



HAL
open science

Additional Clues for a Protective Role of Vitamin D in Neurodegenerative Diseases: 1,25-Dihydroxyvitamin D3 Triggers an Anti-Inflammatory Response in Brain Pericytes

Marie-France Nissou, Audrey Guttin, Cyril Zenga, François Berger, Jean-Paul Issartel, Didier Wion

► To cite this version:

Marie-France Nissou, Audrey Guttin, Cyril Zenga, François Berger, Jean-Paul Issartel, et al.. Additional Clues for a Protective Role of Vitamin D in Neurodegenerative Diseases: 1,25-Dihydroxyvitamin D3 Triggers an Anti-Inflammatory Response in Brain Pericytes. *Journal of Alzheimer's Disease*, 2014, pp.789-99. 10.3233/JAD-140411 . inserm-01199883

HAL Id: inserm-01199883

<https://inserm.hal.science/inserm-01199883>

Submitted on 16 Sep 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Additional Clues for a Protective Role of Vitamin D in Neurodegenerative Diseases:
1,25-Dihydroxyvitamin D₃ Triggers an Anti-Inflammatory Response in Brain Pericytes

Marie-France Nissou^{a,b,d,1}, Audrey Guttin^{b,c,e,1}, Cyril Zenga^a, François Berger^{a,b,c,d},
Jean-Paul Issartel^{b,c,f,1} and Didier Wion^{a,b,1,*}

^aINSERM U1167, CLINATEC, Centre de Recherche Edmond J Safran, MINATEC Campus CEA, Grenoble, France

^bINSERM U836, Grenoble Institut des Neurosciences, Team Nanomedicine and Brain, Grenoble, France

^cUniversity of Grenoble Alpes, Grenoble, France

^dCHU de Grenoble and Institut de Biologie et Pathologie, Grenoble, France

^eCaCyS, EHPE, CNRS, UJF, Grenoble, France

^fCNRS, Grenoble, France

Abstract. Epidemiological and experimental studies suggest that 1,25-dihydroxyvitamin D₃ (1,25D) plays a neuroprotective role in neurodegenerative diseases including Alzheimer's disease. Most of the experimental data regarding the genes regulated by this hormone in brain cells have been obtained with neuron and glial cells. Pericytes play a critical role in brain function that encompasses their classical function in blood-brain barrier control and maintenance. However, the gene response of brain pericytes to 1,25D remains to be investigated. Analyses of the transcriptomic response of human brain pericytes to 1,25D demonstrate that human brain pericytes in culture respond to 1,25D by regulating genes involved in the control of neuroinflammation. In addition, pericytes respond to the pro-inflammatory cytokines tumor necrosis factor α and Interferon γ by inducing the expression of the CYP27B1 gene which is involved in 1,25D synthesis. Taken together, these results suggest that neuroinflammation could trigger the synthesis of 1,25D by brain pericytes, which in turn respond to the hormone by a global anti-inflammatory response. These findings identify brain pericytes as a novel 1,25D-responsive cell type and provide additional evidence for the potential value of vitamin D in the prevention or therapy of Alzheimer's disease and other neurodegenerative/neuropsychiatric diseases associated with an inflammatory component.

INTRODUCTION

Recent data suggest a role for 1,25-dihydroxyvitamin D₃ (1,25D) in the prevention or in the therapy of neurodegenerative and neuropsychiatric diseases including multiple sclerosis and Alzheimer's disease (AD) (for recent reviews, see [1–10]). 1,25D is a secosteroid hormone produced from vitamin D through a two-step hydroxylation process successively producing 25-hydroxyvitamin D₃ (25D) and vitamin D's most active metabolite 1,25D. This last step is catalyzed by the 25-Hydroxyvitamin D₃ 1 α -Hydroxylase named CYP27B1. Inactivation of 1,25D occurs through another hydroxylation step catalyzed by the vitamin D 24-Hydroxylase named CYP24A1, and generating 1,24,25-trihydroxyvitamin D₃ [11, 12]. 1,25D regulates the transcription of its cell-specific target genes by interacting with a protein named vitamin D receptor (VDR). The presence of VDR in brain [13–16], the capacity of 1,25D to regulate the expression of neurotrophic factors (for reviews, see [17]), and its anti-inflammatory potential [2, 18] are strong arguments in favor of a role for 1,25D in brain function. Regarding brain diseases, many recent studies report an association between vitamin D insufficiency and neurodegenerative/neuropsychiatric disorders [5, 7, 10, 19, 20]. Experimental evidence also demonstrates a preventive or therapeutic potential for 1,25D in brain disorders including multiple sclerosis, Parkinson's disease, AD, and traumatic brain injury (for recent reviews, see [21–24]). Investigating the gene response to vitamin D in brain cells is critical for understanding the molecular basis of its neuroprotective effect. Most of the experimental data regarding the potential of 1,25D to regulate the expression of genes able to prevent or delay the progression of neurodegenerative diseases have been obtained *in vitro* using brain-derived cells. These include neurons and macro/microglial cells (see [25–32]). However, little attention has been given to another abundant brain cell type, the brain pericyte. Pericytes are microvessel cells that wrap around endothelial cells [33–35]. The pericyte-to-endothelial ratio is 1 : 3, and the total length of capillaries in human brain is around 400 miles [36, 37]. The edge-to-edge inter-microvessels distance in rat brain is around 20 μ m in gray matter and 30 μ m in white matter [38]. This means that pericytes are very close to every neuronal cell. Because of their perivascular localization, pericytes are components of the neurovascular unit (NVU), an anatomical and functional entity associating neurons, glial and immune cells, endothelial cells, pericytes, and the extracellular matrix [33–35, 39, 40]. How brain pericytes communicate with glial and neuronal cells in NVU is still largely unknown, but they are, however, essential for proper functions of neurons [36, 41]. In addition to their role in angiogenesis, vessel maintenance, and blood-brain barrier formation and functions [33, 35], brain pericytes also contribute to immune and inflammatory responses by synthesizing pro-inflammatory cytokines including CXCL8/IL-8, CXCL11/ITAC, CCL5/Rantes, tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and interleukin-6 (IL-6) [42]. They participate to the adaptive immune response [43] and also have an immunosuppressive function by decreasing T lymphocyte proliferation [44]. The importance of pericytes in brain function is assessed by the observation that age-dependent vascular damage in pericyte-deficient mice precedes neuronal degenerative changes, learning impairment, memory deficiency, and the neuroinflammatory response [41]. Pericyte loss or dysfunction influences multiple steps of AD in several experimental models [36, 41, 45]. Hence, targeting brain pericytes is now considered as a novel therapeutic option for AD [39, 41, 45, 46]. The emerging evidence for a role of vitamin D in brain function and its potential preventive and therapeutic interest in the management of neurodegenerative disease prompted us to analyze the transcriptomic response of human brain pericyte to 1,25D. Our results provide evidence for a functional relationship between vitamin D and pericytes in the control of inflammation that could explain some of the anti-inflammatory and neuroprotective effects of vitamin D.

MATERIAL AND METHODS

Cell culture

Human brain pericytes and human brain endothelial cells were obtained from ScienCell Research Laboratories (San Diego, CA, USA). Cells were cultured on poly-lysine coated culture dishes according to the provider instructions. Briefly, the growth medium for pericytes was made with Pericyte Basal Culture Medium supplemented with 2% fetal bovine serum, with Pericyte Cell Growth Supplement and with penicillin and streptomycin, all from Science-Cell Research Laboratories (San Diego, CA, USA).

Brain endothelial cells were cultured in Endothelial Basal Culture Medium supplemented with Endothelial Growth Supplement and with penicillin and streptomycin, all from ScienCell Research Laboratories. In the absence of a definitive pan-marker for pericytes, immunophenotyping of pericytes relies on the use of a panel of different markers [34]. Positive markers can include PDGFR β , nestin, and CD13/aminopeptidase N, whereas VE-Cad can be used as a negative marker. Immunophenotyping of our cell cultures demonstrated a homogeneous positive labelling for PDGFR β , nestin, CD13/aminopeptidase N and no detectable synthesis of the endothelial marker VE-Cadherin (Supplementary Fig. 1A-D). On the contrary, brain endothelial cells stained positively for the endothelial marker VE-Cadherin (Supplementary Fig. 1E). TNF- α and Interferon- γ (PeproTech, Neuilly-sur-Seine, France) were used at 50 ng/ml.

Western blot analysis

For western blot analysis, cell pellets were resuspended and lysed in RIPA buffer (Cell Signaling Technology, Danvers, USA) supplemented with complete Protease Inhibitor Cocktail (Cell Signaling Technology, Danvers, USA). The proteins (40 μ g) were resolved with 8% polyacrylamide gel and transferred onto the Hybond N+ membrane (Amersham, Velizy-Villacoublay, France) according to standard protocols. Blots were then probed with the VDR antibody N20 (Santa Cruz, sc-1009) or D6 (Santa Cruz, sc-13133), followed by incubation with the corresponding horseradish peroxidase conjugated secondary antibodies (Santa Cruz, sc-2004 or sc-2005).

RNA extraction and RT-qPCR

2 μ g of total RNA were transcribed into cDNA using iScriptTM Reverse Transcription Supermix for RT-qPCR (BioRad Laboratories, Marnes-la-Coquette, France). PCR primers (Eurogentec, Angers, France) for each gene were designed using the Universal Probe Library Assay Design Center (<https://www.roche-applied-science.com/sis/rtqpcr/upl/ezhome.html>) and sequences of the primers used are given as Supplementary Table 1. Then real-time PCRs were performed according to the SYBR Green methodology using the SsoAdvancedTM SYBR Green Supermix (BioRad Laboratories, Marnes-la-Coquette, France), on a CFX96 TouchTM Real-Time PCR Detection System (BioRad Laboratories, Marnes-la-Coquette, France). Reference genes are β -actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), whose expressions were not affected by 1,25D according to our Affymetrix data (data not shown). Analyses were performed as previously described [25] with CFX ManagerTM software (BioRad Laboratories, Marnes-la-Coquette, France) using $\Delta\Delta$ Ct method. Each qPCR was performed in triplicate for PCR yield validation and all reactions were performed on three different biological samples. Data were quantified relative to gene expressions of pericyte cells without 1,25D treatment, which was standardized to 100. The statistical validation was given by the CFX Manager software and $p < 0.005$ was considered significant.

Gene expression profiling

Total RNA (300 ng) extracted as described above was reverse transcribed and labelled with 3 prime IVT Express kit (Affymetrix, High Wycombe, UK) following the manufacturer's instructions. Labelled samples were hybridized on GeneChip Human Genome U133 Plus 2.0 (Affymetrix, High Wycombe, UK). This microarray contains over 47,000 unique transcripts which correspond approximately to 39,000 human genes. Subsequent wash, stain, and scan were performed according to the standard Affymetrix protocols. Raw expression data were normalized using the Robust Multi-array Average method. Experiments were repeated twice using different RNA samples on GeneChip (Affymetrix, High Wycombe, UK) and corroborated by RT-qPCR for genes of interest. The transcriptomic data have been deposited in NCBI's Gene Expression Omnibus and are accessible through the Gene Expression Omnibus accession number GSE54765.

RESULTS

Pericytes express VDR and respond to 1,25D by inducing CYP24A1 expression

1,25D regulates gene expression of its target genes by interacting with the nuclear receptor VDR. We first investigated the presence of VDR in human brain pericyte. Western blot analysis of pericyte cell lysates with the VDR polyclonal antibody N20 detected the presence of a 53 kDa band consistent with the presence of VDR in these cells (Fig. 1A). A similar result was obtained with the VDR monoclonal antibody D6 (Supplementary Fig. 2). We next determined the functionality of the 1,25D/VDR transduction pathway in human brain pericytes by analyzing CYP24A1 regulation by 1,25D (CYP24A1 is a well-known 1,25D-responsive gene [47]). Results presented in Fig. 1B demonstrate the strong induction of CYP24A1 gene expression in pericytes treated for 24 hours with 10^{-8} M 1,25D.

Brain pericyte transcriptomic response to 1,25D

Having demonstrated the presence of VDR in human brain pericytes and the responsiveness of these cells to 1,25D, we analyzed the transcriptomic response of brain pericytes to 1,25D. RNA from pericytes cultured for 24 hours either with 10^{-8} M 1,25D or with vehicle only (ethanol) were extracted and processed for transcriptomic analyses. Experiments were performed on two different RNA preparations for each condition. Twenty genes were found upregulated by a factor of at least 1.7, and 5 down-regulated genes were also detected (expression ratios lower than 0.7) (Table 1). Eight of these genes were already described as 1,25D targets in cells other than pericytes. These genes are cytochrome P450A1 (CYP24A1) [47], elongation factor Tu GTP binding domain (EFTUD1) [48], thrombomodulin (THBD) [49], odd-skipped related 2 (OSR2) [50], Kruppel-like factor 4 (KLF4) [51], insulin-like growth factor binding protein 5 (IGFBP5) [52], fatty acid binding protein 4 (FABP4) [53], and tumor necrosis factor (ligand) superfamily member 4 (TNFS4/OX40L) [54]. An analysis of the 25 genes found regulated by 1,25D was carried out with the GeneDeck analysis tool (<http://www.genecards.org/>). This allowed the identification of 14 disorders associated with this set of genes with a p value lower than 10^{-5} . Six of these disorders were inflammatory diseases: endotheliitis ($p = 2.2 \times 10^{-10}$), asthma ($p = 1.4 \times 10^{-6}$), rheumatoid arthritis ($p = 2 \times 10^{-6}$), arthritis ($p = 5.1 \times 10^{-6}$), inflammation ($p = 6.4 \times 10^{-6}$), and pancreatitis ($p = 1.1 \times 10^{-5}$). The other disorders include various cancers and hypertension. To confirm the relationship existing between the transcriptomic response of brain pericytes to 1,25D and inflammation, a bibliographic search in PubMed associating each of these 25 genes with the keyword "inflammation" was done. Relevant results were obtained for 8 genes. These genes are vascular cell adhesion molecule 1 (VCAM1), THBD, KLF4, tumor necrosis factor alpha-induced protein 6 (TNFAIP6), chordin-like 1 (CHRD1),

chemokine (C-C motif) ligand 2 (CCL2), FABP4, tumor necrosis factor (ligand) superfamily member 4 (TNFSF4). THBD, KLF4, FABP4, and TNFSF4 have already been described as 1,25D-responsive genes in cells other than brain cells or pericytes [49, 51, 53, 54]. Confirmatory RT-qPCR analyses were performed that validated the transcriptomic data for the 4 remaining genes which were not previously described as 1,25D targets (Fig. 2). These are CCL2, CHDRL1, TNFPAIP6, and VCAM-1. KLF4 and TNFSF4 were also included as positive controls in these confirmatory experiments. Importantly the regulations observed are consistent with a global anti-inflammatory response (see discussion below).

Induction of CYP27B1 expression by the pro-inflammatory cytokines TNF- α and Interferon- γ

25D is the most abundant metabolite of vitamin D in the circulation, but the most active metabolite is 1,25D [11, 12]. Hydroxylation of 25D to active 1,25D is required for the induction of the 1,25D-dependent signaling pathway [11, 12]. This reaction is carried out by 25-Hydroxyvitamin D3 1 α -Hydroxylase CYP27B1, the rate limiting enzyme in 1,25D synthesis. Analysis of our transcriptomic data for CYP27B1 failed to detect any expression for this gene in our pericyte cultures (data not shown). Considering the global anti-inflammatory gene response of pericytes to 1,25D, we hypothesized that inflammatory stimuli could upregulate CYP27B1 gene expression in these cells. This would allow pericytes to metabolize 25D into 1,25D and then limit the inflammatory response locally. To investigate this point we treated brain pericytes with TNF- α and Interferon- γ which were recently found to upregulate CYP27B1 in astrocytes [55]. Figure 3 showed that a treatment of pericytes for 24 hours with these two inflammatory cytokines stimulated the expression of CYP27B1.

DISCUSSION

Epidemiological studies reveal a relationship between several neurodegenerative/psychiatric diseases and low vitamin D levels [5, 7, 10, 19–21, 57, 58]. Although association does not mean causation, the possible role of vitamin D as a protective co-factor against brain disorders is further supported by data demonstrating the neuroprotective effect of vitamin D or of its active metabolite 1,25D in several experimental models of brain diseases (for recent reviews, see [1–10]). Understanding the molecular pathways involved in these effects is critical for optimizing clinical trials. At the transcriptomic level, 1,25D is already known to upregulate several genes relevant to neuroprotection in brain cells. Several of these genes encode neurotrophins including NGF and GDNF [59–61]. 1,25D is a potent immunoregulatory agent [2–4, 62, 63]. In neurons or glial cells, 1,25D regulates the expression of genes involved in the control of neuroinflammation, such as cystathionine beta-synthase, which codes for the enzyme producing the neuroprotective agent hydrogen sulfide [25], and gamma-glutamyl transpeptidase (gamma-GT) which is the enzyme that synthesizes the antioxidant glutathione [64]. 1,25D also reduces the expression of the inflammatory cytokines M-CSF and TNF- α in astrocytes challenged with lipopolysaccharides [65], and protects brain cells from nitric oxide excessive production by down-regulating iNOS [30]. However, all these data have been obtained with cells other than pericytes, and the global gene response of pericytes to 1,25D remained to be investigated. This point is critical regarding the importance of pericytes in brain function [33–46]. The present study reveals that eight out of the twenty five genes we found regulated by 1,25D in brain pericytes are related to inflammation. With exception of VCAM1, all of these genes are involved in critical anti-inflammatory processes. This regulation is consistent with a global anti-inflammatory process since pro-inflammatory genes are found down-regulated, while anti-inflammatory genes are upregulated.

Pro-inflammatory genes down-regulated by 1,25D in pericytes

CCL2/MCP-1 is an inflammatory chemokine that is also able to modulate blood-brain barrier permeability. It is upregulated in AD patients and notably in brain microvessels [66]. High plasma concentration of CCL2 are found in mild AD [67] and is associated with a faster rate of cognitive decline during the early stages of AD [68]. CCL2 is also associated with multiple sclerosis patients and experimental autoimmune encephalomyelitis (EAE). Moreover, deficiency in the CCL2 receptor CCR2 confers resistance to EAE [69]. TNFSF4/OX40L is the ligand of the OX40 receptor. The OX40-OX40L interaction is critical for the regulation of T cell tolerance and T-cell mediated inflammatory diseases [70]. Consequently, OX40 signaling is involved in allergic inflammation [71, 72] and in multiple sclerosis, and is also involved in the development of EAE [73]. Down-regulation of TNFSF4/OX40L is suggested to provide a novel approach for treating inflammatory disease [72, 74–76]. The fatty acid binding protein FABP4 acts at the interface of metabolic and inflammatory pathways [77]. It is involved in the development of a chronic metabolic inflammatory state recently referred to as metaflammation that connects obesity with inflammation [77]. FABP4 is associated with inflammatory factors related to obesity [78], and is also implicated in asthma [79] and rheumatoid arthritis [80]. To our knowledge, the involvement of FABP4 and the potential of inhibitors of FABP4 have not yet been investigated in inflammatory neurodegenerative disorders.

Anti-inflammatory genes upregulated by 1,25D in pericytes

Thrombomodulin (THBD) is a well characterized 1,25D responsive gene proposed as a biomarker for evaluating the effect of vitamin D supplementation [49]. The anti-inflammatory properties of thrombomodulin have been recently reviewed [81, 82]. THBD sequesters thrombin which has pro-inflammatory activities, in addition to its role in hemostasis and thrombosis. Note that thrombin is highly expressed in AD brain vessels and is a mediator of cerebral inflammation in AD. Thrombomodulin also promotes the activation of protein C, which, in addition to its anticoagulant activity, also has anti-inflammatory effects by suppressing the production of the pro-inflammatory cytokine TNF- α [83]. Tumor necrosis factor, alpha-induced protein 6 (TNFAIP6/TSG6) is a secreted protein whose expression is upregulated in response to pro-inflammatory cytokines such as TNF and IL-1. It has anti-inflammatory effects and acts as a negative feedback modulator by down-regulating the inflammatory response [84]. The identification of KLF4 as a 1,25D upregulated gene is in agreement with a previous finding obtained with keratinocytes [51] and is in line with a general role of 1,25D and KLF4 in the control of inflammatory responses. KLF4 inhibits endothelial inflammation [85] and represses the expression of histidine carboxylase, which is the enzyme that converts histidine to histamine, a major actor of allergy and inflammation [86]. In macrophage KLF4 promotes the anti-inflammatory macrophage/microglia M2 phenotype at the expense of the M1 pro-inflammatory phenotype [87]. In this regard, it is worth mentioning that M2 microglia is reported to drive oligodendrocyte differentiation during CNS remyelination. Although such effects are observed with macrophages, they may be relevant in the case of pericytes. Indeed, these cells are a source of macrophage activity, macrophage markers, phagocytosis, and antigen presentation [88]. Note, however, that KLF4 also increases the synthesis of several pro-inflammatory cytokines in M1 microglial cells [89]. This suggests that the effects of 1,25D on KLF4 expression could participate to the control of a balanced inflammatory response, thus preventing the development of chronic inflammation. Chordin-like protein 1 (CHRD1), also known as Ventroptin, is a bone morphogenetic protein-4 antagonist [90, 91]. It antagonizes the function of BMP4 by binding to it and preventing its interaction with receptors. Increased expression of BMP4 mRNA within the hippocampus dentate

gyrus is correlated with a decrease in cell proliferation in A_{PP}^{swe/PS1 Δ E9} transgenic mice [92]. BMP4 is upregulated during EAE [93], and this cytokine is also reported to mediate inflammation in endothelial cells [94]. Hence, the synthesis of CHRDL1 by pericytes would protect cells from inflammation. Vascular adhesion molecule-1 (VCAM1) is a cell surface protein with adhesion properties. It is a lymphocyte adhesion molecule induced on human endothelium by inflammatory stimuli [95] and has been initially considered as an inflammatory marker. However, it is not clear whether its function is only directed toward the aggravation of the original insult or if, depending on the context, it is also involved in the restoration of homeostasis. VCAM1 plays a key role in the brain in maintaining subventricular zone adult stem cell niche structure and function [96]. This suggests that VCAM1 could have similar functions in the maintenance of the structure and functions of the NVU by regulating cell-cell junctions. Upregulation of VCAM1 has been suggested to play a protective role in maintaining the integrity of the ependymal zone during neuroinflammation [96]. VCAM-1 depletion leads to the rapid decrease of the subventricular zone neural stem cell population, suggesting that chronic VCAM-1 insufficiency/deficiency would lead to neural stem cell depletion [96]. Collectively, our gene expression data provide strong evidence that the gene response of human brain pericytes to 1,25D is directed toward the control of neuroinflammation.

Expression of CYP27B1 in pericytes can be induced by an inflammatory stimulus

1,25D results from the metabolism of 25D by 25-Hydroxyvitamin D3 1 α -Hydroxylase (CYP27B1). Thus, the availability of 1,25D at the cellular level depends on both circulating levels of 25D, and CYP27B1 expression level. 25D is detected in human cerebrospinal fluid [58]. Therefore, the characterization of stimuli able to induce CYP27B1 is critical. Here we report that TNF- α and Interferon- γ , two inflammatory cytokines recently described to regulate CYP27B1 expression in astrocytes and microglial cells [55], also induce the expression of CYP27B1 in pericytes. Note that pericytes are reported to secrete TNF- α [42]. Our data strongly suggest the existence of a regulatory intracrine/autocrine feedback loop in pericytes balancing the inflammatory potential of TNF- α and Interferon- γ . A paracrine role for the 1,25D produced by pericytes in the NVU during inflammation can also be considered. In conclusion, our results point to brain pericytes and 1,25D as two active players in the regulation of neuroinflammation. They provide additional evidence for a role of vitamin D metabolites in the prevention and the therapy of neurodegenerative/neuropsychiatric diseases through the modulation of neuroinflammation. They also strengthened the interest of targeting brain pericytes for controlling brain disorders such as AD [41, 45, 46, 97, 98].

ACKNOWLEDGMENTS

We thank Dr. A. Hihi for his critical reading of the manuscript.

Authors' disclosures available online (<http://www.jalz.com/disclosures/view.php?id=2286>).

SUPPLEMENTARY MATERIAL

Supplementary material is available in the electronic version of this article:

<http://dx.doi.org/10.3233/JAD-140411>.

REFERENCES

[1] Gezen-Ak D, Yilmazer S, Dursun E (2014) Why vitamin D in Alzheimer's disease? The hypothesis. *J Alzheimers Dis* **40**, 257-269.

- [2] Fernandes de Abreu DA, Eyles D, Féron F (2009) Vitamin D, a neuro-immunomodulator: Implications for neurodegenerative and autoimmune diseases. *Psychoneuroendocrinology***34**(Suppl 1), S265-S277.
- [3] DeLuca GC, Kimball SM, Kolasinski J, Ramagopalan SV, Ebers GC (2013) Review: The role of vitamin D in nervous system health and disease. *Neuropathol Appl Neurobiol***39**, 458-484.
- [4] Smolders J, Moen SM, Damoiseaux J, Huitinga I, Holmøy T (2011) Vitamin D in the healthy and inflamed central nervous system: Access and function. *J Neurol Sci***311**, 37-43.
- [5] Balion C, Griffith LE, Striffler L, Henderson M, Patterson C, Heckman G, Llewellyn DJ, Raina P (2012) Vitamin D, cognition, and dementia: A systematic review and meta-analysis
- [6] Kesby JP, Eyles DW, Burne THJ, McGrath JJ (2011) The effects of vitamin D on brain development and adult brain function. *Mol Cell Endocrinol***347**, 121-127.
- [7] Annweiler C, Montero-Odasso M, Llewellyn DJ, Richard-Devantoy S, Duque G, Beauchet O (2013) Meta-analysis of memory and executive dysfunctions in relation to vitamin D. *J Alzheimers Dis* **37**, 147-171.
- [8] Soni M, Kos K, Lang IA, Jones K, Melzer D, Llewellyn DJ (2012) Vitamin D and cognitive function. *Scand J Clin Lab Invest Suppl***243**, 79-82.
- [9] Stewart A, Wong K, Cachat J, Elegante M, Gilder T, Mohnot S, Wu N, Minasyan A, Tuohimaa P, Kalueff AV (2010) Neurosteroid vitamin D system as a nontraditional drug target in neuropsychopharmacology. *Behav Pharmacol***21**, 420-426.
- [10] Anglin RES, Samaan Z, Walter SD, McDonald SD (2013) Vitamin D deficiency and depression in adults: Systematic review and meta-analysis. *Br J Psychiatry J Ment Sci***202**, 100-107.
- [11] Holick MF (2007) Vitamin D deficiency. *N Engl J Med* **357**, 266-281.
- [12] Norman AW (2008) From vitamin D to hormone D: Fundamentals of the vitamin D endocrine system essential for good health. *Am J Clin Nutr***88**, 491S-499S.
- [13] Cui X, Pelekanos M, Liu P-Y, Burne THJ, McGrath JJ, Eyles DW (2013) The vitamin D receptor in dopamine neurons; its presence in human substantia nigra and its ontogenesis in rat midbrain. *Neuroscience* **236**, 77-87.
- [14] Eyles DW, Smith S, Kinobe R, Hewison M, McGrath JJ (2005) Distribution of the vitamin D receptor and 1 α -hydroxylase in human brain. *J Chem Neuroanat***29**, 21-30.
- [15] Prüfer K, Veenstra TD, Jirikowski GF, Kumar R (1999) Distribution of 1,25-dihydroxyvitamin D₃ receptor immunoreactivity in the rat brain and spinal cord. *J Chem Neuroanat***16**, 135-145.
- [16] Stumpf WE, O'Brien LP (1987) 1,25 (OH)₂ vitamin D₃ sites of action in the brain. An autoradiographic study. *Histochemistry***87**, 393-406.
- [17] Garcion E, Wion-Barbot N, Montero-Menei CN, Berger F, Wion D (2002) New clues about vitamin D functions in the nervous system. *Trends Endocrinol Metab***13**, 100-105.
- [18] Vanoirbeek E, Krishnan A, Eelen G, Verlinden L, Bouillon R, Feldman D, Verstuyf A (2011) The anti-cancer and anti-inflammatory actions of 1,25(OH)₂D₃. *Best Pract Res Clin Endocrinol Metab***25**, 593-604.
- [19] Annweiler C, Llewellyn DJ, Beauchet O (2013) Low serum vitamin D concentrations in Alzheimer's disease: A systematic review and meta-analysis. *J Alzheimers Dis* **33**, 659-674.
- [20] McGrath JJ, Burne TH, Féron F, Mackay-Sim A, Eyles DW (2010) Developmental vitamin D deficiency and risk of schizophrenia: A 10-year update. *Schizophr Bull* **36**, 1073-1078.
- [21] Evatt ML (2014) Parkinson disease: Low vitamin D and Parkinson disease—a causal conundrum. *Nat Rev Neurol***10**, 8-9.

- [22] Eyles DW, Burne THJ, McGrath JJ (2013) Vitamin D, effectson brain development, adult brain function and the linksbetween lowlevels of vitaminDand neuropsychiatric disease. *Front Neuroendocrino***34**, 47-64.
- [23] Mesliniene S, Ramrattan L, Giddings S, Sheikh-AliM(2013)Role of vitamin D in the onset, progression, and severity ofmultiple sclerosis. *EndocrPract***19**, 129-136.
- [24] Stein DG, Cekic MM (2011) Progesterone and vitamin Dhormone as a biologic treatment of traumatic brain injury inthe aged. *PM R* **3**, S100-S110.
- [25] Nissou M-F, Brocard J, El Atifi M, Guttin A, Andrieux A, Berger F, Issartel J-P, Wion D (2013) The transcriptomicresponse ofmixed neuron-glia cell cultures to1,25-dihydroxyvitamin D3 includes genes limiting the progression of neurodegenerative diseases. *J Alzheimers Dis* **35**,553-564.
- [26] Dursun E, Gezen-AkD,Yilmazer S (2013)Anewmechanismfor amyloid- β induction of iNOS: Vitamin D-VDR pathwaydisruption. *J Alzheimers Dis* **36**, 459-474.
- [27] Neveu I, Naveilhan P, Jehan F, Baudet C, Wion D, De LucaHF, Brachet P (1994) 1,25-dihydroxyvitamin D3 regulates thesynthesis of nerve growth factor in primary cultures of glialcells. *Brain Res Mol Brain Res* **24**, 70-76.
- [28] R´emy S, Naveilhan P, Brachet P, Neveu I (2001) Differentialregulation of GDNF, neurturin, and their receptors in primarycultures of rat glial cells. *J Neurosci Res* **64**, 242-251.
- [29] Naveilhan P, Neveu I, Baudet C, Funakoshi H, Wion D, BrachetP, MetsisM(1996) 1,25-Dihydroxyvitamin D3 regulatesthe expression of the low-affinity neurotrophin receptor. *Brain Res Mol Brain Res* **41**, 259-268.
- [30] Garcion E, Nataf S, Berod A, Darcy F, Brachet P (1997) 1,25-Dihydroxyvitamin D3 inhibits the expression of inducible nitric oxide synthase in rat central nervous system during experimental allergic encephalomyelitis. *Brain Res Mol BrainRes* **45**, 255-267.
- [31] Correale J, Ysraelit MC, Gait´an MI (2011) Vitamin Dmediatedimmune regulation in multiple sclerosis. *J NeuroSci***311**, 23-31.
- [32] Lefebvre d’Hellencourt C, Montero-Menei CN, BernardR, Couez D (2003) Vitamin D3 inhibits proinflammatorycytokines and nitric oxide production by the EOC13microglial cell line. *J Neurosci Res* **71**, 575-582.
- [33] Ballabh P, Braun A, Nedergaard M (2004) The blood-brainbarrier: An overview: Structure, regulation, and clinical implications. *Neurobiol Dis* **16**, 1-13.
- [34] Dore-Duffy P, Cleary K (2011) Morphology and propertiesof pericytes. *Methods Mol***686**, 49-68.
- [35] D´iaz-Flores L, Guti´errez R, Madrid JF,VarelaH,Valladares F,Acosta E, Mart´ın-VasalloP,D´iaz-Flores L Jr (2009) Pericytes.Morphofunction, interactions and pathology in a quiescent and activated mesenchymal cell niche. *HistolHistopathol***24**,909-969.
- [36] Winkler EA, Bell RD, Zlokovic BV (2011) Central nervoussystem pericytes in health and disease. *Nat Neurosci***14**, 1398-1405.
- [37] Zlokovic BV (2008) The blood-brain barrier in health andchronic neurodegenerative disorders. *Neuron* **57**, 178-201.
- [38] Schlageter KE, Molnar P, Lapin GD, Groothuis DR(1999) Microvessel organization and structure in experimentalbrain tumors: Microvessel populations with distinctivestructural and functional properties. *Microvasc Res* **58**,312-328.
- [39] Stanimirovic DB, Friedman A (2012) Pathophysiology of theneurovascular unit: Disease cause or consequence? *J CerebBlood Flow Metab***32**, 1207-1221.
- [40] S´a-Pereira I, Brites D, Brito MA (2012) Neurovascular unit:A focus on pericytes. *MolNeurobiol***45**, 327-347.

- [41] Bell RD, Winkler EA, Sagare AP, Singh I, LaRue B, Deane R, Zlokovic BV (2010) Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging. *Neuron* **68**, 409-427.
- [42] Alcendor DJ, Charest AM, Zhu WQ, Vigil HE, Knobel SM (2012) Infection and upregulation of proinflammatory cytokines in human brain vascular pericytes by human cytomegalovirus. *J Neuroinflammation* **9**, 95.
- [43] Pober JS, Tellides G (2012) Participation of blood vessel cells in human adaptive immune responses. *Trends Immunol* **33**, 49-57.
- [44] Tu Z, Li Y, Smith DS, Sheibani N, Huang S, Kern T, Lin F (2011) Retinal pericytes inhibit activated T cell proliferation. *Invest Ophthalmol Vis Sci* **52**, 9005-9010.
- [45] Sagare AP, Bell RD, Zhao Z, Ma Q, Winkler EA, Ramanathan A, Zlokovic BV (2013) Pericyte loss influences Alzheimer-like neurodegeneration in mice. *Nat Commun* **4**, 2932.
- [46] Zlokovic BV (2011) Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nat Rev Neurosci* **12**, 723-738.
- [47] Naveilhan P, Neveu I, Baudet C, Ohya M, Brachet P, Wion D (1993) Expression of 25(OH) vitamin D3 24-hydroxylase gene in glial cells. *Neuroreport* **5**, 255-257.
- [48] Milani C, Katayama MLH, de Lyra EC, Welsh J, Campos LT, Brentani MM, Maciel M, do S, Roela RA, del Valle PR, Góes JCGS, Nonogaki S, Tamura RE, Folgueira MAAK (2013) Transcriptional effects of 1,25 dihydroxyvitamin D(3) physiological and supra-physiological concentrations in breast cancer organotypic culture. *BMC Cancer* **13**, 119.
- [49] Carlberg C, Seuter S, de Mello VDF, Schwab U, Voutilainen S, Pulkki K, Nurmi T, Virtanen J, Tuomainen T-P, Uusitupa M (2013) Primary vitamin D target genes allow a categorization of possible benefits of vitamin D3 supplementation. *PLoS One* **8**, e71042.
- [50] Verlinden L, Kriebitzsch C, Eelen G, Van Camp M, Leyssens C, Tan BK, Beullens I, Verstuyf A (2013) The odd-skipped related genes *Osr1* and *Osr2* are induced by 1,25-dihydroxyvitamin D3. *J Steroid Biochem Mol Biol* **136**, 94-97.
- [51] Lu J, Goldstein KM, Chen P, Huang S, Gelbert LM, Nagpal S (2005) Transcriptional profiling of keratinocytes reveals a vitamin D-regulated epidermal differentiation network. *J Invest Dermatol* **124**, 778-785.
- [52] Schmid C, Schläpfer I, Gosteli-Peter MA, Hauri C, Froesch ER, Zapf J (1996) 1 alpha,25-dihydroxyvitamin D3 increases IGF binding protein-5 expression in cultured osteoblasts. *FEBS Lett* **392**, 21-24.
- [53] Lee H, Bae S, Yoon Y (2012) Anti-adipogenic effects of 1,25-dihydroxyvitamin D3 are mediated by the maintenance of the wingless-type MMTV integration site/ β -catenin pathway. *Int J Mol Med* **30**, 1219-1224.
- [54] Nguyen NLH, Chen K, McAleer J, Kolls JK (2013) Vitamin D regulation of OX40 ligand in immune responses to *Aspergillus fumigatus*. *Infect Immun* **81**, 1510-1519.
- [55] Smolders J, Schuurman KG, van Strien ME, Melief J, Hendrickx D, Hol EM, van Eden C, Luchetti S, Huitinga I (2013) Expression of vitamin D receptor and metabolizing enzymes in multiple sclerosis-affected brain tissue. *J Neuropathol Exp Neurol* **72**, 91-105.
- [56] Afzal S, Bojesen SE, Nordestgaard BG (2014) Reduced 25-hydroxyvitamin D and risk of Alzheimer's disease and vascular dementia. *Alzheimers Dement* **10**, 296-302.
- [57] Dickens AP, Lang IA, Langa KM, Kos K, Llewellyn DJ (2011) Vitamin D, cognitive dysfunction and dementia in older adults. *CNS Drugs* **25**, 629-639.

- [58] Johansson P, Almqvist EG, Johansson J-O, Mattsson N, Andreasson U, Hansson O, Wallin A, Blennow K, Zetterberg H, Svensson J (2013) Cerebrospinal fluid (CSF) 25-hydroxyvitamin D concentration and CSF acetylcholinesterase activity are reduced in patients with Alzheimer's disease. *PLoS One* **8**, e81989.
- [59] Wion D, MacGrogan D, Neveu I, Jehan F, Houlgatte R, Brachet P (1991) 1,25-Dihydroxyvitamin D₃ is a potent inducer of nerve growth factor synthesis. *J Neurosci Res* **28**, 110-114.
- [60] Brown J, Bianco JL, McGrath JJ, Eyles DW (2003) 1,25-dihydroxyvitamin D₃ induces nerve growth factor, promotes neurite outgrowth and inhibits mitosis in embryonic rat hippocampal neurons. *Neurosci Lett* **343**, 139-143.
- [61] Naveilhan P, Neveu I, Wion D, Brachet P (1996) 1,25-Dihydroxyvitamin D₃, an inducer of glial cell line-derived neurotrophic factor. *Neuroreport* **7**, 2171-2175.
- [62] Griffin MD, Xing N, Kumar R (2003) Vitamin D and its analogs as regulators of immune activation and antigen presentation. *Annu Rev Nutr* **23**, 117-145.
- [63] Mathieu C, van Etten E, Decallonne B, Guilietti A, Gysemans C, Bouillon R, Overbergh L (2004) Vitamin D and 1,25-dihydroxyvitamin D₃ as modulators in the immune system. *J Steroid Biochem Mol Biol* **89-90** 449-452.
- [64] Garcion E, Sindji L, Leblondel G, Brachet P, Darcy F (1999) 1,25-dihydroxyvitamin D₃ regulates the synthesis of gamma glutamyl transpeptidase and glutathione levels in rat primary astrocytes. *J Neurochem* **73**, 859-866.
- [65] Furman I, Baudet C, Brachet P (1996) Differential expression of M-CSF, LIF, and TNF-alpha genes in normal and malignant rat glial cells: Regulation by lipopolysaccharide and vitamin D. *J Neurosci Res* **46**, 360-366.
- [66] Grammas P, O'Vase R (2001) Inflammatory factors are elevated in brain microvessels in Alzheimer's disease. *Neurobiol Aging* **22**, 837-842.
- [67] Galimberti D, Fenoglio C, Lovati C, Venturelli E, Guidi I, Corra B, Scalabrini D, Clerici F, Mariani C, Bresolin N, Scarpini E (2006) Serum MCP-1 levels are increased in mild cognitive impairment and mild Alzheimer's disease. *Neurobiol Aging* **27**, 1763-1768.
- [68] Westin K, Buchhave P, Nielsen H, Minthon L, Janciauskiene S, Hansson O (2012) CCL2 is associated with a faster rate of cognitive decline during early stages of Alzheimer's disease. *PLoS One* **7**, e30525.
- [69] Izikson L, Klein RS, Charo I, Weiner HL, Luster AD (2000) Resistance to experimental autoimmune encephalomyelitis in mice lacking the CC chemokine receptor (CCR)2. *J Exp Med* **192**, 1075-1080.
- [70] Ishii N, Takahashi T, Soroosh P, Sugamura K (2010) OX40-OX40 ligand interaction in T-cell-mediated immunity and immunopathology. *Adv Immunol* **105**, 63-98.
- [71] Xiao X, Balasubramanian S, Liu W, Chu X, Wang H, Taparowsky EJ, Fu Y-X, Choi Y, Walsh MC, Li XC (2012) OX40 signaling favors the induction of TH9 cells and airway inflammation. *Nat Immunol* **13**, 981-990.
- [72] Wang Y-H, Liu Y-J (2007) OX40-OX40L interactions: A promising therapeutic target for allergic diseases? *J Clin Invest* **117**, 3655-3657.
- [73] Carboni S, Aboul-Enein F, Waltzinger C, Killeen N, Lassmann H, Pena-Rossi C (2003) CD134 plays a crucial role in the pathogenesis of EAE and is upregulated in the CNS of patients with multiple sclerosis. *J Neuroimmunol* **145**, 1-11.
- [74] Kaur D, Brightling C (2012) OX40/OX40 ligand interactions in T-cell regulation and asthma. *Chest* **141**, 494-499.

- [75] Karulf M, Kelly A, Weinberg AD, Gold JA (2010) OX40ligand regulates inflammation and mortality in the innate immune response to sepsis. *J Immunol***185**, 4856-4862.
- [76] Redmond WL, Weinberg AD (2007) Targeting OX40 and OX40L for the treatment of autoimmunity and cancer. *Crit Rev Immunol***27**, 415-436.
- [77] Furuhashi M, Ishimura S, Ota H, Miura T (2011) Lipid chaperones and metabolic inflammation. *Int J Inflamm***2011**, 642612.
- [78] Terra X, Quintero Y, Auguet T, Porrás JA, Hernández M, Sabench F, Aguilar C, Luna AM, Del Castillo D, Richart C (2011) FABP 4 is associated with inflammatory markers and metabolic syndrome in morbidly obese women. *Eur J Endocrinol***164**, 539-547.
- [79] Shum BOV, Mackay CR, Gorgun CZ, Frost MJ, Kumar RK, Hotamisligil GS, Rolph MS (2006) The adipocyte fatty acid-binding protein aP2 is required in allergic airway inflammation. *J Clin Invest* **116**, 2183-2192.
- [80] Andres Cerezo L, Kuklova M, Hulejova H, Vernerova Z, Pesakova V, Pecha O, Veigl D, Haluzík M, Pavelka K, Vencovsky J, Senolt L (2013) The level of fatty acid-binding protein 4, a novel adipokine, is increased in rheumatoid arthritis and correlates with serum cholesterol levels. *Cytokine* **64**, 441-447.
- [81] Morser J (2012) Thrombomodulin links coagulation to inflammation and immunity. *Curr Drug Targets* **13**, 421-431.
- [82] Conway EM (2012) Thrombomodulin and its role in inflammation. *Semin Immunopathol***34**, 107-125.
- [83] Grey ST, Tsuchida A, Hau H, Orthner CL, Salem HH, Hancock WW (1994) Selective inhibitory effects of the anticoagulant activated protein C on the responses of human mononuclear phagocytes to LPS, IFN-gamma, or phorbol ester. *J Immunol***153**, 3664-3672.
- [84] Milner CM, Day AJ (2003) TSG-6: A multifunctional protein associated with inflammation. *J Cell Sci***116**, 1863-1873.
- [85] Shen B, Smith RS Jr, Hsu Y-T, Chao L, Chao J (2009) Kruppel-like factor 4 is a novel mediator of Kallistatin in inhibiting endothelial inflammation via increased endothelial nitric-oxide synthase expression. *J Biol Chem***284**, 35471-35478.
- [86] Ai W, Liu Y, Langlois M, Wang TC (2004) Kruppel-like factor 4 (KLF4) represses histidine decarboxylase gene expression through an upstream Sp1 site and downstream gastrin responsive elements. *J Biol Chem***279**, 8684-8693.
- [87] Liao X, Sharma N, Kapadia F, Zhou G, Lu Y, Hong H, Paruchuri K, Mahabeleshwar GH, Dalmas E, Venteclef N, Flask CA, Kim J, Doreian BW, Lu KQ, Kaestner KH, Hamik A, Clément K, Jain MK (2011) Kruppel-like factor 4 regulates macrophage polarization. *J Clin Invest* **121**, 2736-2749.
- [88] Thomas WE (1999) Brain macrophages: On the role of pericytes and perivascular cells. *Brain Res Brain Res Rev* **31**, 42-57.
- [89] Kaushik DK, Mukhopadhyay R, Kumawat KL, Gupta M, Basu A (2012) Therapeutic targeting of Kruppel-like factor 4 abrogates microglial activation. *J Neuroinflammation***9**, 57.
- [90] Sakuta H, Suzuki R, Takahashi H, Kato A, Shintani T, Iemura S, Yamamoto TS, Ueno N, Noda M (2001) Vntroptin: ABMP-4 antagonist expressed in a double-gradient pattern in the retina. *Science* **293**, 111-115.
- [91] Kane R, Godson C, O'Brien C (2008) Chordin-like 1, a bone morphogenetic protein-4 antagonist, is upregulated by hypoxia in human retinal pericytes and plays a role in regulating angiogenesis. *Mol Vis* **14**, 1138-1148.

- [92] Li D, Tang J, Xu H, Fan X, Bai Y, Yang L (2008) Decreased hippocampal cell proliferation correlates with increased expression of BMP4 in the APP^{swe}/PS1^{DeltaE9} mouse model of Alzheimer's disease. *Hippocampus* **18**, 692-698.
- [93] Ara J, See J, Mamontov P, Hahn A, Bannerman P, Pleasure D, Grinspan JB (2008) Bone morphogenetic proteins 4, 6, and 7 are up-regulated in mouse spinal cord during experimental autoimmune encephalomyelitis. *J Neurosci Res* **86**, 125-135.
- [94] Jo H, Song H, Mowbray A (2006) Role of NADPH oxidases in disturbed flow- and BMP4- induced inflammation and atherosclerosis. *Antioxid Redox Signal* **8**, 1609-1619.
- [95] Osborn L, Hession C, Tizard R, Vassallo C, Luhowskyj S, Chi-Rosso G, Lobb R (1989) Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. *Cell* **59**, 1203-1211
- [96] Kokovay E, Wang Y, Kusek G, Wurster R, Lederman P, Lowry N, Shen Q, Temple S (2012) VCAM1 is essential to maintain the structure of the SVZ niche and acts as an environmental sensor to regulate SVZ lineage progression. *Cell Stem Cell* **11**, 220-230.
- [97] Sengillo JD, Winkler EA, Walker CT, Sullivan JS, Johnson M, Zlokovic BV (2013) Deficiency in mural vascular cells coincides with blood-brain barrier disruption in Alzheimer's disease. *Brain Pathol* **23**, 303-310.
- [98] Pimentel-Coelho PM, Rivest S (2012) The early contribution of cerebrovascular factors to the pathogenesis of Alzheimer's disease. *Eur J Neurosci* **35**, 1917-1937.

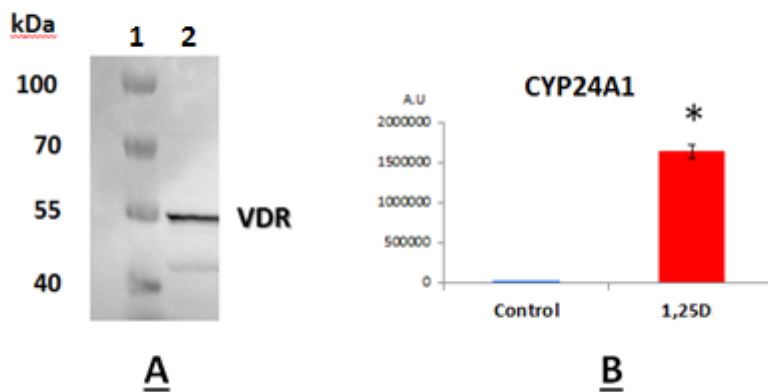


Fig. 1. Human pericyte cells express VDR and respond to 1,25D3. A) Immunoblot showing the presence of VDR in human pericyte brain cell cultures. Lane 1, molecular weight markers; lane 2: pericyte cell lysate. The blot was incubated with the VDR antibody N20. B) RT-qPCR assay of the mRNA of the 1,25D inducible gene vitamin D 24-Hydroxylase (CYP24A1) demonstrates a significant increase in CYP24A1 mRNA amount when pericytes are cultured for 24 hours with 10⁻⁸ M 1,25D3. (**p* < 0.005); A.U: arbitrary units.

Probe set ID	Gene Name		Exp 1	Exp 2	Average	Ref
206504_at	cytochrome P450, family 24, subfamily A,	CYP24A1	158,2	355,8	237,2	[24]
203868_s_at	vascular cell adhesion molecule 1	VCAM1	3,4	3,2	3,4	
218973_at	elongation factor Tu GTP binding domain	EFTUD1	3,0	3,8	3,4	[25]
203887_s_at	thrombomodulin	THBD	2,8	2,8	2,8	[26]
213568_at	odd-skipped related 2 (Drosophila)	OSR2	2,0	3,7	2,7	[27]
221841_s_at	Kruppel-like factor 4 (gut)	KLF4	2,3	3,1	2,7	[28]
224762_at	serine incorporator 2	SERINC2	2,2	3,4	2,7	
206025_s_at	tumor necrosis factor, alpha-induced prot.	TNFAIP6	2,4	2,8	2,6	
210002_at	GATA binding protein 6	GATA6	2,4	2,4	2,4	
206765_at	potassium inwardly-rectifying channel, s	KCNJ2	2,1	2,6	2,3	
225532_at	Cdk5 and Abl enzyme substrate 1	CABLES1	2,3	2,0	2,1	
204345_at	collagen, type XVI, alpha 1	COL16A1	1,5	2,4	1,9	
229581_at	extracellular leucine-rich repeat and fi	ELFN1	1,7	2,1	1,9	
209763_at	chordin-like 1	CHRD1	1,6	2,3	1,9	
219025_at	CD248 molecule, endosialin	CD248	1,7	1,9	1,8	
212190_at	serpin peptidase inhibitor, clade E	SERPINE2	1,9	1,6	1,7	
211958_at	insulin-like growth factor binding protein	IGFBP5	1,7	1,8	1,7	[29]
221872_at	retinoic acid receptor responder (tazarot	RARRES1	2,0	1,5	1,7	
209840_s_at	leucine rich repeat neuronal 3	LRRN3	1,7	1,7	1,7	
200878_at	endothelial PAS domain protein 1	EPAS1	1,6	1,7	1,7	
216598_s_at	chemokine (C-C motif) ligand 2	CCL2	0,6	0,7	0,7	
203980_at	fatty acid binding protein 4, adipocyte	FABP4	0,6	0,7	0,6	[30]
207426_s_at	tumor necrosis factor (ligand) superfam	TNFSF4	0,6	0,6	0,6	[31]
222862_s_at	adenylate kinase 5	AK5	0,6	0,6	0,6	
239909_at	ADAMTS-like 1	ADAMTSL1	0,4	0,4	0,5	

Table 1:List of differentially expressed genes in human brain pericyte cultures in the presence of 10^{-8} M 1,25D (average induction fold ≥ 1.7 or ≤ 0.7 compared to control cells). Fold changes are the average of two independent experiments. Published references for genes previously reported to be modulated by 1,25D are indicated.

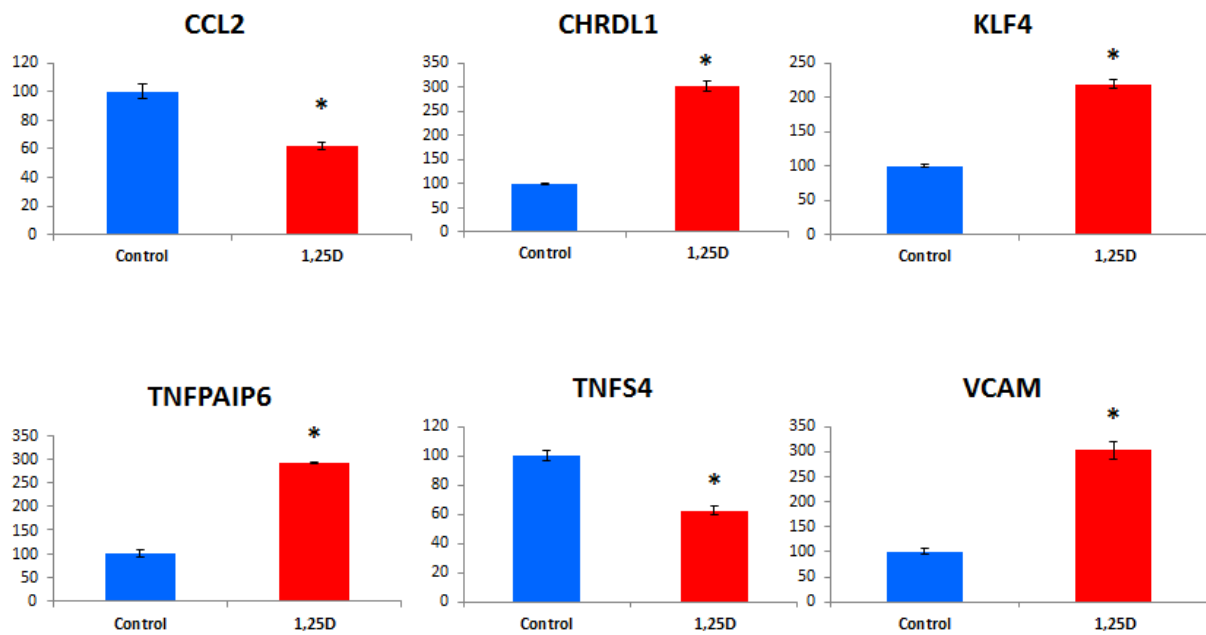


Fig. 2. Confirmatory RT-qPCR of the transcriptomic data for the genes newly identified as upregulated by 1,25D and involved in the regulation of inflammation. Results are depicted relative to control and normalized to actin and GAPDH mRNA. (* $p < 0.005$ compared to control). KLF4 and TNFSF4, two genes previously described as induced by 1,25D [51, 54], were included as positive control. CCL2, Chemokine (C-C motif) ligand 2; CHRDL1, chordin-like 1; KLF4, Kruppel-like factor 4; TNFAIP6, Tumor necrosis Factor, alpha-induced protein 6; TNFSF4, Tumor necrosis factor (ligand) superfamily member 4; VCAM, Vascular cell adhesion molecule 1.

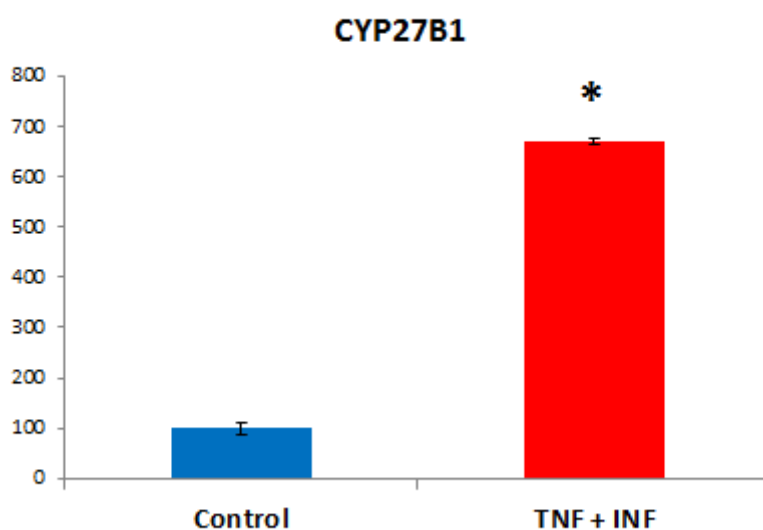


Fig. 3. TNF- α and Interferon- γ induce the expression of 25-Hydroxyvitamin D3 1 α -Hydroxylase (CYP27B1) in human brain pericytes. Human brain pericytes were cultured in the presence or absence of TNF- α (50 ng/ml) and Interferon- γ (50 ng/ml) for 24 hours. Then RNA were extracted and CYP27B1 expression quantified by RT-qPCR. (* $p < 0.005$ compared to control).