr* **95**, 163-178.
- **Association between vitamin D binding protein (GC, VDBP) polymorphisms and Parkinson's disease:**
  - Gezen-Ak D, et al (2016) GC and VDR SNPs and Vitamin D Levels in Parkinson's Disease: The Relevance to Clinical Features. Neuromolecular Med.

Serum 25OHD levels

The relation between vitamin D and neurodegeneration

- Vitamin D deficiency and **cognitive performance** (2006-2010)
  - Wilkins CH, Sheline YI, Roe CM, Birge SJ, Morris JC (2006) Vitamin D deficiency is associated with low mood and worse cognitive performance in older adults. *Am J Geriat Psychiat* **14, 1032-1040.**
  - Annweiler C, Schott AM, Allali G, Bridenbaugh SA, Kressig RW, Allain P, Herrmann FR, Beauchet O (2010) Association of vitamin D deficiency with cognitive impairment in older women: cross-sectional study. *Neurology* ***74, 27-32***.
- Vitamin D levels and **cognitive decline** (2009-2010)
  - Llewellyn DJ, Langa KM, Lang IA (2009) Serum 25- hydroxyvitamin D concentration and cognitive impairment. *J Geriatr Psychiatry Neurol* ***22, 188-195***.
  - Llewellyn DJ, Lang IA, Langa KM, Muniz-Terrera G, Phillips CL, Cherubini A, Ferrucci L, Melzer D (2010) Vitamin D and risk of cognitive decline in elderly persons. *Arch Intern Med* **170, 1135-1141.**

Vitamin D deficiency and Alzheimer's disease

- **Meta analysis:** Serum 25OHD levels of AD patients are **significantly lower than** that of healthy controls!
  - Annweiler C. et al **2013**. Low serum vitamin D concentrations in Alzheimer's disease: A systematic reviewand meta-analysis. *J Alzheimers Dis* **33**, 659-674.

- **Vitamin D deficiency increses the risk of developing AD and vascular dementia (VaD)!**
  - Afzal S, et al. **2013**. Reduced 25-hydroxyvitamin D and risk of Alzheimer's disease and vascular dementia. *Alzheimers Dement*. doi: 10.1016/j.jalz.2013.05.1765.
  - A longitudinal study
  - **30 years follow up**
  - **10,186 individulas**

- **Vitamin D induces amyloid beta clearance of macrophages in AD patinets!**
  - Masoumi A, et al. 2009 1alpha,25-dihydroxyvitamin D3 interacts with curcuminoids to stimulate amyloid-beta clearance by macrophages of Alzheimer's disease patients. *J Alzheimers Dis* **17**, 703-717.
  - Mizwicki MT, et al. 2011. Genomic and nongenomic signaling induced by 1,25(OH)2-vitamin D3 promotes the recovery of amyloid-phagocytosis by Alzheimer's disease macrophages. *J Alzheimers Dis* **29**, 51- 62.

- **Annweiler and Beauchet (AD-IDEA)**
  - Annweiler C, et al. 2011 Alzheimer's disease–input of vitaminD with mEmantine assay (AD-IDEA trial): Study protocol for a randomized controlled trial. *Trials* **12**, 230
  - A combined treatment of both vitamin D and memantine (a well known AD drug).
  - Gave significantly better results compared with the memantine alone treated patients.

Littlejohns TJ. et al.
Vitamin D and the risk of dementia and Alzheimer disease.
Neurology. 2014

- In elderly people, increased risk of developing AD or dementia is sigificantly associated with vitamin D deficiency!

  - **University of Exeter Medical School, UK**
  - **David Llywellyn**
  - **1,658 elderly individulas**
  - White Americans
  - **Over 65 years old**
  - No dementia
  - No signs of any cardiovascular disases
  - No stroke
  - **6 years of follow up**
  - **171 individulas develop dementia**
  - **107 of them converts to AD**

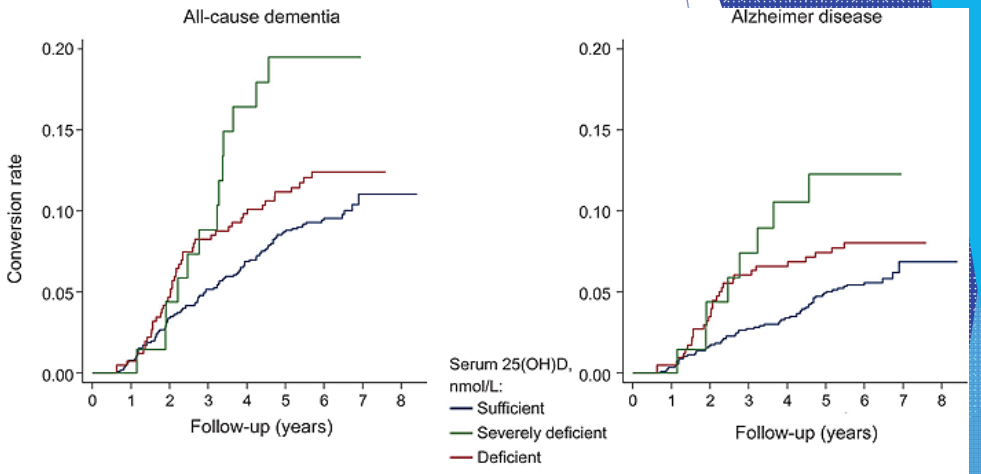


Fig. 1. *Kaplan-Meier curves for unadjusted rates of all-cause dementia and Alzheimer disease by serum 25-hydroxyvitamin D (25(OH)D) concentrations.*

- Conslusion:
  - **Mild vitamin D deficiency** increases the risk of developing **dementia by 53% !**
  - **Severe vitamin D deficiency** increases the risk of developing **dementia by 125% !**

Nutrient Biomarkers for Dementia

- 666 individuals with no dementia
- Plasma levels of 22 nutrient biomarkers
- 12 years follow up

- Low levels of plasma vitamin D, karotenoids and polysaturated fats are associated with significantly high risk of dementia

ELSEVIER

CrossMark

Alzheimer's & Dementia 13 (2017) 1125-1132

Alzheimer's & Dementia

Featured Article

Nutrient biomarker patterns and long-term risk of dementia in older adults

Camille Amadieu[a], Sophie Lefèvre-Arbogast[a], Cécile Delcourt[a], Jean-François Dartigues[a], Catherine Helmer[a], Catherine Féart[a], Cécilia Samieri[a,*]

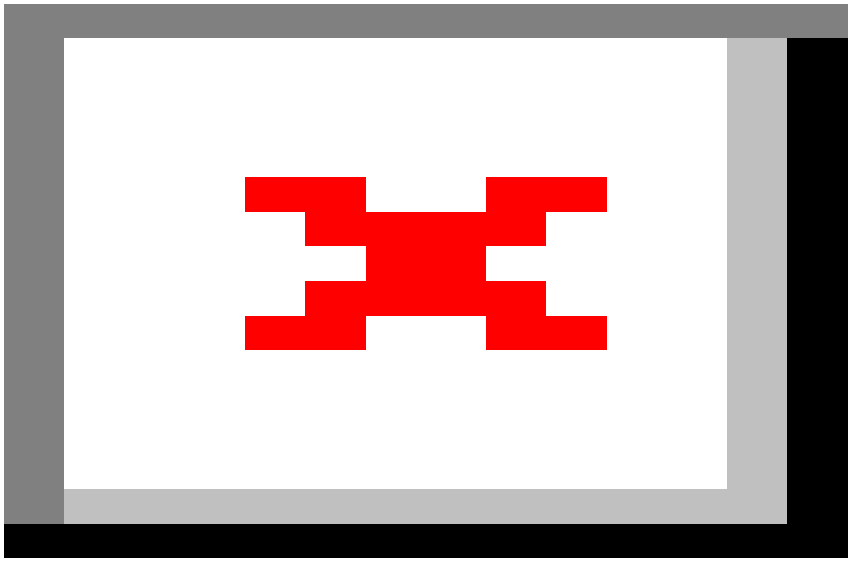
[a]University of Bordeaux, INSERM, Bordeaux Population Health Research Center, UMR 1219, Bordeaux, France

Table 2
Baseline plasma concentrations of the 22 candidate nutrient biomarkers according to incident dementia over 12 years in the Bordeaux sample of the Three-City study (N = 666)

Nutrient biomarkers	Incident dementia (n = 110)	No dementia (n = 556)	P
25(OH)D, nmol/L	28.4 (13.1)	36.3 (18.9)	<.001
Carotenoids, μg/L			
α carotene	86.6 (64.4)	99.0 (78.1)	.06
β carotene	338.6 (226.0)	407.2 (304.2)	.005
Lycopene	232.2 (150.9)	274.9 (170.9)	.75
Lutein	161.9 (84.3)	168.1 (87.9)	.19
Zeaxanthin	38.3 (26.1)	40.2 (23.6)	.21
β Cryptoxanthin	150.9 (107.8)	168.4 (127.7)	.11
Vitamin E, mg/L			
α Tocopherol	13.9 (3.6)	13.4 (3.3)	.17
γ Tocopherol	0.6 (0.4)	0.6 (0.3)	.32
Retinol, μg/L	502.2 (135.8)	511.0 (145.3)	.72
Fatty acids, % of total fats			
Saturated fatty acids			
Myristic acid (14:0)	1.3 (0.4)	1.2 (0.5)	.13
Palmitic acid (16:0)	28.4 (5.7)	27.9 (5.7)	.16
Stearic acid (18:0)	11.8 (3.8)	11.6 (3.3)	.34
Monounsaturated fatty acids			
Palmitoleic acid (16:1 n-7)	2.3 (0.9)	2.3 (0.9)	.85
Oleic acid (18:1)	20.2 (3.8)	20.3 (3.7)	.53
Polyunsaturated fatty acids			
Linoleic acid (18:2 n-6)	24.6 (5.5)	25.1 (5.3)	.20
γ linolenic acid (18:3 n-6)	0.4 (0.2)	0.4 (0.3)	.72
Arachidonic acid (20:4 n-6)	6.6 (2.1)	6.9 (1.8)	.17
α linolenic acid (18:3 n-3)	0.4 (0.3)	0.4 (0.2)	.58
Eicosapentaenoic acid (20:5 n-3)	1.0 (0.6)	1.0 (0.6)	.40
Docosapentaenoic acid (22:5 n-3)	0.5 (0.1)	0.5 (0.2)	.98
Docosahexaenoic acid (22:6 n-3)	2.4 (0.8)	2.4 (0.8)	.81

NOTE. Values are mean (standard deviation). P values were estimated using univariate Cox proportional hazard models with delayed entry (and age as a time scale).

CSF Vitamin D – CSF AMYLOID BETA CORRELATION



- The correaltion of CSF vitamin D (25OHD) and CSF amyloid beta 1-42 levels in 50 patients with dementia (AD or Non-AD) (N=50; r = 0.3726, p=0.0077)
- *Season adjusted 25OHD levels
- *Unpublished data*

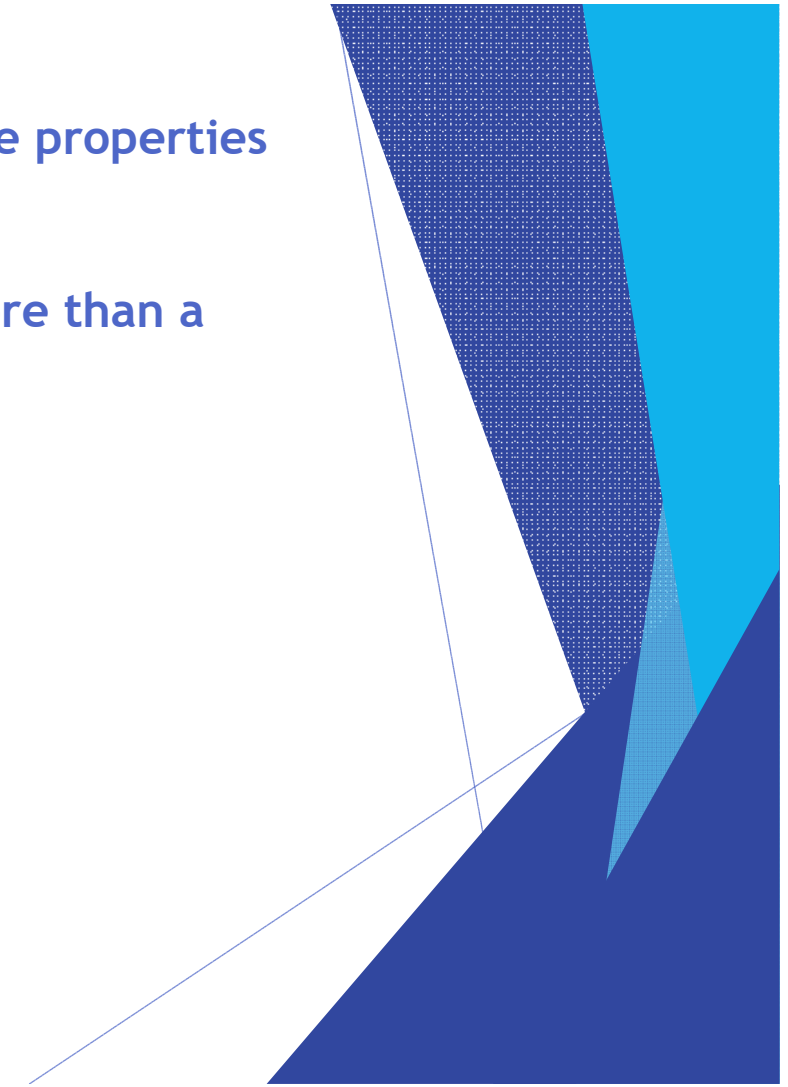
Growing evidence suggests a neurosteroid like properties for vitamin D.

Yet sceptics are asking the same question more than a decade:

Is it really there?

In other words:

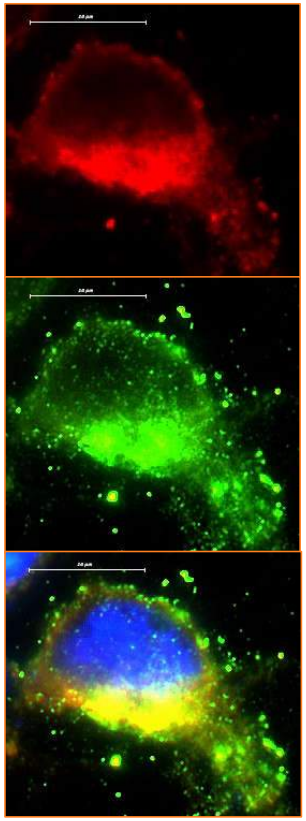
(Does vitamin D have an action in brain as we know it?)



The cerebral expression of vitamin D-associated enzymes and receptors?

VITAMIN D RECEPTORS

- Vitamin D, regulates over 1.000 genes in different tissues and in different conditions via a nuclear hormone receptor which is **vitamin D receptor (VDR)** and via its suggested membrane receptor **(1,25MARRS)**
  - Vitamin D receptor
    - Location: membrane lipid rafts?, cytoplasm and nucleus
    - Genomic function - Transcription factor (cytoplasmic or nuclear VDR)
    - Fast non genomic function - Induction of various signalling pathways (membrane VDR)
  - Membrane receptor (membrane associated rapid response steroid binding protein-1,25 MARRS), ERp57, Grp58, Pdia3
    - Location: membrane lipid rafts, ER and nucleus
    - Genomic function - Transcription factor
    - Fast non genomic function - Induction of various signalling pathways
    - Protein folding



Magnification x100, I/3 filter, Alexafluor 488 tagged anti-VDR (green); TX filter, Alexafluor 568 tagged anti-1,25-MARRS (red). Overlay picture: VDR and 1,25-MARRS colocalization (yellow)

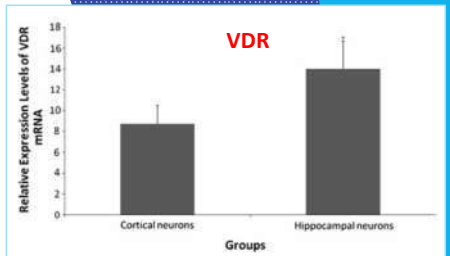


Fig. 1 VDR expression levels in cortical and hippocampal neurons. VDR mRNA level in hippocampal neurons was higher than cortical neurons ($p = 0.0012$). Data are presented as a mean SD

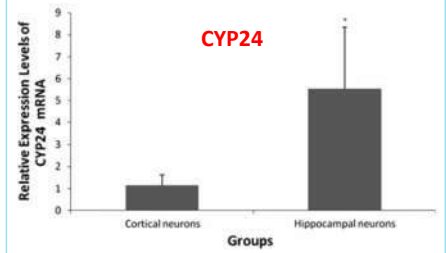
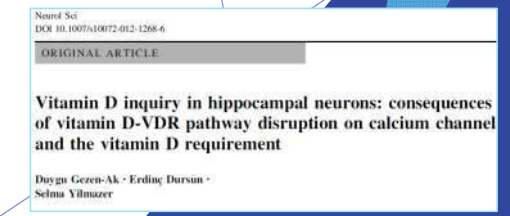


Fig. 2 CYP24 expression levels in cortical and hippocampal neurons. CYP24 mRNA level in hippocampal neurons was higher than cortical neurons ($p = 0.0038$, adjusted with Welch correction). Data are presented as a mean SD



Neurol Sci
DOI 10.1007/s10072-012-1268-6

ORIGINAL ARTICLE

Vitamin D inquiry in hippocampal neurons: consequences of vitamin D-VDR pathway disruption on calcium channel and the vitamin D requirement

Duygu Gezen-Ak · Erdinç Dursun · Selma Yilmazer

Long before us:

- Bidmon et al., 1991; Musiol et al., 1992; Stumpf and O'Brien, 1987
  - ***Initial identification of the cells that contains VDR in the brains*** of rats and hamsters
  - Radiolabeled 1,25(OH)2D3 and autoradiography

- The presence of the VDR was confirmed in the brains of mice, rats, chicks and humans
  - when a specific antibody against the VDR was developed
  - Eyles et al., 2005; Prufer et al., 1999; Sutherland et al., 1992; Veenstra et al., 1998; Walbert et al., 2001; Zanello et al., 1997.

- In the adult rodent brain,
- the VDR is located within different cell types, including
  - neurons, astrocytes (Brown et al., 2003; Cui et al., 2013; Eyles et al., 2005),
  - oligodendrocytes (Baas et al., 2000)
  - in multiple brain regions (Prufer et al., 1999; Veenstra et al., 1998).

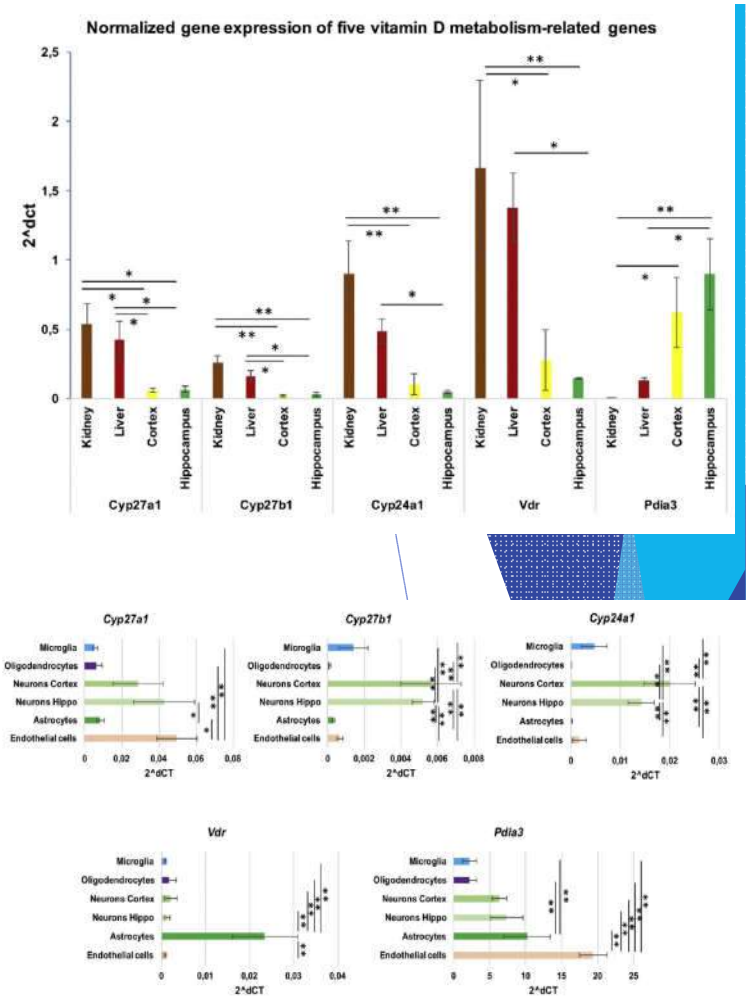
Landel study (2018)

- Compared the transcript expression of
  - Cyp27a1, Cyp27b1, Cyp24a1, VDR and Pdia3
  - in purified cultures of ***astrocytes***, ***endothelial cells***, ***microglia***, ***neurons*** and ***oligodendrocytes***.

- Observed that
- **Endothelial cells** and **neurons** can possibly **transform the inactive cholecalciferol into 25(OH)D3.**
- **Neurons or microglia** can metabolise **25(OH)D3** into **1,25(OH)2D3**,

- Alternatively,
- **1,25(OH)2D3** can induce **autocrine or paracrine rapid non-genomic actions** via **PDIA3** whose transcript is abundantly expressed in all cerebral cell types.

- Their data indicate that,
- within the brain,
- vitamin D may trigger **major auto-/paracrine non genomic actions**, in addition to **its well documented activities as a steroid hormone**.

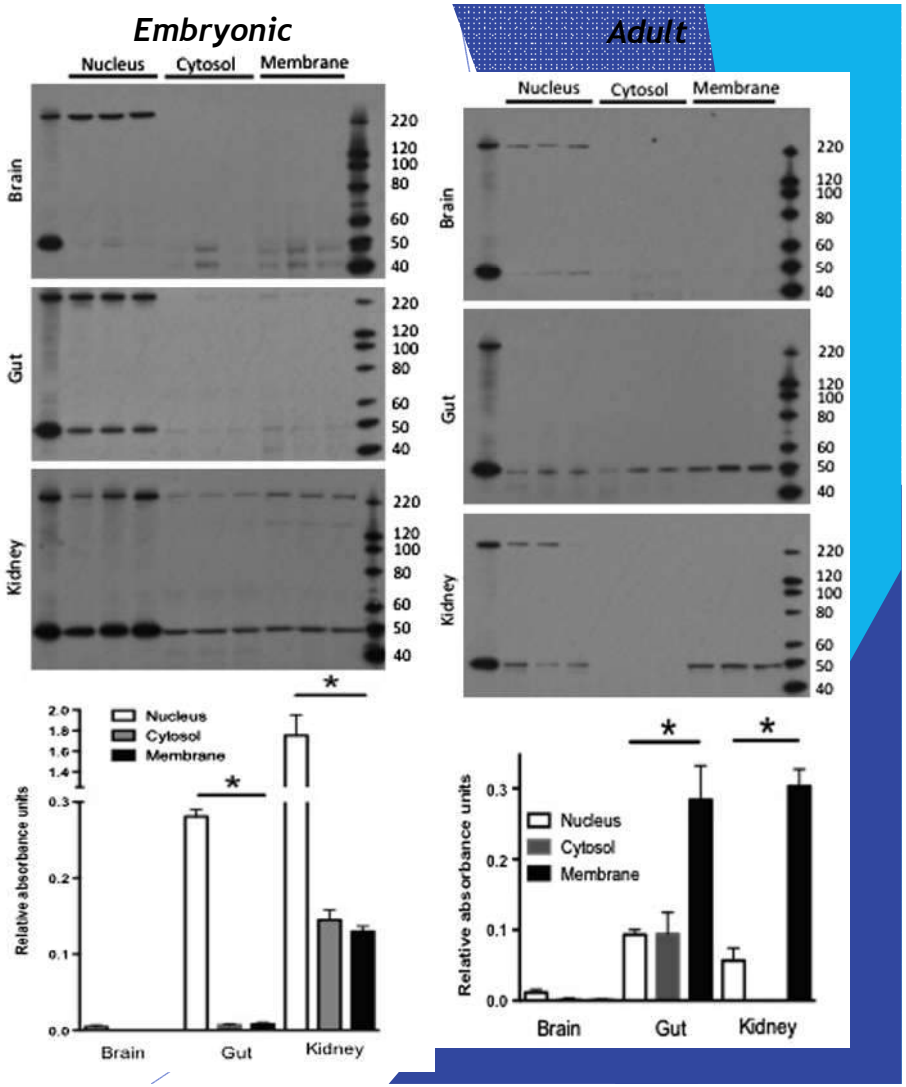


Landel V. et al. Journal of Steroid Biochemistry and Molecular Biology 177 (2018) 129-134

The subcellular location of VDR

- 2014. Eyles D.W. et al. demonstrated that,
- in all **embryonic tissues**
  - VDR distribution **is mostly nuclear**,
- however by **adulthood**
  - at least in the gut and kidney,
  - VDR presence **in the plasma membrane** is more prominent
  - (indicating some change in VDR function with the maturation of these tissues?)
- The subcellular distribution of VDR in the embryo
- did not appear to be altered by vitamin D deficiency
  - indicating that perhaps there are other mechanisms at play in vivo to stabilize this receptor in the absence of its ligand.

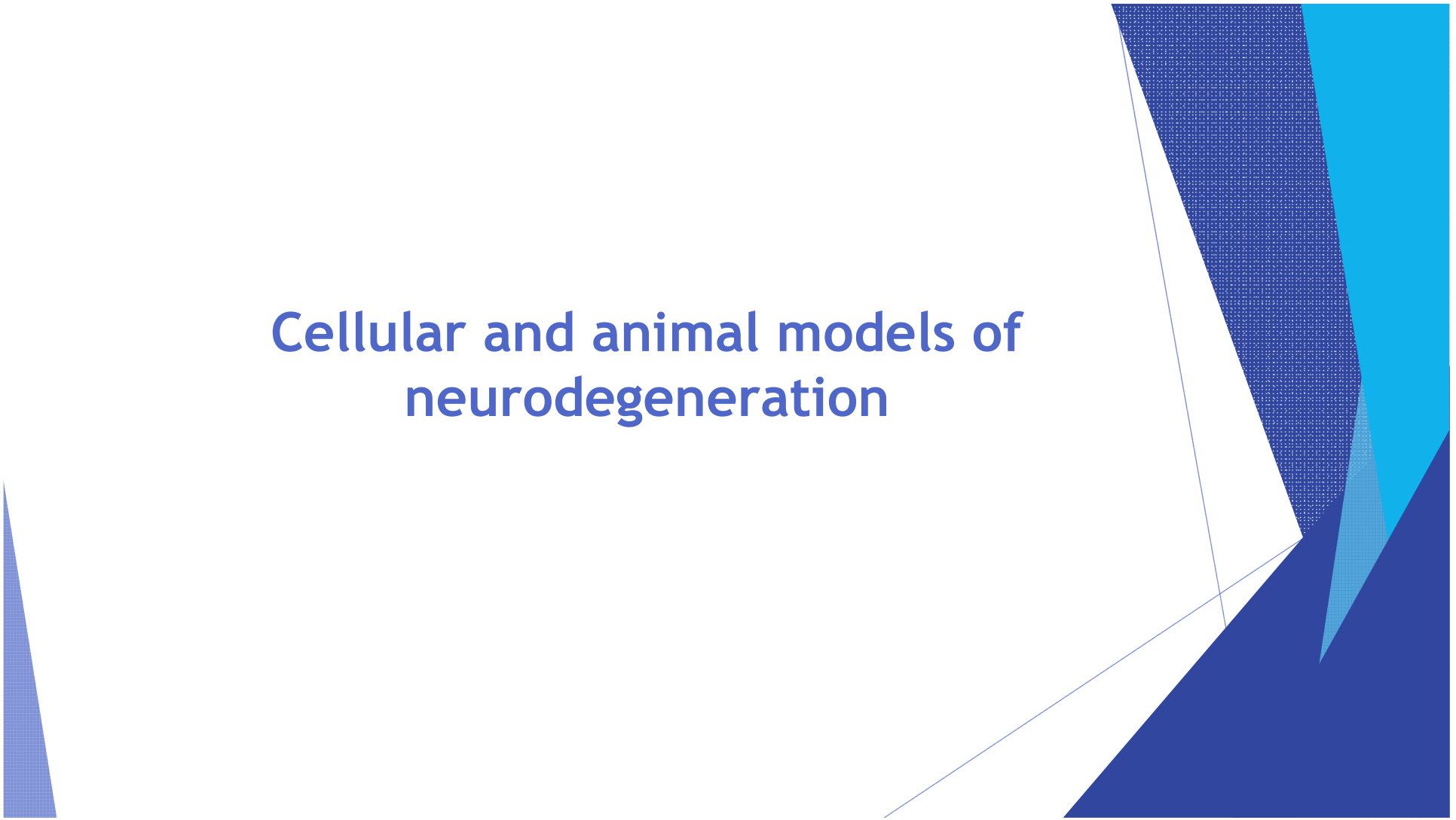
Eyles D.W. et al. Neuroscience 268 (2014) 1-9

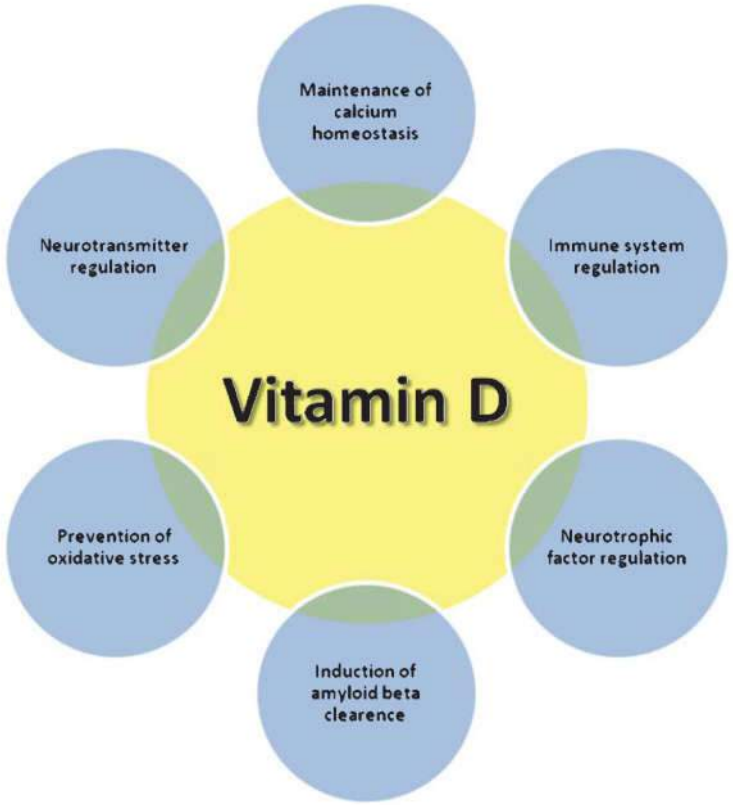


Subsection conclusion:

- The location of VDR and PDIA3 is well established in CNS,
- The location and action of vitamin D metabolism related enzymes including **Cyp27a1, Cyp27b1, Cyp24a1** are demonstrated in major cell types of CNS
- Vitamin D has **major auto-/paracrine non genomic actions**, in addition to **its well documented activities as a steroid hormone in CNS**

Cellular and animal models of neurodegeneration





Journal of Alzheimer's Disease 40 (2014) 257–269
DOI 10.3233/JAD-131970
IOS Press

Hypothesis

Why Vitamin D in Alzheimer's Disease? The Hypothesis

Duygu Gezen-Ak*, Selma Yılmazer and Erdinç Dursun*

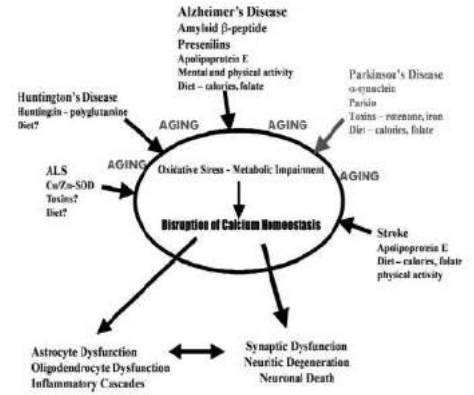
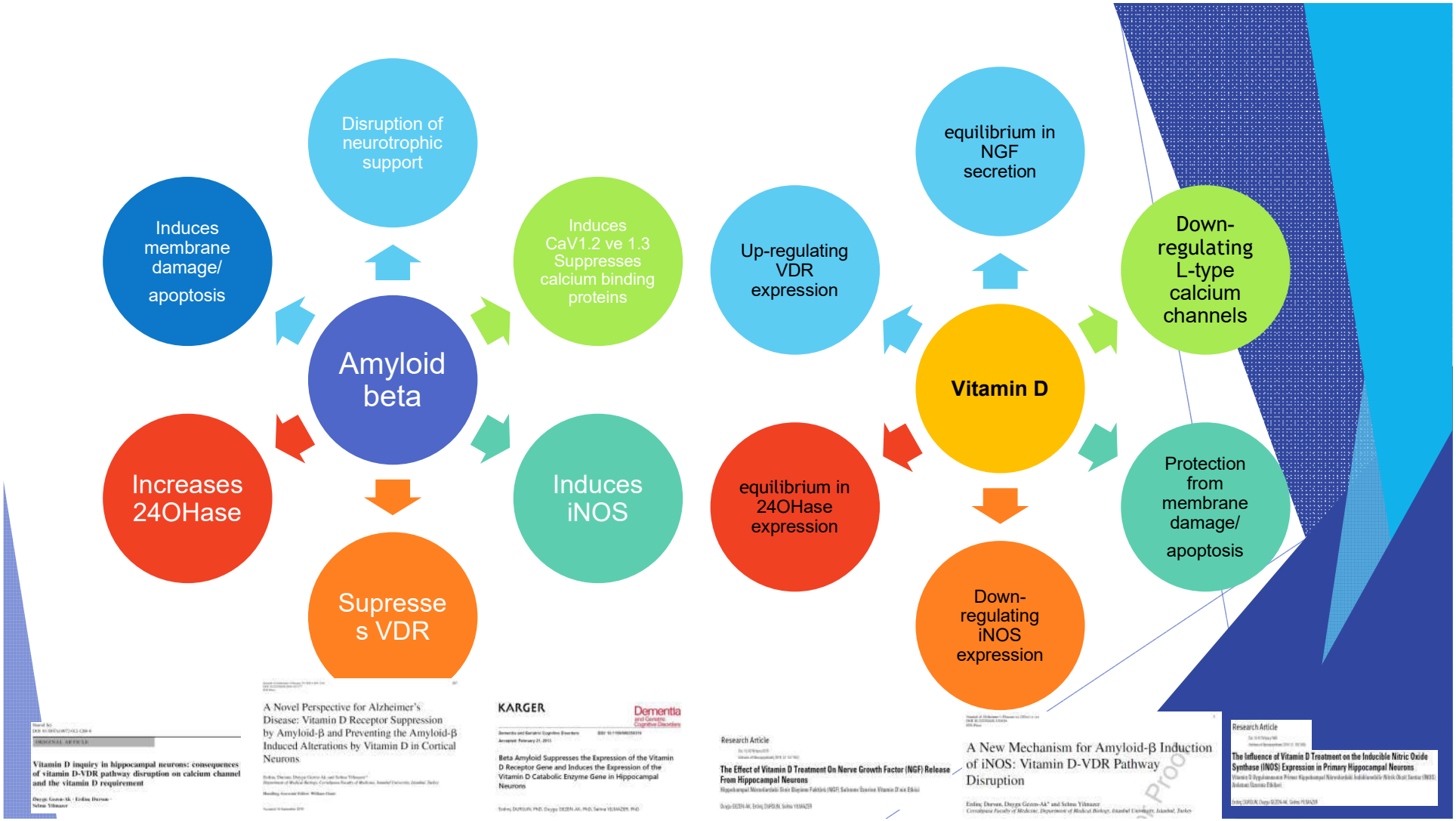
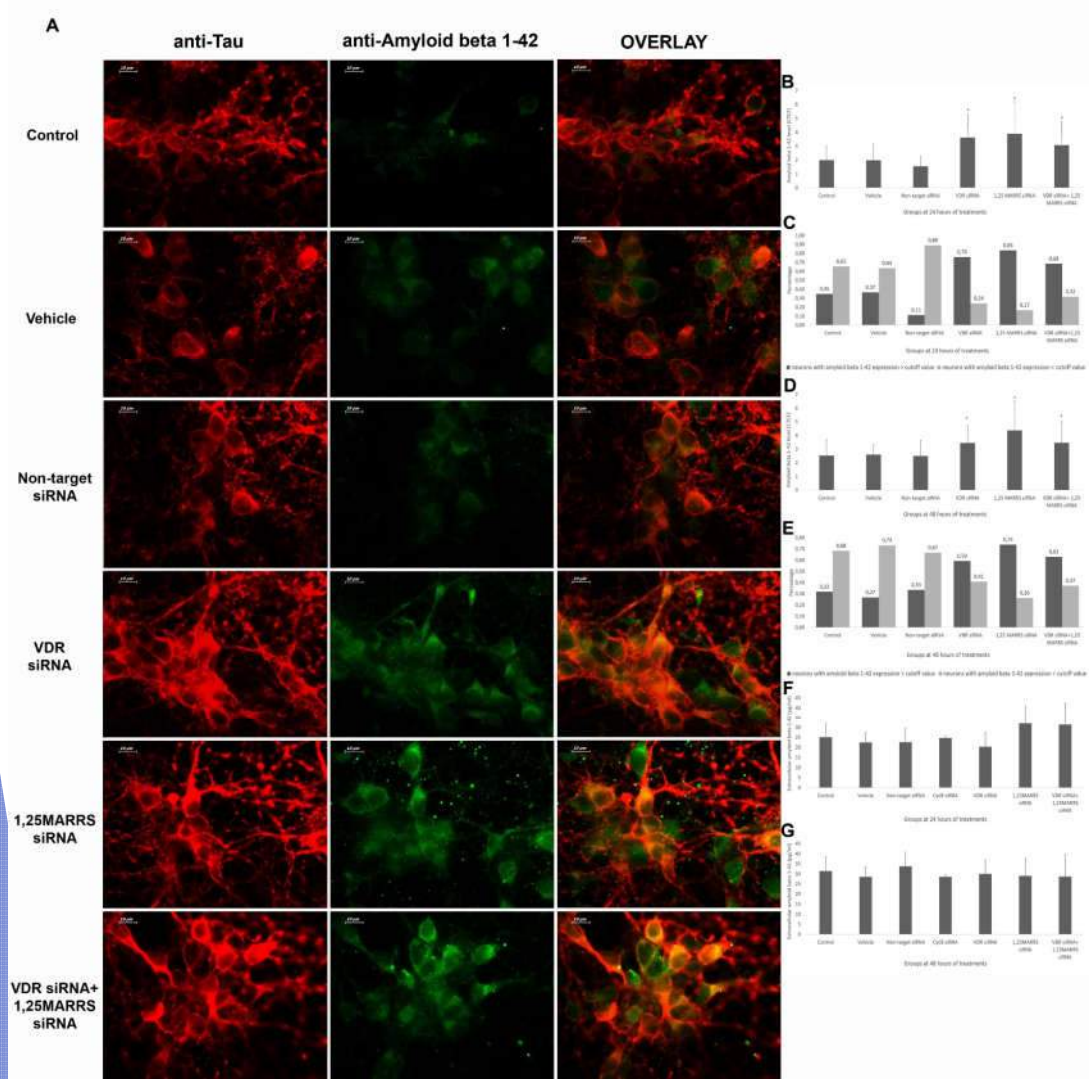


Fig. 1. Central role of altered cellular calcium homeostasis in the pathogenesis of different age-related neurodegenerative disorders. Different genetic and environmental factors may cause or increase the risk of specific neurodegenerative disorders. However, each factor cooperates with age-related increases in oxidative stress and metabolic compromise to disrupt neuronal calcium homeostasis resulting in synaptic dysfunction and cell death. Alterations in glial cell calcium homeostasis may occur and contribute to inflammatory processes and white matter damage in neurodegenerative disorders.


Amyloid beta
Disruption of neurotrophic support
Induces membrane damage/ apoptosis
Induces CaV1.2 ve 1.3 Suppresses calcium binding proteins
Increases 24OHase
Induces iNOS
Supresse s VDR
Vitamin D
equilibrium in NGF secretion
Up-regulating VDR expression
Down-regulating L-type calcium channels
equilibrium in 24OHase expression
Protection from membrane damage/ apoptosis
Down-regulating iNOS expression
Vitamin D inquiry in hippocampal neurons: consequences of vitamin D-VDR pathway disruption on calcium channel and the vitamin D requirement
A Novel Perspective for Alzheimer's Disease: Vitamin D Receptor Suppression by Amyloid-β and Preventing the Amyloid-β Induced Alterations by Vitamin D in Cortical Neurons
KARGER
Dementia and Geriatric Cognitive Disorders
Beta Amyloid Suppresses the Expression of the Vitamin D Receptor Gene and Induces the Expression of the Vitamin D Catabolic Enzyme Gene in Hippocampal Neurons
Research Article
The Effect of Vitamin D Treatment On Nerve Growth Factor (NGF) Release From Hippocampal Neurons
A New Mechanism for Amyloid-β Induction of iNOS: Vitamin D-VDR Pathway Disruption
Research Article
The Influence of Vitamin D Treatment on the Inducible Nitric Oxide Synthase (iNOS) Expression in Primary Hippocampal Neurons



ACS Chemical Neuroscience
Research Article
pubs.acs.org/chemneuro

Vitamin D Receptor Regulates Amyloid Beta 1−42 Production with Protein Disulfide Isomerase A3

Duygu Gezen-Ak,[†] Irem L. Atasoy,[‡] Esin Candas,[†] Merve Alaylioglu,[†] Selma Yilmazer,[†] and Erdinç Dursun[*,†]

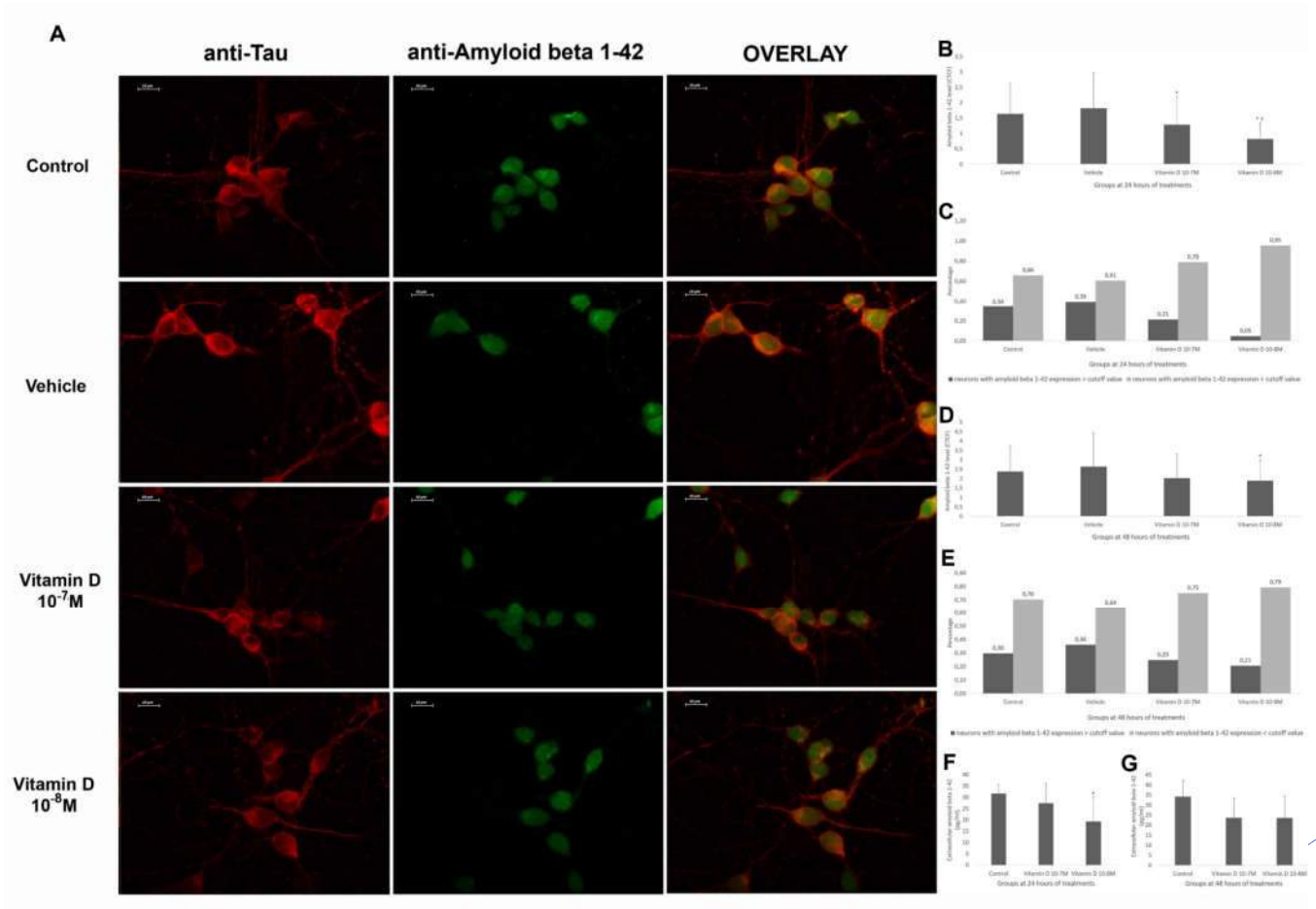
The corrected total cell fluorescence (CTCF) was determined and calculated as
CTCF = integrated density (area of selected cell × mean fluorescence of background readings)

Groups in 24h	The percentage of induction in amyloid beta 1-42 levels
VDR siRNA treated neurons	189% induction
1,25MARRS (PDIA3) siRNA treated neurons	205% induction
VDR siRNA + 1,25MARRS (PDIA3) siRNA treated neurons	163% induction

Groups in 24h	The percentage of cells that express amyloid beta 1-42 higher than the cuttoff value
VDR siRNA treated neurons	76% of the cells
1,25MARRS (PDIA3) siRNA treated neurons	83% of the cells
VDR siRNA + 1,25MARRS (PDIA3) siRNA treated neurons	68% of the cells

Groups in 48h	The percentage of induction in amyloid beta 1-42 levels
VDR siRNA treated neurons	136% induction
1,25MARRS (PDIA3) siRNA treated neurons	172% induction
VDR siRNA + 1,25MARRS (PDIA3) siRNA treated neurons	136% induction

Groups in 48h	The percentage of cells that express amyloid beta 1-42 higher than the cuttoff value
VDR siRNA treated neurons	59% of the cells
1,25MARRS (PDIA3) siRNA treated neurons	74% of the cells
VDR siRNA + 1,25MARRS (PDIA3) siRNA treated neurons	63% of the cells



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Research Article
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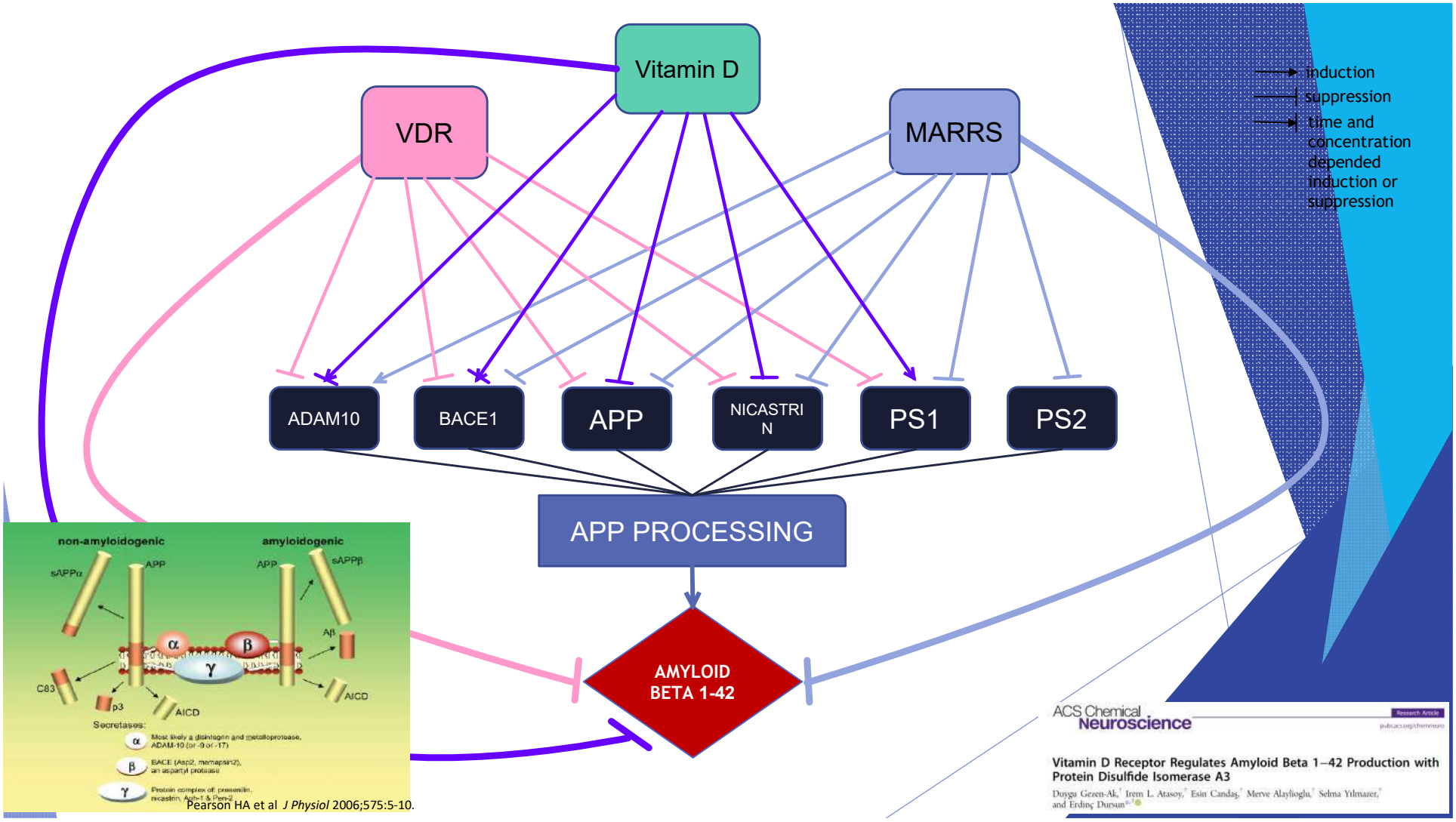
Vitamin D Receptor Regulates Amyloid Beta 1−42 Production with Protein Disulfide Isomerase A3

Duygu Gezen-Ak,† Irem L. Atasoy,† Esin Candas,† Merve Alaylioglu,† Selma Yilmazer,† and Erdinç Dursun*,†

the corrected total cell fluorescence (CTCF) was determined and calculated as

CTCF = integrated density − (area of selected cell × mean fluorescence of background readings)

Groups in 24h	The percentage of reduction in amyloid beta 1-42 levels
10^{-7}M 1,25(OH)$_2$D$_3$ treated neurons	27% reduction
10^{-8}M 1,25(OH)$_2$D$_3$ treated neurons	50% reduction
Groups in 24h	The percentage of cells that express amyloid beta 1-42 lower than the cuttoff value
10^{-7}M 1,25(OH)$_2$D$_3$ treated neurons	79% of the cells
10^{-8}M 1,25(OH)$_2$D$_3$ treated neurons	95% of the cells
Groups in 48h	The percentage of reduction in amyloid beta 1-42 levels
10^{-7}M 1,25(OH)$_2$D$_3$ treated neurons	17% reduction
10^{-8}M 1,25(OH)$_2$D$_3$ treated neurons	21% reduction
Groups in 48h	The percentage of cells that express amyloid beta 1-42 lower than the cuttoff value
10^{-7}M 1,25(OH)$_2$D$_3$ treated neurons	75% of the cells
10^{-8}M 1,25(OH)$_2$D$_3$ treated neurons	79% of the cells



Pearson HA et al *J Physiol* 2006;575:5-10.

ACS Chemical Neuroscience

Research Article

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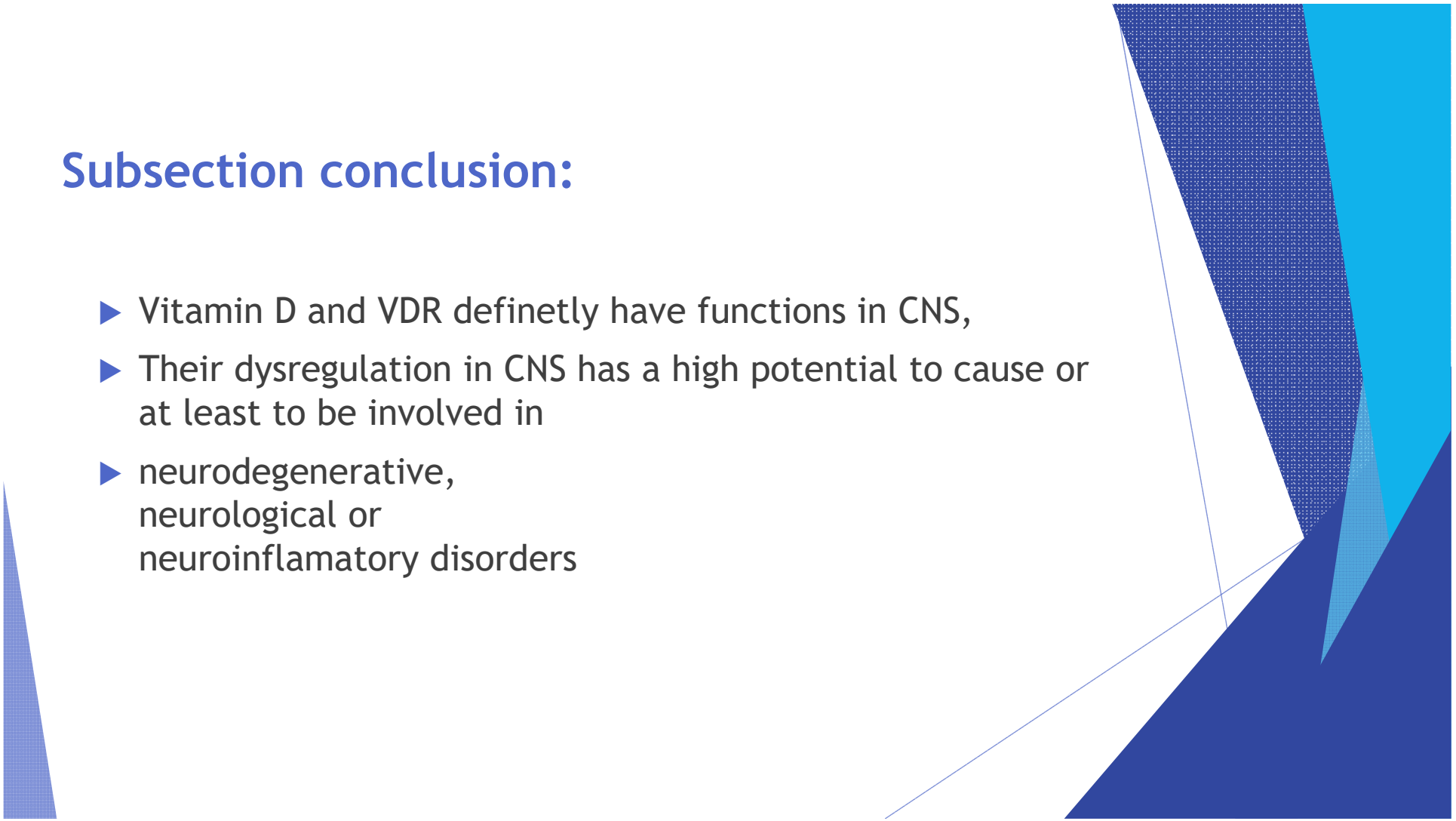
Subsection conclusion:

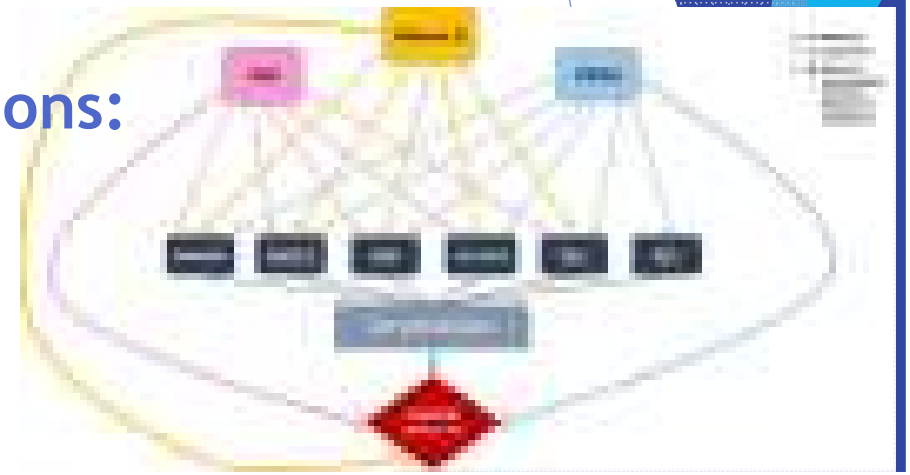
- Vitamin D and VDR definetly have functions in CNS,
- Their dysregulation in CNS has a high potential to cause or at least to be involved in
- neurodegenerative,
  neurological or
  neuroinflamatory disorders



Neurodegeneration:
Loss of function - death of a neuron

What does vitamin D do in a neuron?
How does it do that?

Future Directions:



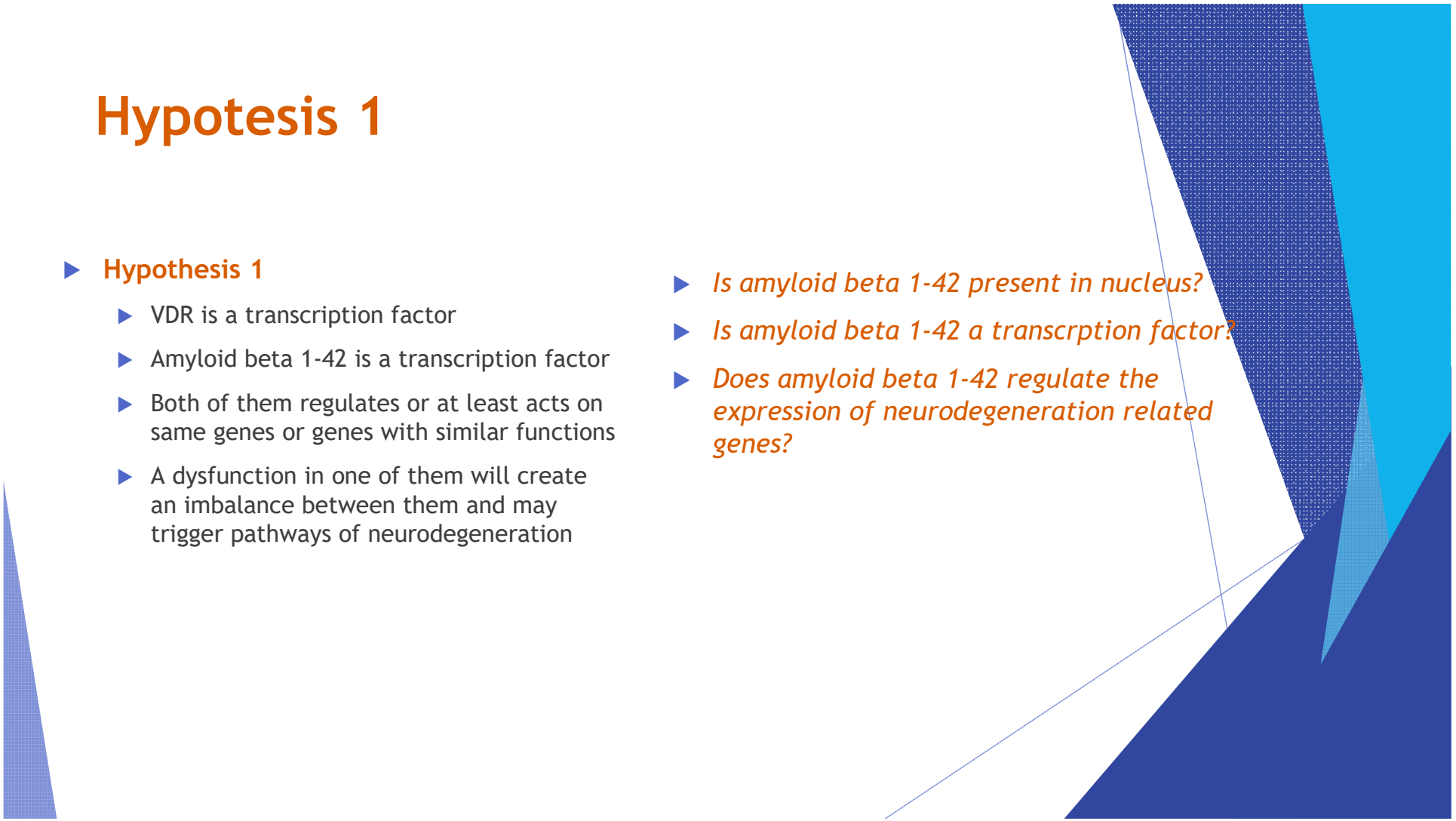
Novel properties of vitamin D and its receptors may emerge from the relation between amyloid beta and vitamin D?

- **Hypothesis 1**
  - VDR is a transcription factor!
  - Amyloid beta 1-42 is a transcription factor?
  - Both of them regulates or at least acts on same genes or genes with similar functions?
  - A dysfunction in one of them will create an imbalance between them and may trigger pathways of neurodegeneration?
- **Hypothesis 2**
  - VDR is located on neuronal plasma membrane!
  - VDR contributes to the action of the proteins involved in amyloidogenic or non-amyloidogenic pathways located in neuornal plasma membrane!
  - Vitamin D deficiency or VDR dysfunction may contribute to dysfunction of these pathways!

Hypotesis 1

- **Hypothesis 1**
  - VDR is a transcription factor
  - Amyloid beta 1-42 is a transcription factor
  - Both of them regulates or at least acts on same genes or genes with similar functions
  - A dysfunction in one of them will create an imbalance between them and may trigger pathways of neurodegeneration

- *Is amyloid beta 1-42 present in nucleus?*
- *Is amyloid beta 1-42 a transcrption factor?*
- *Does amyloid beta 1-42 regulate the expression of neurodegeneration related genes?*



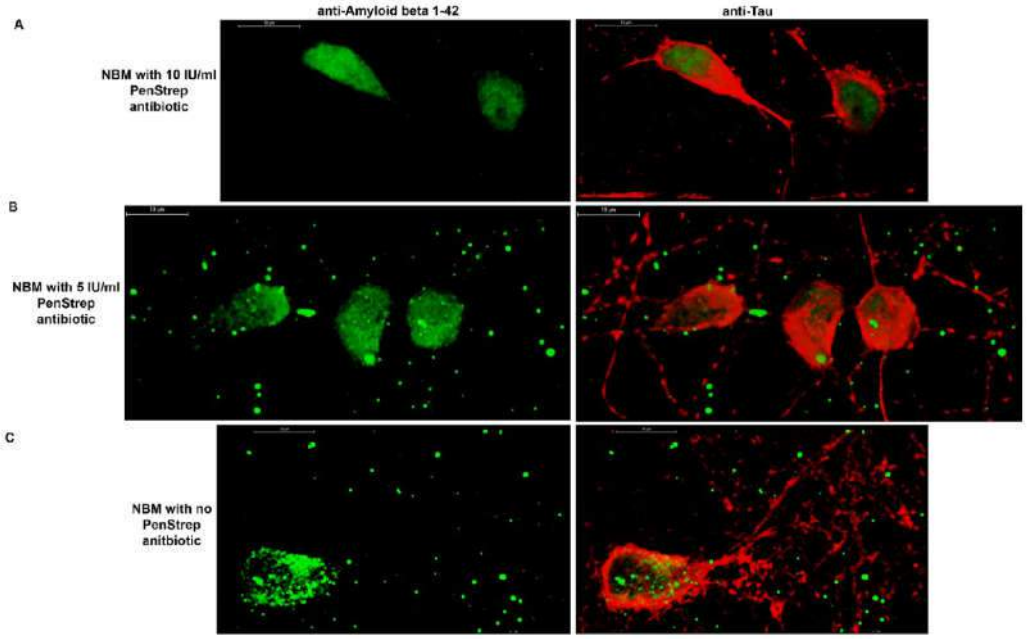


Figure 5. Aβ 1−42 localization depends on antibiotic (PenStrep) administration. Immunofluorescent labeling of Aβ 1−42 (green). Tau46 (red) was counter-labeled as a neuronal marker, 63× (confocal microscopy images). (A) Neurons treated with 10 IU/ml PenStrep. Aβ 1−42 is localized both in the cytoplasm and nucleus. The immunoreactivity was strong in the nucleus. (B) Neurons treated with 5 IU/mL PenStrep. Aβ 1−42 is localized in the cytoplasm and nucleus. The immunoreactivity was moderate in the nucleus compared with the 10 IU/ml PenStrep-treated neurons. (C) Untreated neurons. Aβ 1−42 is mostly localized in the cytoplasm, and weak expression was detected in the nucleus. The data indicated that the localization of Aβ 1−42 changed in 10 IU/mL PenStrep-treated neurons compared to neurons that were not treated with PenStrep, but no significant difference in the CTCF of Aβ 1−42 was found in these groups.

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THE ALZHEIMER'S AMYLOID β–PEPTIDE (Aβ) BINDS A SPECIFIC DNA Aβ–INTERACTING DOMAIN (AβID) IN THE *APP*, *BACE1*, AND *APOE* PROMOTERS IN A SEQUENCE–SPECIFIC MANNER: CHARACTERIZING A NEW REGULATORY MOTIF

Bryan Maloney[1] and **Debomoy K. Lahiri**[1,2,*]

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Abstract

Deposition of extracellular plaques, consisting of amyloid β peptide (Aβ), in the brain is the confirmatory diagnostic of Alzheimer's disease (AD); however, the physiological and pathological role of Aβ is not fully understood. Herein, we demonstrate novel Aβ activity as a putative transcription factor upon AD–associated genes. We used oligomers from 5'–flanking regions of the apolipoprotein E (*APOE*), Aβ–precursor protein (*APP*) and β–amyloid site cleaving enzyme–1 (*BACE1*) genes for electrophoretic mobility shift assay (EMSA) with different fragments of the Aβ peptide. Our results suggest that Aβ bound to an Aβ–interacting domain (AβID) with a consensus of "KGGRKTGGGG". This peptide–DNA interaction was sequence specific, and mutation of the first "G" of the decamer's terminal "GGGG" eliminated peptide–DNA interaction. Furthermore, the cytotoxic Aβ25–35 fragment had greatest DNA affinity. Such specificity of binding suggests that the AβID is worth of further investigation as a site wherein the Aβ peptide may act as a transcription factor.

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Nuclear Translocation Uncovers the Amyloid Peptide Aβ42 as a Regulator of Gene Transcription*•

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Christian Barucker[‡§], **Anja Harmeier**[‡1], **Joerg Weiske**[¶2], **Beatrix Fauler**[‖], **Kai Frederik Albring**[¶**], **Stefan Prokop**[‡‡], **Peter Hildebrand**[§§], **Rudi Lurz**[‖], **Frank L. Heppner**[‡‡], **Otmar Huber**[¶**], **and Gerhard Multhaup**[‡§3]

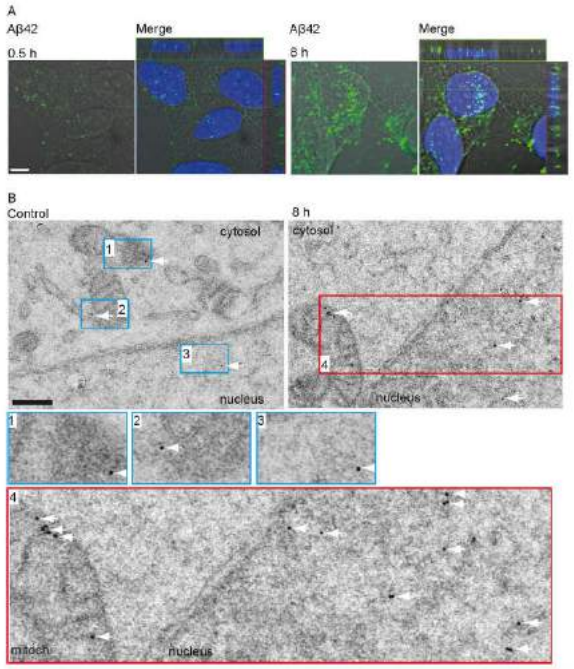


FIGURE 4. **Microscopic demonstration of intranuclear Aβ *in vitro*.** *A*, confocal laser scanning of SH-SY5Y cells treated with N-terminally biotinylated Aβ42. Z-scan images were taken from the top to the bottom of the cells to generate a pseudo three-dimensional image. Images taken in the center of a Z-scan, indicated by the cross in the orthogonal construction (*merge*), revealed Aβ42 peptides in the nucleus after 0.5 and 8 h of incubation time. Particularly after 8 h, the accumulation of Aβ is visible in the cytoplasm as well as in the nucleus. *Scale bar*, 5 μm. *B*, electron micrographs of SH-SY5Y cells that were either untreated (control) or treated with Aβ42 for 8 h. Staining shows endogenous Aβ in the cytoplasm (*1*), mitochondria (*2*), and the nucleus (*3*) of untreated SH-SY5Y cells; treatment of cells with Aβ42 for 8 h resulted in stronger signals of Aβ in the nucleus as well as in cytoplasm and mitochondria (*4*); see also enlarged images *1*–*4* for better visibility. *Scale bar*, 500 nm.

PLOS ONE

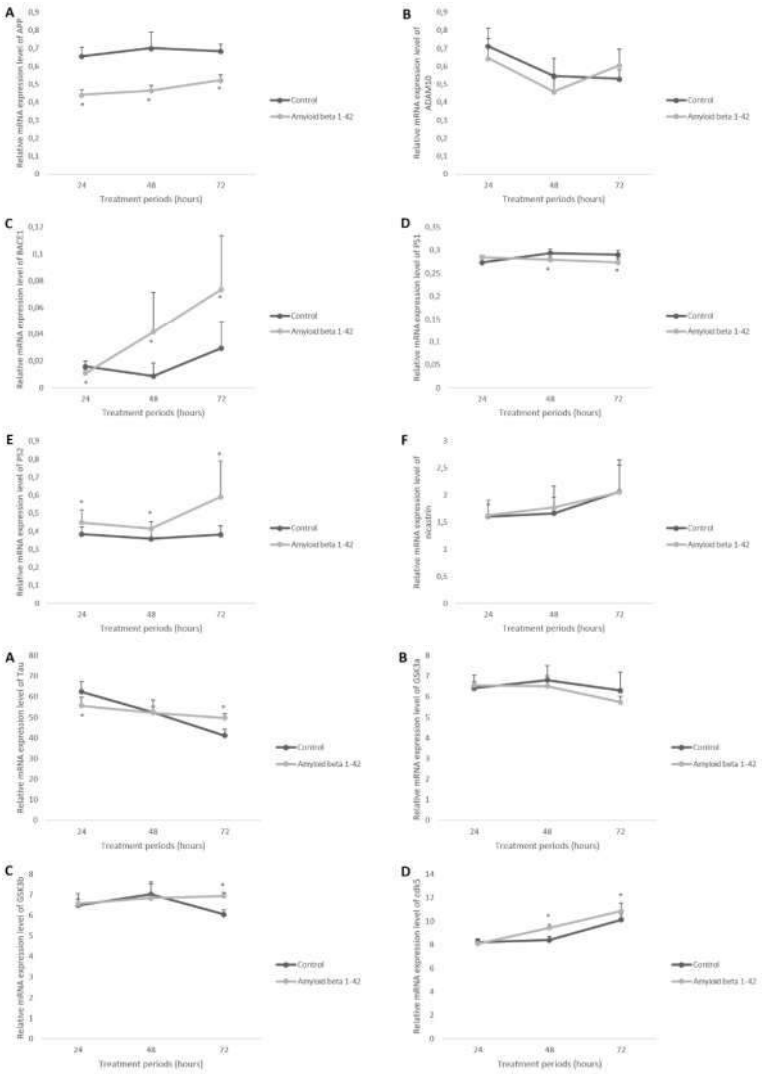
RESEARCH ARTICLE

Vitamin D receptor is present on the neuronal plasma membrane and is co-localized with amyloid precursor protein, ADAM10 or Nicastrin

Erdinç Dursun, Duygu Gezen-Ak*

Supplementary Table 1: The FpClass PPI prediction tool was used to identify partner proteins for both APP and VDR. The tool predicted 1133 partners for APP and 583 partners for VDR. An analysis of the FpClass tool data indicated that 153 of these partners interacted with both APP and VDR. A total of 153 proteins were classified according to their functions.

Protein Translation/ Modification	MEMBRANE/membrane related proteins	TRANSCRIPTION FACTORS/ REGULATION		NFκbeta pathway	Nuclear receptors	Cell cycle/ Apoptosis	Cytokines/ Immune response	Intracellular signalling pathways		Chaperons	Proteosome pathway	Cytoskeleton
RPL11	NUMB	JUN	IRF1	RELA	PPARG	CDK1	TGFBR1	SRC	ZFYVE9	HSP90AA1	COPS5	VIM
EIF2AK2	CTNNB1	FOS	IRF3	TAB2	AHR	CDK5	TRAF6	MAPK1	STRAP	HSP90B1	UBE2I	ACTB
SUMO1	NOTCH1	NR3C1	HIF1A	IKBKB	ESR1	PTEN	IL8	MAPK8	SRGAP3	HSF1	PSME3	
	CDH1	ATF2	PARP1	IKBKG	(ERalpha)	TP53	PTMA	MAPK9	PTK2	HSPA1B	PSMC5	
	GHR (Somatotropin receptor)	ATF3	EDF1	NFKB1	NR2F2	RB1	IFNB1	MAPK11	PIK3R1	HSPB1	MDM2	
	FHL2	ETS1	HNF1A	CHUK	NCOA1	TP53BP2		MAPK14	VRK1			
		ETS2	TOP1	BCL3	NCOA3	CDKN1A		STAT1	AXIN1			
		ATF4	FOXO1			CDC25C		STAT3	RPS6KA1			
		JUNB	NFE2			MYB		PIAS1	RPS6KA3			
		POLR2A	NKX2-1			MYC		GSK3B	S100B			
		MED28	NFATC2			NDN		PIN1	TNIK			
		NCOR2	CREBBP			RB1CC1		ABL1	STK4			
		SIRT1	SMYD2			WWOX		PRKCA	STK11			
		RUNX1	SNAI1			BRCA1		PRKCD	PPP1CC			
		RUNX2	YBX1			BCL2		CSNK1A1	(PP-1G)			
		EP300	XRCC6			BCL2L1		CSNK1D	TXN			
		NCL	GTF2B			DAXX		CSNK2A1	CTSL1			
		RELB	SMARCA4			CCND1		CSNK2A2				
		PAX6	SMARCE1			CCNE1		CSK				
		FANCA	RAD51			NOL3		SMAD2				
		SP1	SPEN			ING1		SMAD3				
		NFYA	CTBP2			PLK1						
		ELK1	(Synaptic ribbon)									
		TBP	RFWD2									
		HDAC4	KHDRBS1									
		HDAC6	BAZ2A									
		HDAC7										
		NFE2L2										
		BCL6										



NeuroMolecular Medicine (2018) 20:363–375
https://doi.org/10.1007/s12017-018-8498-6

ORIGINAL PAPER

The Transcriptional Regulatory Properties of Amyloid Beta 1–42 may Include Regulation of Genes Related to Neurodegeneration

Duygu Gezen-Ak[1] · İrem L. Atasoy[1] · Esin Candaş[1] · Merve Alaylıoğlu[1] · Erdinç Dursun[1]

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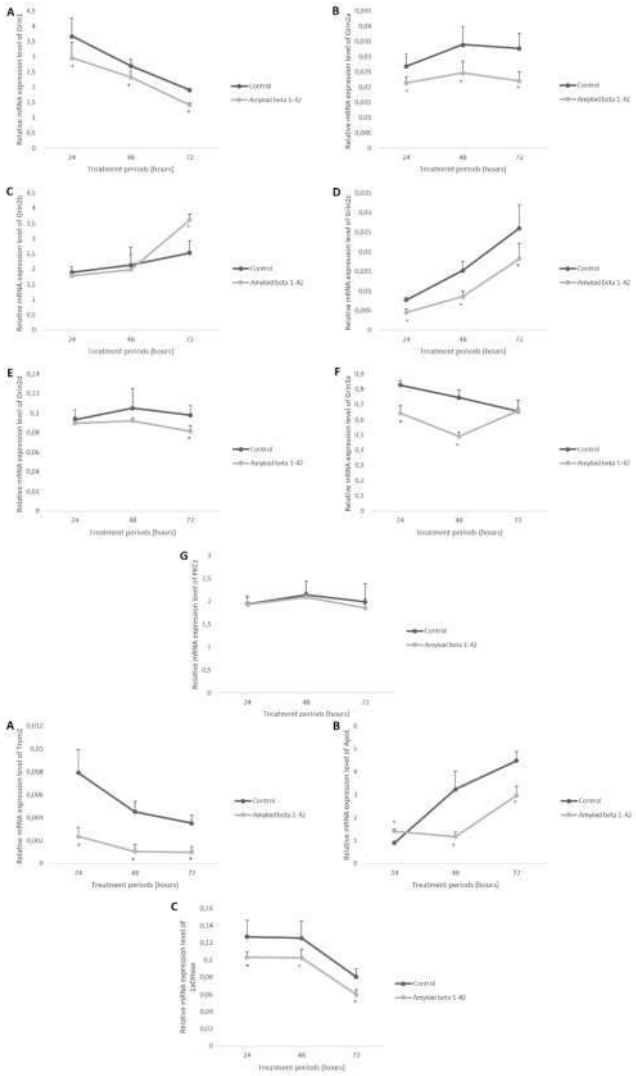
- *alpha secretase (ADAM10),*
- *beta secretase (BACE1),*
- *the gamma secretase complex (PS-1, PS-2, Nicastrin),*
- *the substrate APP,*
- *APOE* (the significant risk factor for sporadic form of the AD),
- *TREM2* (recently indicated as a contributor to AD risk), the
- *NMDR genes Grin1, Grin2a, Grin2b, Grin2c, Grin2d, Grin3*
- *PKCzeta* as contributors of memory and learning,
- key elements of tau pathology such as *tau*, *GSK3α*, *GSK3β* and *Cdk5*,
- cholecalciferol metabolism-related enzyme 1α hydroxylase (1α OHase-encoded by *CYP27b1 gene*).

- **Needs confirmation with ChIP**



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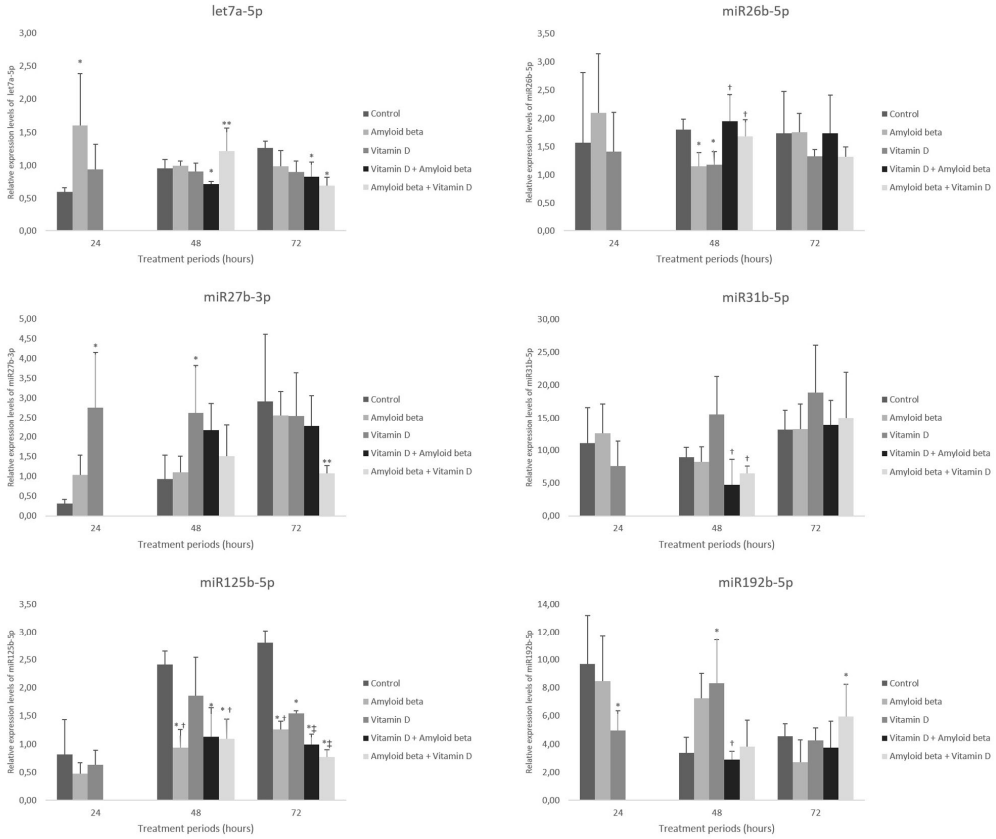
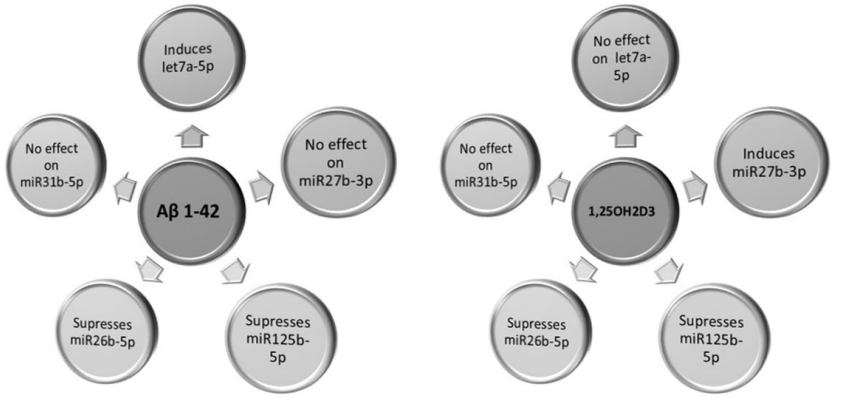
- If amyloid beta 1-42 is a trancription factor then it may have important functions beyond todays knowledge.
- If that is the case then treatments targeting total elimination of amyloid beta might be reconsidered!
- High amount of amyloid beta 1-42 may increase its production working as a transcription factor and change the expression of neurodegeneration promoting genes?
- We know that VDR regulates most of these genes that is foretold.
- If VDR and amyloid beta 1-42 effects the transcription of the same genes then the absence of one may disrupt the balance in neurons.
- Vitamin D deficiency or VDR dysfunction may promote this imbalance
- The presence of amyloid beta 1-42 it self reduces VDR expression and vitamin D production and induces vitamin D catabolism.

Besides transcriptional regulation...
Vitamin D and amyloid beta have a cross talk over post transcriptional regulation via miRNAs



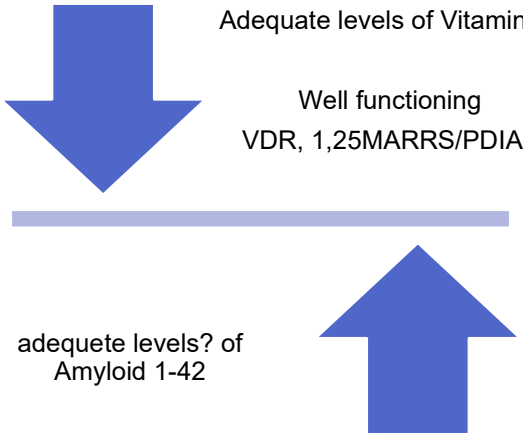


let-7a-5p, mir-26b-5p, mir-27b-3p, mir-31-5p, mir-125b-5p, mir-192-5p,

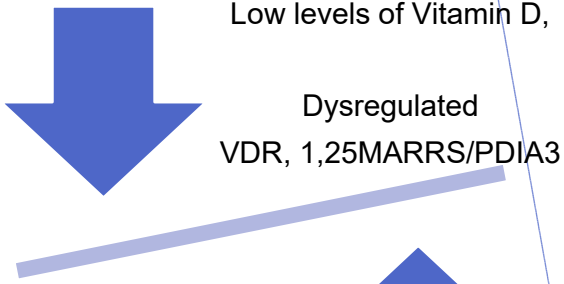
are suggested to be related with

- vitamin D metabolism,
- neuronal differentiation,
- development
- and memory

Healthy cell, healthy cell maintanence and aging



Loss of cell maintanence, neurodegeneration

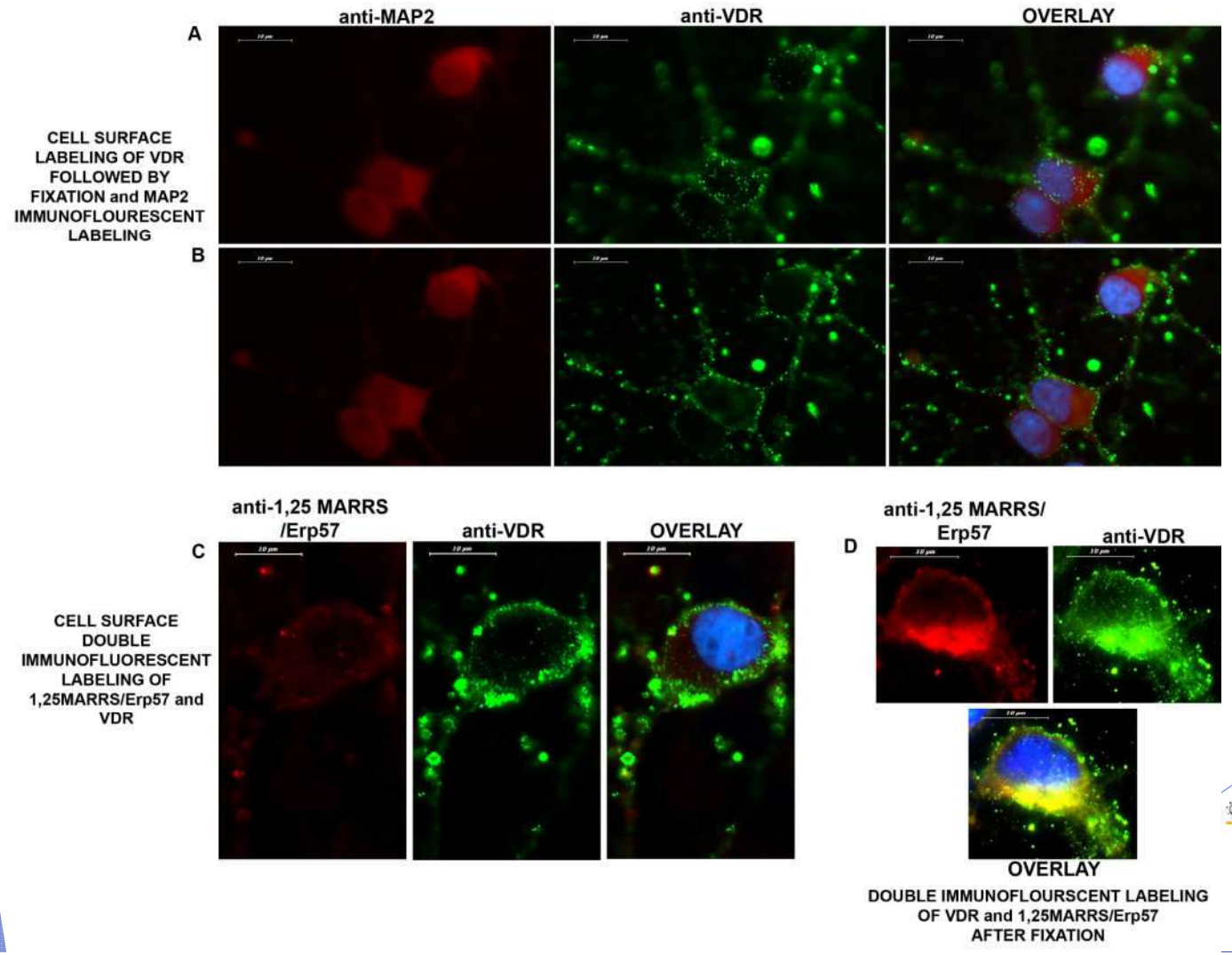


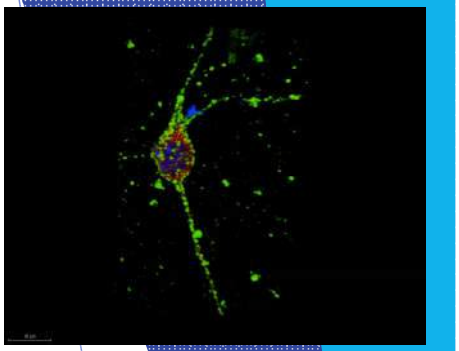
- **If VDR and amyloid beta 1-42 effects the transcription or post-transcriptional regulation of the same genes then the absence of one may disrupt the balance in neurons.**
- **Vitamin D deficiency or VDR dysfunction may promote this imbalance**

Hypothesis 2

- **Hypothesis 2**
  - VDR is located on neuronal plasma membrane!
  - VDR contributes to the action of the proteins involved in amyloidogenic or non-amyloidogenic pathways located in neuornal plasma membrane!
  - Vitamin D deficiency or VDR dysfunction may contribute to dysfunction of these pathways!

- *Is VDR present in neuronal plasma membranes?*
- *Is VDR colocalized with the proteins of amyloidogenic or non- amyloidogenic pathways?*





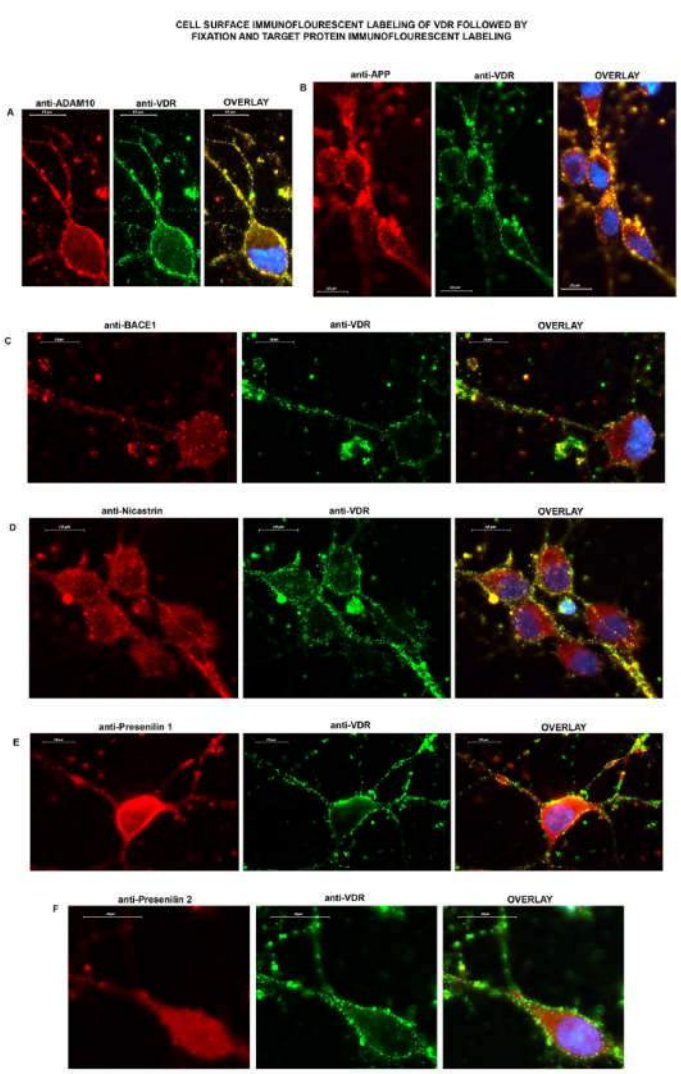
x63, 1/3. Alexafluor 488, anti-VDR (green); TX, Alexafluor 568, anti- MAP2 (red) was used as neuronal marker

PLOS ONE

RESEARCH ARTICLE

Vitamin D receptor is present on the neuronal plasma membrane and is co-localized with amyloid precursor protein, ADAM10 or Nicastrin

Erdinç Dursun, Duygu Gezen-Ak*



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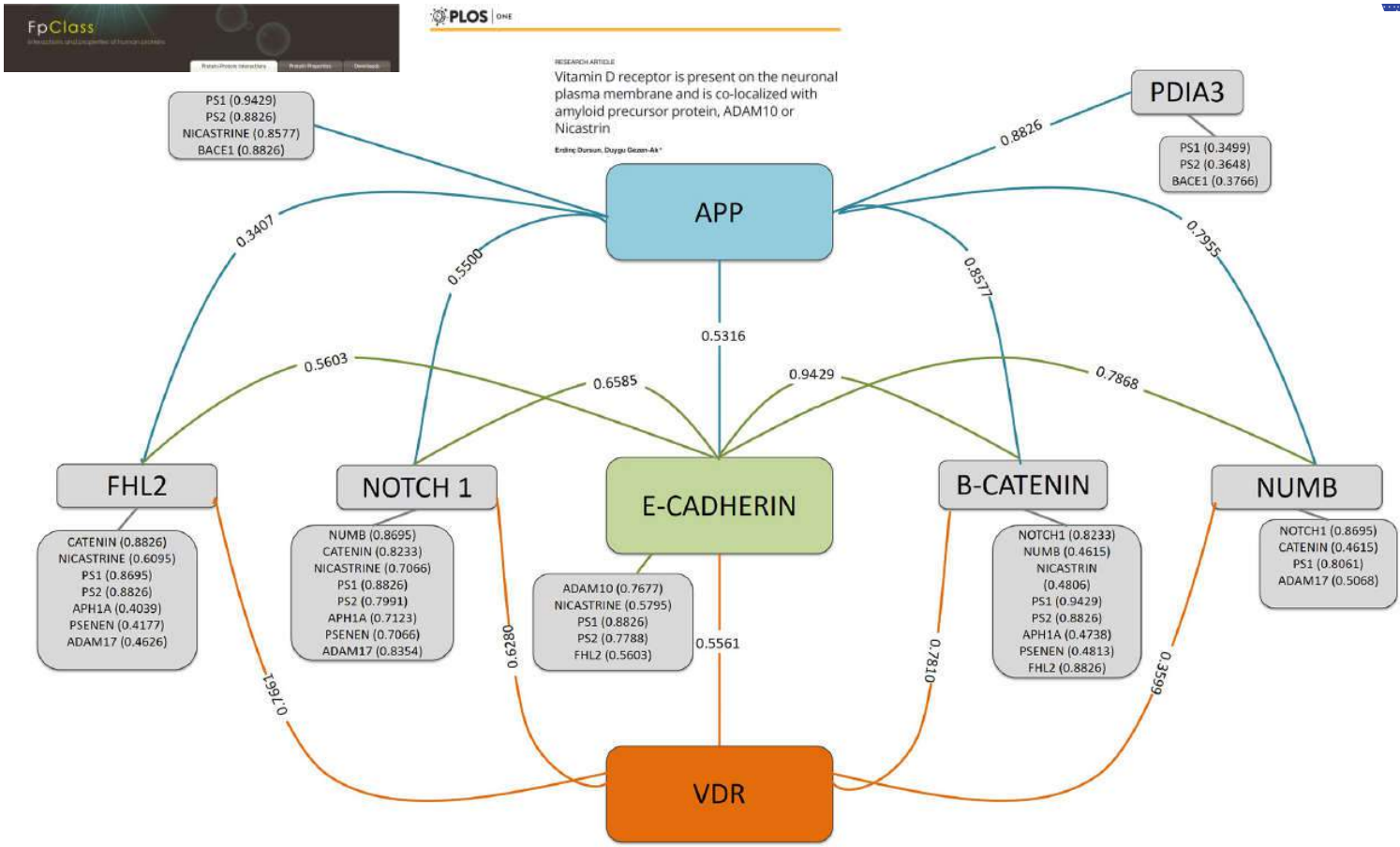


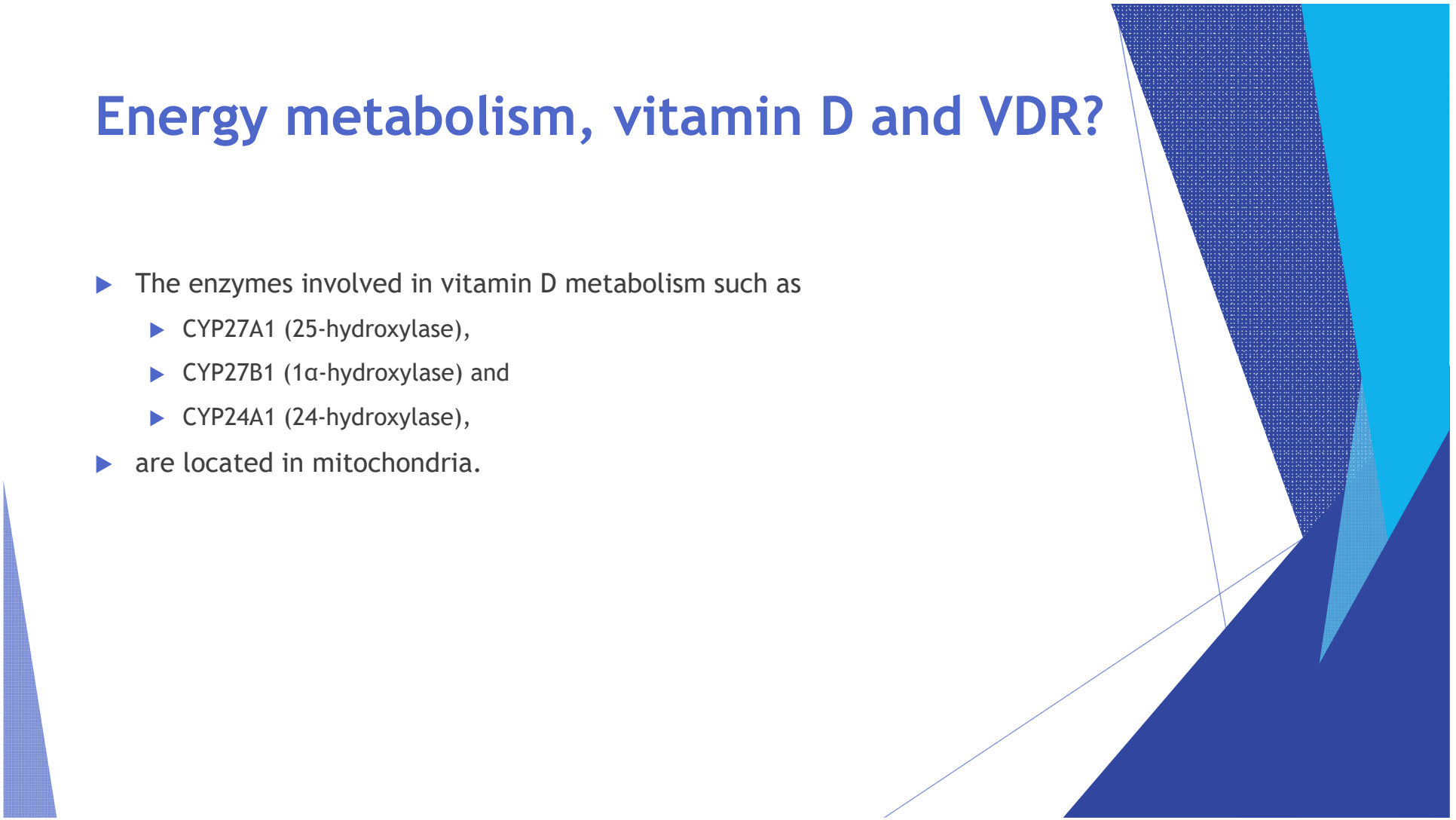
Fig 3. Summary of FpClass PPI prediction tool data. The FpClass PPI prediction tool was used to identify partner proteins for both APP and VDR. The tool predicted 1133 partners for APP and 583 partners for VDR. An analysis of the FpClass tool data indicated that 153 of these partners interacted with both APP and VDR. A total of 153 proteins were classified according to their functions in S1 Table. Five of these proteins (NUMB, catenin (CTNNB1), NOTCH1, E-cadherin (CDH1), and FHL2) were membrane or membrane-related proteins. These 5 proteins were used for further analyses with the target proteins (PS1, PS2, Nicastrin, BACE1, ADAM10) and PDIA3. The software predicted 5244 partners for these proteins, and the proteins that are the most relevant to plasma membrane interactions are presented in the figure with their PPI total score.

- How does VDR translocates into plasma membrane?
- Which proteins are interatcing with VDR directly in plasma membranes?
- What does VDR do in neuronal plasma membrane?

Investigations are ongoing…

Energy metabolism, vitamin D and VDR?

- The enzymes involved in vitamin D metabolism such as
  - CYP27A1 (25-hydroxylase),
  - CYP27B1 (1α-hydroxylase) and
  - CYP24A1 (24-hydroxylase),
- are located in mitochondria.



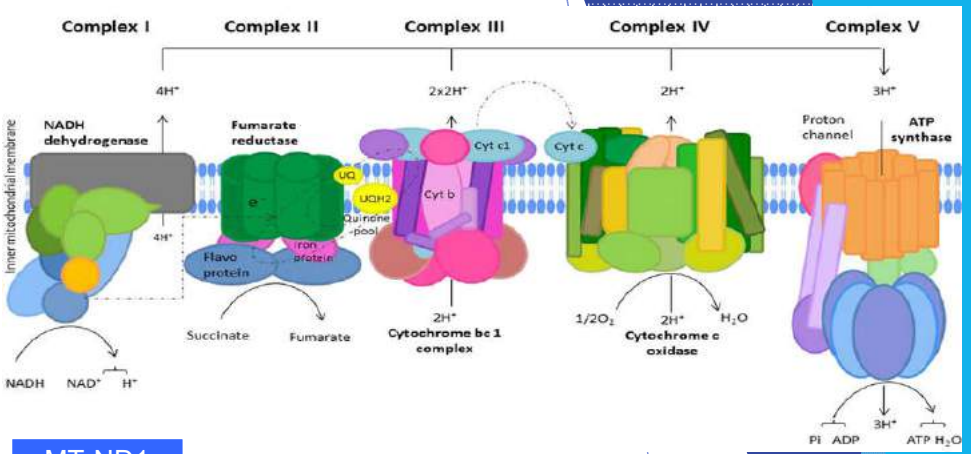
OXPHOS

- Our results indicate that

1. vitamin D or the disruption of vitamin D pathway have effects on mitochondrial gene expression.

2. vitamin D receptor might have a role as a transcription factor in mitochondria.

3. vitamin D deficiency or the disruption of vitamin D pathway might cause mitochondrial dysfunction which is accepted as one of the major reason in the development of neurodegenerative disorders.



MT-ND1
MT-ND2
MT-ND3
MT-ND4
MT-ND4L
MT-ND5
MT-ND6

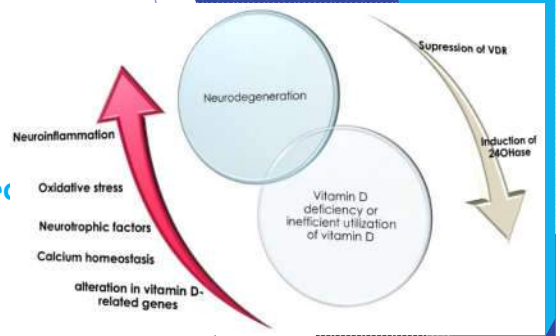
CYTB

MT-CO1
MT-CO2
MT-CO3

ATP 6
ATP 8

Conclusion

- The location of **vitamin D receptors or vitamin D metabolism related enzyemes** is **well established in CNS**,
- Vitamin D and VDR **definetly have functions in CNS**,
- Their **dysregulation** in CNS has a **high potential to cause or at least to be involved**
  - **neurodegenerative,**
    neurological or
    neuroinflamatory disorders
- Vitamin D has **major auto-/paracrine non genomic actions**, in addition to **its well documented activities as a steroid hormone in CNS**
- **Vitamin D and VDR** might be a part of **signal relaying complex in neuronal plasma membrane**
- **Vitamin D and VDR** may regulate gene expression together with **amyloid fragments**
  - The balance in such regulation might be a key for preventing neurodegeneration
- **Vitamin D and VDR** regulate **mitochondiral gene expression** and thus **energy metabolism**



BRAIN AND NEURODEGENERATIVE DISORDERS RESEARCH LAB

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ONAP-21712
ONAP-28651
27781
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24360